Respiratory tract infection: prevention, early detection and attenuation of immune response

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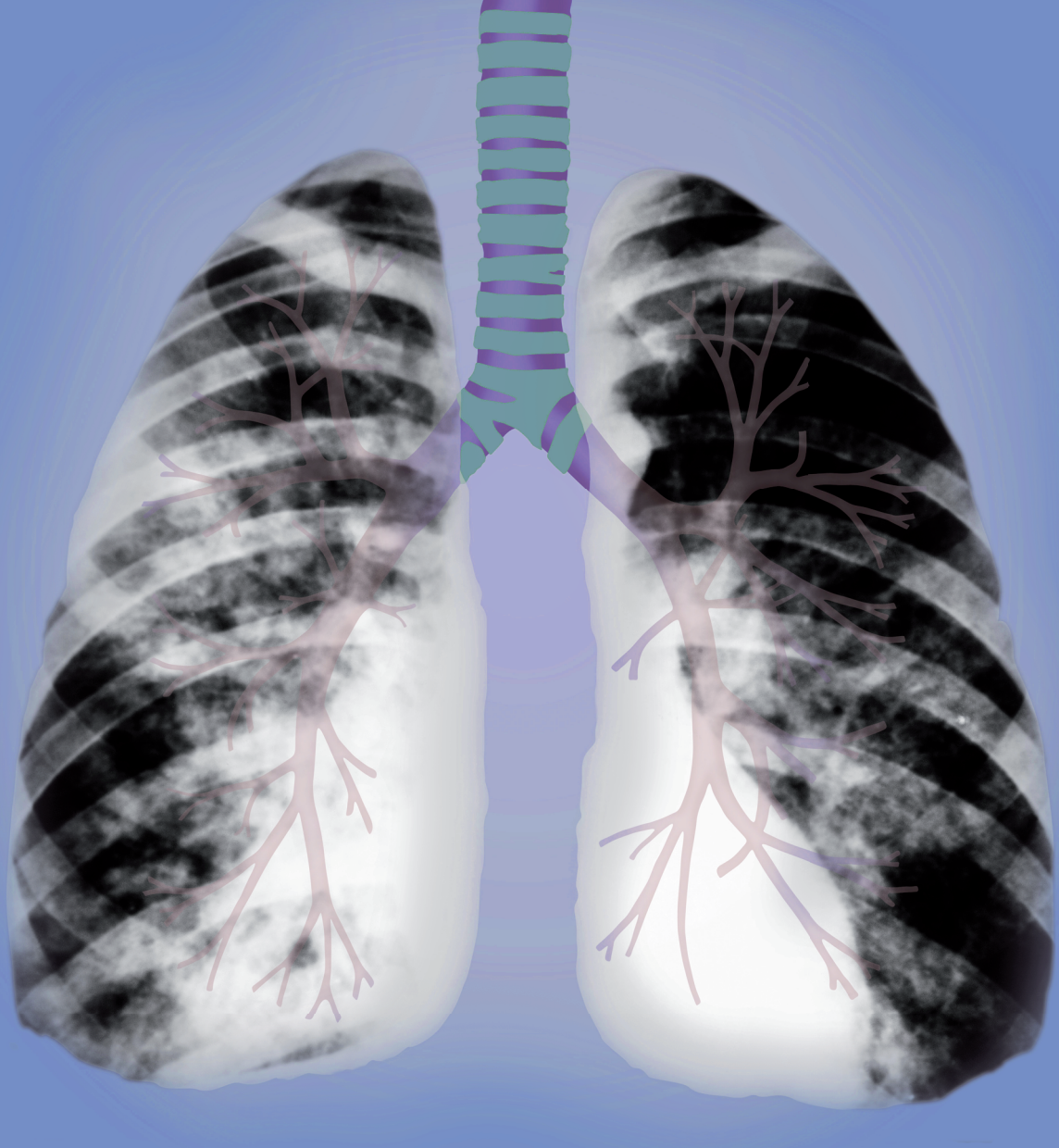

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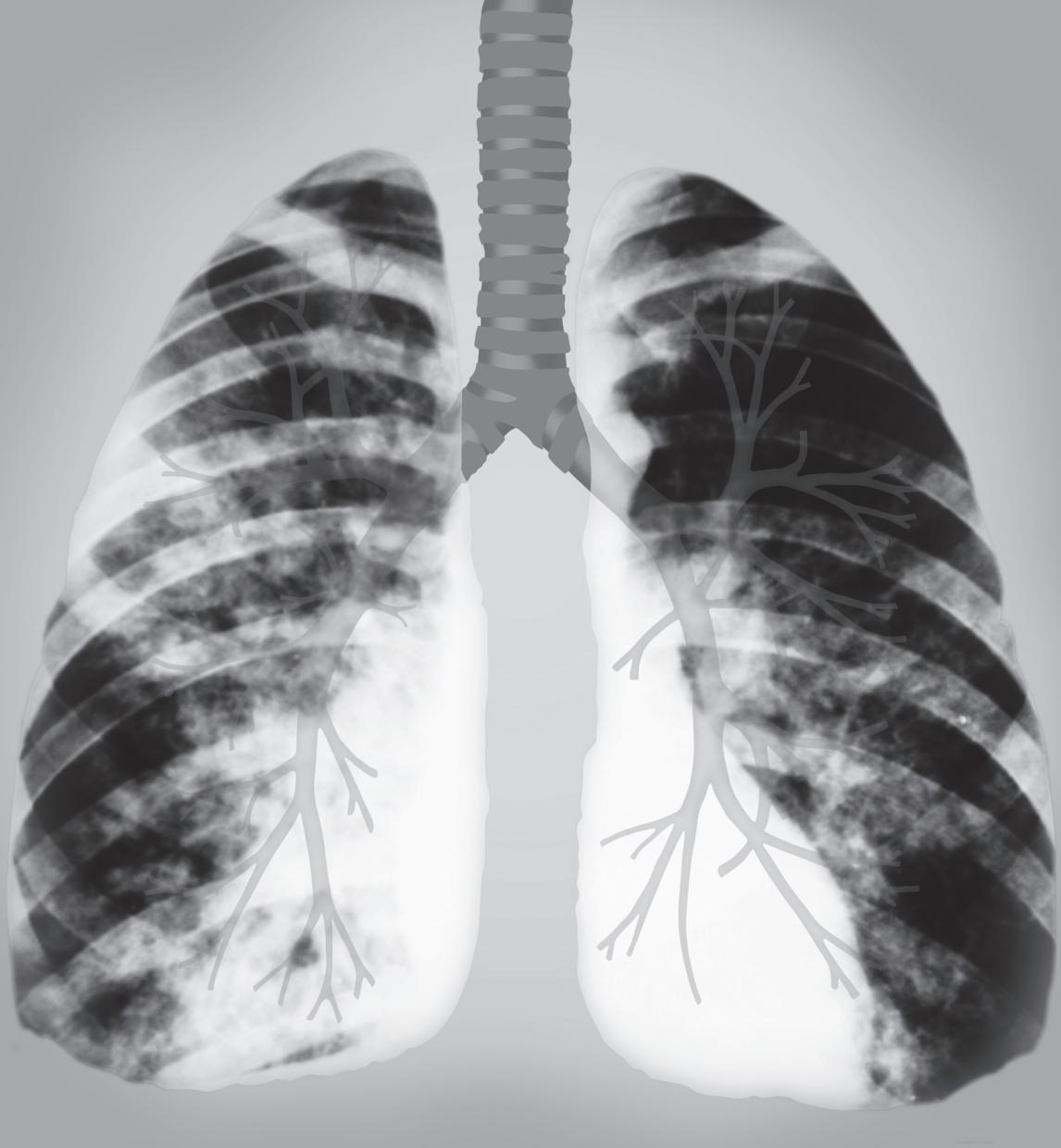
Contents



1

Introduction and outline of this thesis

Respiratory tract infections

Respiratory tract infections are among the most common infections treated by health care practitioners. In this respect, a distinction is made between upper and lower respiratory tract infections. The tract is divided in an upper part above the vocal cords (including the nose and nasal passages, paranasal sinuses, the pharynx, and the portion of the larynx above the vocal cords) and a lower part, below the vocal cords (including the larynx below the vocal folds, trachea, bronchi, bronchioles, and alveoli). Lower respiratory tract infections (LRTI) occur frequently and can have severe consequences: they accounted for the largest part of infectious disease mortality in the United States (1) and globally (2), with only limited improvement in recent decades.

Two aspects characterise an infectious disease such as LRTI: the establishment of a microorganism on or within a host and (tissue) damage due to the replicating microorganism itself, its toxins, or the inflammatory response of the host to its presence. The combination of these aspects in the lower respiratory tract leads to bronchitis, bronchiolitis or pneumonia. Patients may present with acute disease (e.g., pneumococcal pneumonia) or chronic respiratory tract infection (e.g., tuberculosis). Acute lower respiratory tract infections are mostly defined by complaints lasting for less than three weeks. The host inflammatory response indirectly causes symptoms such as fever, and the local tissue damage and extension of the pulmonary infiltrate determine the intensity of coughing, production of sputum, shortness of breath, and sometimes, pleural pain.

Microbial aetiology

Viruses are the most common etiologic microorganisms causing acute lower respiratory tract infections. Various types of viruses may cause bronchitis, whereas some of these are also able to cause bronchiolitis or pneumonia. For example, human rhinovirus most often causes mild (upper respiratory tract infection or) bronchitis. Influenza virus and respiratory syncytial virus (RSV) may cause more severe bronchiolitis and pneumonia, but can also be found in the upper respiratory tract.

Viral infections may pave the way for bacterial ('super') infection, in particular in patients already colonized with bacteria because of an underlying disease such as COPD or bronchiectasis. Superinfection may result in pneumonia, and such bacterial infections may progress rapidly and result in severely ill patients. Common bacterial etiologic microorganisms are *Streptococcus pneumoniae*, *Haemophilus influenzae, Legionella pneumophila,* and *Mycoplasma pneumoniae*. Finally, fungi and parasites may cause pneumonia as well. However, these last infections occur predominantly in susceptible, immunocompromised hosts – due to underlying disease or immunosuppressive treatment – or are linked to exposure in a specific geographic area.

In the Netherlands, the average patient with a community-acquired acute lower respiratory tract infection most likely has a viral or bacterial cause of infection or a combined infection with both a virus and a bacterium (3-6). Yearly, such infections account for the admittance of about 50.000 individuals to hospitals in the Netherlands (www.zorgatlas.nl).

Host inflammatory response

Most body cells can detect the presence of microbes with pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) which recognize pathogen-associated molecular patterns (PAMPs) on the invading microorganisms. In respiratory epithelial cells, typically among the first cell types to become involved in a respiratory tract infection, the activation of these PRRs is essential in limiting the spread of pathogens and in triggering an immune response through the release of proinflammatory mediators (cytokines and chemokines). These mediators initiate host innate and adaptive immune responses that – in most cases – succeed in containing or killing a tissue-invading microorganism (7).

This host inflammatory response comes, however, at a cost, as in most cases at least some collateral damage to the tissue occurs that must be restored and may result in some form of permanent scar (e.g., bronchiectasis, pleural adhesions). An uncontrolled and/or exacerbated inflammatory response to the microorganism may be associated with severe acute lung injury (such as Acute Respiratory Distress Syndrome) and consequently, severe morbidity and mortality (8, 9). Ideally, the host response to infection should accomplish sterilisation of a local site of infection without causing collateral damage in the form of destruction of lung tissue and lung architecture (9). Therefore, a delicate balance between an adequate inflammatory response to eradicate the causative microorganism followed or accompanied by an anti-inflammatory response, causing cytokine neutralization and inhibition of macrophage recruitment to dampen the host immune response, is paramount.

Prevention of the most vulnerable by vaccination

Rather than treating and coping with consequences of respiratory tract infections, in many cases, it is possible to vaccinate against pathogens causing LRTI and thus prevent infection and its consequences altogether. For respiratory tract infections, vaccines against influenza, *Bordetella pertussis*, *Streptococcus pneumonia,* and *Haemophilus influenzae* are used in various target groups. Vaccination mimics natural infection in the sense that it primes the host immune system for an adequate enhanced response upon re-exposure to the pathogen, but this is accomplished without the morbidity and risks of natural infection. In addition to providing individual protection, vaccination – if the vaccination rate surpasses a certain level determined by the ease of transmission of the pathogen – may also protect individuals not yet immunized (or too young or unable to respond to vaccination) by

herd immunity. In addition, transplacental transfer of antibodies helps protect new-born infants whose mothers were vaccinated (against influenza and *Bordetella pertussis*) (10, 11). Finally, vaccination of close contacts of certain patients may prevent the introduction of a pathogen in the proximity of these patients, thus shielding off ("cocooning") the most vulnerable in a group or family. This strategy is relevant because vaccination of immunocompromised, frail, and elderly patients may be less effective in preventing infection as compared with their young and healthy close contacts that provide a 'cordon sanitaire'.

In this way, vaccination of household contacts and healthcare professionals against easily transmissible pathogens such as the influenza virus may help protect vulnerable patients by minimizing, or at least reducing, exposure to these pathogens. Already, influenza vaccination coverage is higher among persons living with or caring for vulnerable patients, as compared to the ones without the proximity of vulnerable patients (12). However, in healthcare settings, professionals – both physicians and nurses alike – are often not inclined to get the flu vaccination. In fact, influenza vaccine coverage among healthcare professionals taking care of the most vulnerable patients is very low. Among healthcare workers in Europe, the coverage was below 30%, and in Dutch hospitals in 2012, median vaccination coverage amounted to 13% (13, 14) while more recently the coverage in Dutch hospitals appears to equal European levels. Still, the low acceptance of vaccination such as the one against influenza goes against the principle of always delivering safe care and puts vulnerable patients at risk of acquiring influenza infection during hospital care (15, 16).

In the case of health care providers, influenza vaccination also touches upon other issues, such as absenteeism from work, need for fellow workers to fill in, and the continuation of care at a time it is needed most. During the yearly influenza season, especially in severe seasons, hospitals struggle to meet the demand for care. Due to influenza virus infection and the complications thereof, increased numbers of patients present to hospital for admission in acute care settings. Unfortunately, healthcare staff absenteeism is highest during the peak of the influenza season, and this reduces capacity in hospitals and other (health) care facilities. To meet the demand for, and deliver adequate care without potentially harming patients, an improvement of influenza vaccination coverage among health care workers is urgently needed.

Prevention by vaccination in patients with a stimulated immune system

Besides anti-infective properties, the immune system plays a critical role in fighting off cancer, for example, by detecting and controlling the proliferation of malignant cells (17, 18). T-cells are key players in the anti-tumor immune response, and these cells have, therefore, been an important target for immunotherapeutic interventions. Tumor cells

interfere with immune checkpoints on activated T cells to trigger inhibitory pathways that downregulate the intensity and the extent of the immune response, thus providing tumor cells with the chance to proliferate. In recent years, it was shown that the anti-tumor response of the immune system could be enhanced by blocking these immune checkpoints, thereby 'unlocking' the cell-mediated anti-tumor activity. Immunotherapy has become standard treatment for several malignancies across all tumor stages, for example, for lung cancer, melanoma, and head and neck cancer. Interestingly, the pro-inflammatory potential of checkpoint inhibitors leads to various (auto-)immune-related adverse events (e.g., colitis, encephalitis, pneumonitis).

Influenza vaccination can prevent not only respiratory tract infection but also cardiovascular (and all-cause) mortality in patients with heart failure (19) and the need for interruption of chemotherapy treatment in cancer patients (20). Therefore, patients under treatment for cancer may benefit from yearly influenza vaccination and are elected to get this vaccine by the Health Council of the Netherlands and the Dutch National Institute for Public Health and the Environment (21, 22).

In 2018, a Swiss research group described an increased incidence of checkpoint inhibitors-use related side effects after influenza vaccination in a small cohort (n=23) and they discussed the potential causality between these two interventions (23).

Uncertainty as to the consequences of the findings of the Swiss study led to reluctance of pulmonologists and oncologists to advise patients treated with checkpoint inhibitors to get their yearly influenza vaccination. A reduced influenza vaccination coverage in these patients may increase influenza infections among them and, with that, may cause more interruptions of cancer treatment and more admissions for influenza-like illness in these vulnerable patients (24), and by consequence, worse treatment outcomes.

From an immunological standpoint, one can argue that T cell enhancement during influenza vaccination should increase immunization and protection. That way, this immunotherapy with checkpoint inhibitors may be regarded as a vaccine adjuvant. Clearly, mechanistic studies in this field are urgently needed. Likewise, a potential causal relation between immune-related adverse events after influenza vaccination in patients treated with various immunotherapies for cancer needs to be examined.

Outbreak control by improved detection and monitoring

Once a contagious infectious disease occurs within a community, measures to monitor and contain the impact and dissemination of the disease must be put into effect. In the Netherlands, this is taken up by various parties. Locally, by public health care authorities

that survey and notify specific diseases, and nationally, by the Centre for Infectious Disease Control at the National Institute for Public Health and the Environment that is responsible for organizing year-round surveillance and prepare-and-response to potential outbreak signals notified by the local authorities. In this way, the early detection of outbreaks is possible. After the large Legionella outbreak at the flower show in Bovenkarspel in 1999 (with 188 cases, 163 hospital admissions, and 21 deaths; case-fatality rate of 11%) (25), improvements in outbreak detection were implemented. Still, at the local and regional level, there are ample pitfalls of these systems that may delay outbreak detection:

- Microbiological diagnostics are time-consuming, especially in patients with uncommon presentations of common diseases.
- The list of notifiable diseases is limited.
- New, emerging infectious diseases may present as unknown syndrome, and this may delay outbreak detection.
- Patients of an outbreak may present at different health care institutions. The (extent of an) outbreak is not evident for an individual institution.

The Dutch Public Health Act obliges medical doctors to notify local public health authorities in case of an unusual number of patients with an infectious disease (26). In daily practice, this reason for notification is hardly ever used.

Most of these notifications are labor intensive and depend on the swift action taken by doctors or representatives of microbiological laboratories. With curative care and public health increasingly digitized in the last 20 years, the outbreak control methods should be modernised as well. For instance, signs and warnings of a (potential) local outbreak of infectious disease might be derived straight from the curative care record, and preferentially should appear “automatically” at the desk of local public health care authorities, without delay. Unfortunately, a tool like that is not available in the Netherlands but if developed, would improve early detection of infectious disease outbreaks and thereby, preclude spread at the earliest possible stage.

Some outbreaks tend to occur yearly at predictable seasons, as is the case with acute respiratory tract infections such as influenza and RSV. To manage and mitigate the potential impact of these outbreaks on curative care institutes, both early detection, and monitoring of the outbreaks are important. Until recently, surveillance of acute respiratory tract infections in the Netherlands consisted of monitoring the prevalence of influenza-like illness in primary care and through influenza-associated mortality surveillance (27, 28). Surveillance of Severe Acute Respiratory Infections (SARI), the infections for which hospitalization is necessary (29), was regarded as the missing link in the surveillance pyramid (30). SARI surveillance is deemed necessary to be able to execute preventive measures

timely, to assess the impact of these measures in the high risk (hospitalized) population, and to inform policymakers about the start, progress, and the extent of an outbreak in secondary care.

The World Health Organization (WHO) and the European Centre for Disease Prevention and Control (ECDC) guided countries to set up SARI surveillance (31, 32). SARI surveillance is a relatively new surveillance method, and experience with these data is limited. To evaluate the potential added value of SARI surveillance, a comparison between primary care influenza-like illness surveillance and SARI surveillance should identify strengths and weaknesses, and determine the added value of the SARI surveillance on top of the current surveillance in primary care. It is, for example, currently unclear whether the incidence of influenza virus infection in general practice corresponds with the incidence in hospital admission for acute respiratory tract infection. Moreover, based on SARI surveillance hospitals may be urged to pursue additional hygienic measures for patients with influenza virus infection, perhaps segregate these patients, and empirically prescribe oseltamivir (a neuraminidase inhibitor) when there is a reasonable pre-test probability that a patient has an influenza virus infection. Thus, any discrepancy in the occurrence of the start and peak of influenza infections in general practice and the hospital influenza season could have major implications for the timing of these measures.

Detection of lower respiratory tract infection in primary care

In an individual patient with an acute respiratory tract infection, clinical signs and symptoms cannot distinguish between the various causative agents of the infection (33, 34). In primary care, microbiological diagnostics are not recommended because it is difficult to obtain an adequate sputum sample for culture, (pneumococcal) urinary antigen tests lack sensitivity, all diagnostic procedures take time, and most often, because even advanced, intense testing may fail to identify the causative agent. Also, and perhaps most significantly in this respect, the outcome of microbiological diagnostics will not affect patient treatment (which is based on clinical presentation and knowledge of etiological microbial agents) and prediction of clinical outcome (35-38). Therefore, the focus in primary care is not on finding the causative agent but on the assessment of the risk of a complicated course.

The current strategy aims at identifying patients with pneumonia or lower respiratory tract infection with an increased risk for a complicated course (39). This strategy focuses on the evaluation of the intensity of the host inflammatory response. In this line of reasoning, mildly ill patients likely have limited inflammation only, and thus a small chance of having extensive tissue involvement and significant pneumonia, and by consequence, a favourable outcome. In these patients, lower respiratory tract infection is caused most

often by a virus, and withholding antibiotic treatment does not pose a risk. On the other side of the spectrum, severely ill patients are likely suffering an extensive inflammatory response, have a bigger chance that the infection is caused by bacterial pathogens, and are at an increased risk of a complicated and unpredictable course of pneumonia. These patients should receive antibiotic treatment. Obviously, those in between, i.e., the group of patients that appear ill but are not acutely, severely ill, provide the clinician with the biggest challenge. From these moderately ill patients, it is less clear who will go on to develop a potentially life-threatening infection and would benefit from antibiotic treatment, and who will not. Even in this group, the majority of patients will have a viral cause of acute respiratory tract infection and self-limiting disease. Treating all of the patients in this intermediate group with antibiotics, therefore, would result in unnecessary side-effects, promote antimicrobial resistance, and results in high costs.

Unfortunately, for a diagnosis of pneumonia (most often defined as new infiltrate on the chest X-ray), taking a medical history and performing a physical examination lack sensitivity and specificity (40, 41). In most instances, taking a chest X-ray in patients with an acute respiratory tract infection is neither feasible nor cost-effective, and many general practitioners (GPs) rely on readily available point-of-care testing of biomarkers to help guide their therapeutic decisions. To improve diagnosis and help making therapeutic decisions the general practitioner (GP) can order a chest X-ray or rely on readily available C-reactive protein (CRP) point-of-care testing.

First, the chest X-ray is considered by many the gold standard in defining pneumonia. However, a chest X-ray in outpatients does not improve outcome, and therefore this is not routinely recommended for patients attending their GP with suspicion of acute respiratory tract infection or community-acquired pneumonia (42, 43). In addition, in general, primary care patients must be referred to hospital for an X-ray of the chest, making this a far from attractive first-line diagnostic test. Still, in some patients, a definite conclusion on the presence or absence of pneumonia (as determined by chest X-ray) would help physicians in decision making. For example, in patients not improving on empirical antibiotic treatment or in moderately ill patients with specific underlying diseases, a chest X-ray could help guide the general practitioner in deciding about antibiotic treatment, a wait-and-see policy, or prompt referral to a hospital. Until now, research into the effects and the effectiveness of chest X-rays by the general practitioner for subgroups of patients with an acute lower respiratory tract infection is lacking.

Secondly, CRP point-of-care tests are available. CRP is a biomarker of inflammation, and the results of the point-of-care test are directly available at the time and place of patient care. Studies have shown that a low CRP (< 20 mg/l) can with reasonable certainty ex-

clude pneumonia, irrespective of medical history, comorbidity, and findings on physical examination, while an elevated CRP (> 100 mg/l) dramatically increases the chance of pneumonia warranting antibiotic treatment (44, 45). A meta-analysis ascertained that even when clinical variables are taken into account, the CRP test can help confirm or exclude the presence of pneumonia (46). Thus, this inflammatory biomarker can help GP decision making in daily practice. In a large European cohort, CRP added to clinical signs and symptoms improved assessment of presence or absence of pneumonia in 29% of patients with acute cough (45). However, these are not necessarily the patients suspected of having pneumonia and the ones for whom diagnostic challenges remain. Although the CRP test helps to fill in some of the grey areas of patients with sickness of intermediate severity, it is not surprising that in turn, this new assay creates a new area of uncertainty. In particular, for moderately ill patients with an acute respiratory tract infection and intermediate CRP levels (between 20 and 100 mg/l), evidence-based practice guidelines are lacking. Furthermore, studies that evaluated whether the CRP point-of-care test reduced the number of antibiotic prescriptions showed variable results (47, 48).

There is a continuous search for an optimal biomarker which will distinguish unequivocally viral from bacterial causes of infection and potential inadvertent outcomes from favourable ones, and thus, provide a holy grail on guidance for antimicrobial therapy. So far, besides CRP, procalcitonin (PCT) and mid-regional pro-adrenomedullin (MR-proADM) have shown some promise as biomarkers for the severity of inflammation. Strong evidence which of these markers best predicts outcome in patients with an acute respiratory tract infection is however still lacking. The evidence-based use of these types of additional diagnostics is not established and many worries remain about inappropriate use of these tests that rapidly become more popular. A critical assessment of the current use of these additional diagnostics in daily general practice, the interpretation, and consequences of the test results, will help researchers and clinicians to identify knowledge gaps. Respiratory tract infections are frequently seen in primary care; therefore evidence-based guidelines regarding the use of additional diagnostics are urgently needed, in particular for use in moderately ill patients.

Detection of lower respiratory tract infection at the emergency department

To determine the presence or absence of pneumonia in the setting of the emergency department is less challenging than in primary care. The prevalence of pneumonia among coughing patients at the emergency department (i.e., pre-test chance) is much higher than among primary care patients, and chest X-ray and other radiological diagnostics are readily available at the emergency department. Also, a choice can be made of various microbiological diagnostic tests, tailored to an individual patient`s need (49).

More challenging are the treatment decisions. As physicians at the emergency department cannot determine causative agent on the spot, empirical treatment is – just like in primary care – guided by epidemiological setting (e.g., specific exposure) and severity of disease (i.e., host immune response) (50, 51). The pneumonia severity index has been developed to stratify patients according to prognosis and to guide empirical treatment (50). The studies into predicting pneumonia severity also found that the less severely ill patients, with a limited host inflammatory response, have only a marginal 30-day mortality (50, 51). Thus, these patients can safely be treated with small spectrum antimicrobial therapy in combination with starting microbiological diagnostic tests aimed to adjust the empiric treatment if necessary, and secure follow up. More severely ill patients with an abundant inflammatory response, have an increased 30-day mortality risk and should, therefore, be treated with broad-spectrum antimicrobial therapy. In this group of patients, antimicrobial therapy alone might not be enough. Also, the timing of antimicrobial therapy is important and, without interfering with the effect of the antimicrobial therapy, interventions aimed at attenuation of the inflammatory host response may be necessary, as this can cause collateral damage.

Timing of initiation of antimicrobial therapy at the emergency department

Timely administration of antimicrobial treatment is important to obtain the optimal effect. Early and prompt treatment will prevent microbes from replicating, thereby preventing an even more extensive inflammatory response. Indicators of timing as the door-to-needle time have been used to improve timely administration of appropriate antibiotic treatment. This door-to-needle time is derived from acute ischemic events where timely administration of reperfusion therapy could save organ tissue (52). In patients with pneumonia, early initiation of treatment has been shown to improve outcome (53). Still, administration of antibiotics inappropriate for the etiologic pathogen (e.g., lacking activity against the microorganism) is, however, the strongest predictor for mortality in patients with severe infection (54). Therefore, a short assessment time at the emergency department is essential to minimize the risk of potential harm to patients who eventually did not have a (bacterial) infection, and to determine the adequate empiric antimicrobial treatment if needed (55). If the presence of bacterial infection is likely, the differential diagnosis of possible etiologic microorganisms must be made and the benefit of antimicrobial therapy options considered, and appropriate antimicrobial treatment should be started as soon as possible.

In patients with severe bacterial infections, early treatment is better than late treatment, but late treatment is still better than an ill-directed or no treatment. In contrast, in influenza virus-infected patients in outpatient settings, the time window for treatment with

antiviral neuraminidase inhibitors seems very small, and it was shown that treatment of healthy volunteers ≥48 hours after first symptoms had no added benefit over no treatment (56, 57). Patients hospitalized for influenza may represent a distinct group with continuing viral replication and an extended therapeutic window. For instance, younger hospitalised patients with H1N1pdm09 influenza virus infection had reduced mortality when neuraminidase inhibitor treatment was initiated within 48 hours after the start of symptoms, but this effect remained, although less pronounced, until treatment initiation within five days after symptom onset (58).

In patients at the emergency department, who are elderly, frail, or immunocompromised and at high risk for developing complications, this time window for antiviral treatment is not clear. In daily practice, the majority of these patients present to a hospital with symptoms that started more than 48 hours earlier (58, 59). Evidence for benefits of neuraminidase inhibitor treatment of these patients admitted with seasonal influenza is scarce and is often extrapolated from studies in other patient groups. The benefit of late initiation of treatment (that is >48 hours after symptom onset) has been questioned (60, 61). In addition, negative reporting about the neuraminidase inhibitor oseltamivir has further increased the uncertainty of oseltamivir´s potential benefit (62). The uncertainty about the effectiveness of oseltamivir treatment in patients admitted with influenza virus infection remains a daily challenge during the yearly influenza season.

Attenuation of host inflammatory response to improve outcome

What can we do, besides early initiation of antimicrobial therapy in patients with severe pneumonia (i.e., characterized by an intense inflammatory response), to attenuate inflammatory response without interfering with the antimicrobial properties of such response? Adjunctive anti-inflammatory therapeutic options are being studied (63). For community-acquired pneumonia, macrolides have been proposed to have a positive immune modulatory effect by enhancement of the antibacterial effect of neutrophils and by quashing the immune response after bacterial killing, potentially improving outcome (64, 65). However, this in vitro effect was not observed in a clinical trial in which β-lactam monotherapy was found to be non-inferior to macrolide with β-lactam combination therapy (5).

Corticosteroid therapy in community-acquired pneumonia would improve short term outcome measures but has a large number of side effects (66, 67). Consequently, primary and secondary care guidelines do not recommend to add corticosteroid or other immunosuppressive treatments to the therapy of patients with community-acquired pneumonia. However, in other life-threatening infections such as pneumococcal meningitis, dampening the host inflammatory response has been recognized as an important adjunct treatment. Most treatment protocols dictate that dexamethasone is given to patients with

pneumococcal meningitis before an antibiotic is administered. This approach has been shown to decrease the inflammatory response and to improve treatment outcome (68).

Another approach would be to limit the initiation of the host immune response, i.e., to decrease the quantity of immune-reactive components that are released following the start of antimicrobial treatment. Some research into this approach has been done in pneumococcal infections. *Streptococcus pneumoniae* is the most frequent bacterial causative agent of pneumonia, and pneumococcal infections have substantial morbidity and mortality, mostly driven by an intense host inflammatory response (69). As in other infections with Gram-positive bacteria, this inflammatory response is primarily induced by the release of large quantities of immune-reactive bacterial cell wall components (e.g., lipoteichoic acid) and intracellular proteins (70). Specific antibiotics that lyse the pneumococci enhance this release. Previous in vitro and animal studies, showed a decreased lipoteichoic acid release and subsequent dampened inflammatory response when *Streptococcus pneumoniae* is killed with non-lytic antibiotics, that is rifampicin as opposed to for instance β-lactams (71, 72). To our knowledge, studies in humans to evaluate this effect on inflammatory response and outcome are currently lacking.

Moreover, the H1N1 influenza pandemic gave insight into a rare but potentially lethal inflammatory complication of flu: Acute Respiratory Distress Syndrome (ARDS) (73, 74). ARDS risk factors and pathophysiological mechanisms are currently investigated. ARDS is an inflammatory response with epithelial and alveolar cell damage leading to bilateral opacities on chest radiograph with marked hypoxia occurring within seven days after a clinical insult (75, 76). Since the 2009 H1N1 influenza outbreak, numerous reports appeared indicating that influenza virus infection may, in rare cases, cause ARDS (73, 77). Of note, ARDS can also occur after many other unrelated triggers, for example, sepsis, trauma, inhalation of exogenous toxins, or major surgery (78). Likely, ARDS is caused by the occurrence of several, sequential hits to the lung (79, 80). Little knowledge exists about whether such sequential hits are relevant clinically, for instance, whether elective surgery would lead to a higher percentage of ARDS cases during seasons with respiratory viruses circulating. In this respect, it is of interest that it was found that different viruses are associated with ARDS in critically ill patients (81, 82).

In conclusion, care for patients at risk for an acute respiratory tract infection can likely be optimized by improved application of vaccination strategies, early detection of specific etiologic microorganisms (or at least distinguish a viral from bacterial cause), adequate assessment of the host immune response, and attenuation of an excessive host inflammatory response.

OUTLINE OF THIS THESIS

The current thesis aims to optimize care for patients at risk for or with an acute respiratory tract infection, in several clinical domains, with a focus on prevention, early detection of outbreaks, early diagnosis of lower respiratory tract infection, and strategies to attenuate the inflammatory response and improve clinical outcome in these patients.

In **chapter 2**, the ICARES (Integrated Crisis Alert and REsponse System) project is described, a new automated real-time tool for the detection of clusters of infectious diseases. Besides respiratory tract infections we also analysed two other infectious diseases with the ICARES project: infectious hepatitis and meningitis/encephalitis.

Chapter 3 focuses on the difference in severe acute respiratory infection (SARI) surveillance in secondary care and influenza-like illness (ILI) surveillance in primary care. These surveillance systems are important to detect and monitor the yearly outbreaks**.** The added value of SARI surveillance upon ILI surveillance is discussed.

The use of additional diagnostics by general practitioners in patients with an acute respiratory tract infection is described in **chapter 4**. Use of C-reactive protein for acute respiratory infections is well established in current guidelines. On the contrary, the use of X-rays of the chest is only briefly mentioned in guidelines, and usually without clear indications. We evaluate the current clinical use of both diagnostics and discuss ways to improve strategies.

Chapter 5 describes a cohort of patients with an acute respiratory tract infection for whom their general practitioner orders a chest X-ray. With this cohort, we determine predictive clinical parameters and biomarkers for the presence of pneumonia.

Vaccination is vital in preventing respiratory infections in the general population. Although most agree on the urgent need for an improved, more effective influenza vaccine, currently it is all there is available to prevent influenza. In the healthy and various subgroups of patients, influenza vaccination has shown effectiveness, though limited. Anecdotal reports about side effects withhold caregivers from vaccinating patients who would benefit from influenza vaccination. In **chapter 6**, we describe the absence of excessive immune-related adverse events of influenza vaccination in lung cancer patients treated with immunotherapy. In **chapter 7**, we discuss influenza vaccination in health care workers during the severe influenza season 2017/2018.

The burden of influenza virus infection in three large hospitals in the Netherlands and the analysis of the treatment effect of oseltamivir are topics in **chapter 8**. Can we prevent mortality and in-hospital complications by initiating treatment at admission?

Chapter 9 deals with a complication of influenza virus infection: pneumococcal pneumonia. The PRISTINE (Pneumonia treated with RIfampicin aTtenuates Inflammation) study is the first pilot clinical trial in humans to determine the feasibility of adding rifampicin to standard treatment with β-lactams in patients with community-acquired pneumonia. This combined treatment is done to reduce the release of pro-inflammatory bacterial compounds within the first hours of therapy, and to thereby attenuate the host inflammatory response and improve outcome.

Pneumococcal pneumonia is an obvious complication of influenza virus infection, but there might be more complications related to influenza. As most influenza infections are asymptomatic, we hypothesized that asymptomatic influenza infection in cardiac surgery patients is a risk factor for postoperative Acute Respiratory Distress Syndrome (ARDS). The results of an investigation into the prevalence of ARDS after elective surgery within and outside the influenza season are made available in **chapter 10**.

In **chapter 11**, the findings of this thesis are summarized and discussed in light of the literature and future directions of research.

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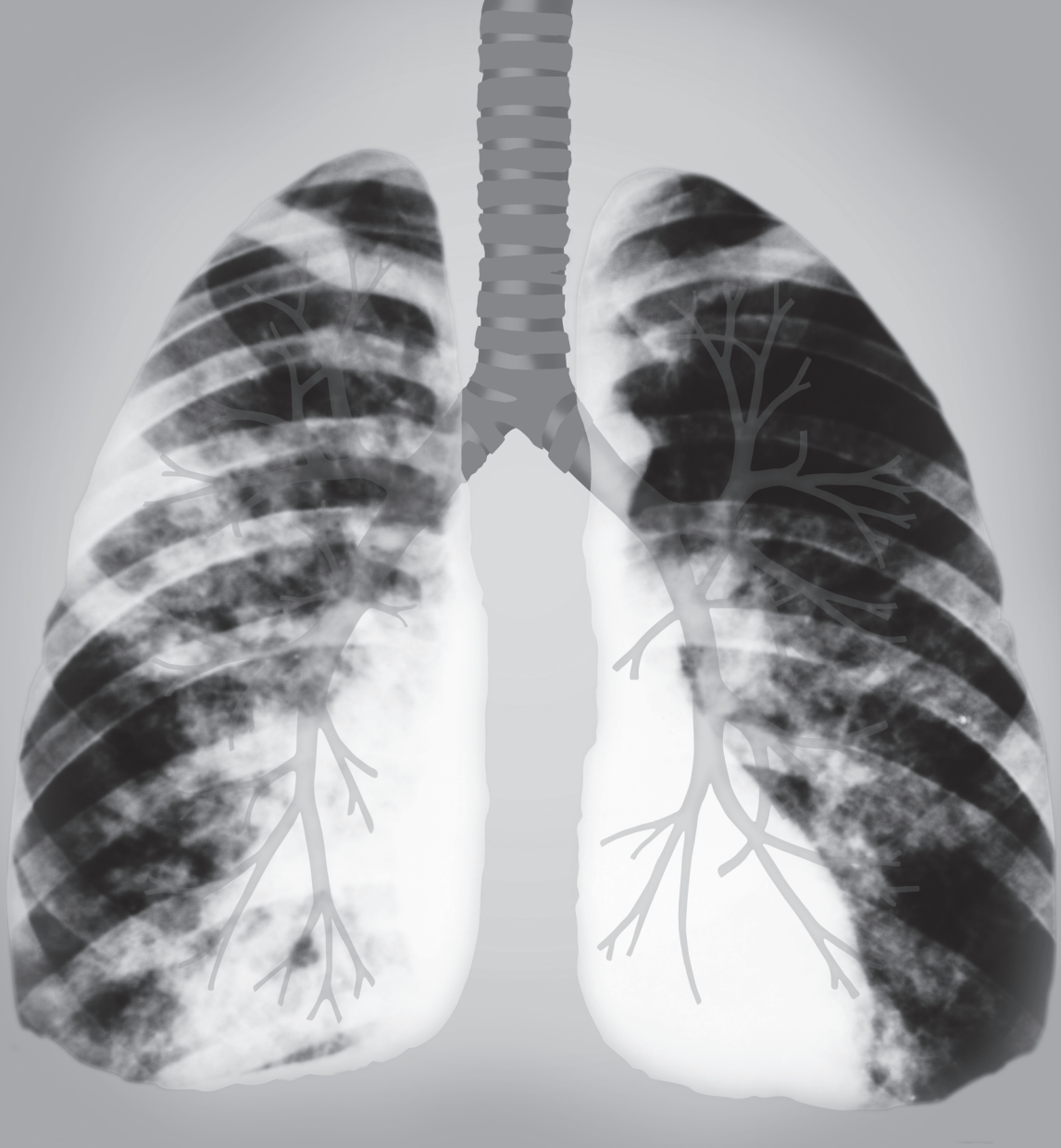

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ICARES: a real-time automated detection tool for clusters of infectious diseases in the Netherlands.

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ABSTRACT

Background

Clusters of infectious diseases are frequently detected late. Real-time, detailed information about an evolving cluster and possible associated conditions is essential for local policy makers, travelers planning to visit the area, and the local population. This is currently illustrated in the Zika virus outbreak.

Methods

In the Netherlands, ICARES (Integrated Crisis Alert and Response System) has been developed and tested on three syndromes as an automated, real-time tool for early detection of clusters of infectious diseases. From local general practices, General Practice Out-of-Hours services and a hospital, the numbers of routinely used syndrome codes for three piloted tracts i.e. respiratory tract infection, hepatitis and encephalitis/meningitis, are sent on a daily basis to a central unit of infectious disease control. Historic data combined with information about patients' syndromes, age cohort, gender and postal code area have been used to detect clusters of cases.

Results

During the first two years, two out of eight alerts appeared to be a real cluster. The first was part of the seasonal increase in Enterovirus encephalitis and the second was a remarkably long lasting influenza season with high peak incidence.

Conclusions

This tool is believed to be the first flexible automated, real-time cluster detection system for infectious diseases, based on physician information from both general practitioners and hospitals. ICARES is able to detect and follow small regional clusters in real time and can handle any diseases entity that is regularly registered by first line physicians. Its value will be improved when more health care institutions agree to link up with ICARES thus improving further the signal-to-noise ratio.

BACKGROUND

Worldwide, the number of infectious disease outbreaks is increasing (1). Consequently, the early detection of and response to clusters of infectious diseases is becoming more important.

Past experience shows that many outbreaks of infectious diseases are detected late. For example, in the Netherlands in 1999, a point source outbreak of Legionnaire's disease was detected 14 days after the first patient was admitted to hospital. At that time, another 70 patients had already been admitted to various hospitals throughout the Netherlands (2;3).

There are many similar examples where retrospective analysis of data clearly indicates that clusters of infectious diseases are not detected until relatively late. This hampers the identification of the source of the outbreak, the control of the associated transmission route(s) and the identification of associated conditions. For example, delayed detection of hemolytic uremic syndrome (HUS) and bloody diarrhea of Shiga Toxin-producing Escherichia coli outbreak in Germany in 2011 had significant and long-lasting impacts (4;5). The speculation about the association between the Zika virus outbreak and microcephaly gave rise to conflicting advice to women of childbearing age (6;7).

Such delayed detections and lack of detailed insight in possible related conditions are costly in terms of the disease burden but also have impact on the social and economic aspects of the communities affected (8).

Reasons for late detection can be attributed to the non-specificity of the detection systems. For example, Google Flu Trends was developed to find a potential flu cluster as soon as possible. Critical analysis revealed that it has overestimated the number of flu cases and Google Flu Trends has discontinued to publish current estimates (9;10).

This large amount of data noise can be overcome by medical doctors being the data source. Medical doctors define a working diagnosis at first patient contact. Such primary data yield more specific results in comparison with lay persons based systems as Google Flu Trends.

On the other hand, using disease syndromes in outbreak surveillance frequently lacks specificity and commonly refers to a broader categorisation, e.g. respiratory tract infection or gastro-enteritis. Additionally, General Practitioners (GPs) do not, for instance, regularly request microbiological testing for these syndromes. This can easily result in a missed opportunity to successfully identify a possible cluster that could represent the first sign of a much larger potential outbreak.

To overcome this information gap, the Dutch Public Health Law (Wet Publieke Gezondheid), based on the International Health Regulations (IHR) (11), obliges medical doctors to report unusual clusters of infectious diseases with possible serious public health consequences. The criteria for reporting under this heading are not well specified and in practice medical doctors hardly ever report such clusters. Still, individual physicians will miss clusters in overlapping physician catchment areas. This is clearly exemplified by the aforementioned examples.

The gaps in surveillance intelligence described above highlight the urgent need for a surveillance tool to capture and analyse regional clusters of infectious diseases. This tool should ideally be automated, real-time and based on diseases identified by medical doctors without adding to the administrative burden of medical professionals (12;13). This will prompt public health professionals to investigate further when certain upper limits of incidence for a given syndrome have been reached. Detailed information about the extent of an outbreak will help public health authorities to inform and advice the involved population adequately. Our case study addresses this gap specifically.

METHODS

From 1 October 2013 to 1 October 2015, a pilot ICARES (Integrated Crisis Alert and Response System) case study was conducted in the Leiden-The Hague region in the western part of the Netherlands. This area has approximately 1.25 million inhabitants, six hospitals, eight GP Out-of-Office-Hours services and 380 individual GP practices.

This study was approved by the Medical Ethical Committee of the Leiden University Medical Center on 18 April 2012. The aim of the case study was to design, develop and test an automated surveillance tool capable of providing early signals of potential clusters that could escalate into outbreaks. The complete spectrum of front-line health care organisations contributed to this case study and included General Practices, Out-of-Hours General Practitioner services, and one hospital (emergency department, ward and intensive care unit admissions and outpatient department consultations). For the hospital, DBC/DOT (Diagnose Behandel Code Op weg naar Transparantie) codes were used to map to the corresponding syndrome. Hospital physicians routinely enter codes during the first evaluation of a patient. These DBC/DOT codes are developed for hospitals to reimburse the costs of patient care at health care insurers and represent the patient's diagnosis.

Diagnostic information from General Practitioner (GP) patient records is obtained using the International Classification of Primary Care (ICPC) (14), according to the guidelines

of the Dutch College of General Practitioners (15). Nowadays, both in daily practice and during out-of-office hours, GPs routinely enter these codes in the electronic patient file at first patient presentation.

Any disease entity that is routinely coded and entered in the patient record can be selected. In this case study, we focused on respiratory tract infection, infectious hepatitis and meningoencephalitis. Trigger diagnostic codes (Table 1) are collected and sent to ICARES every 24 hours, yielding a near real-time snapshot of what is happening in the community and its burden on health care institutions.

Together with these diagnostic codes, the minimal data set (MDS) of patient sex, age range, the four digits of the postal district (i.e. not the full postal code), identification of the participating health care facility and date of consultation are captured for transmission to ICARES. For reasons of data confidentiality, privacy and security, no specific patient identifiable information is collected from the GP systems. With hospital data, an encrypted patient identification number is added, with only the principal investigator at the hospital being able to decrypt these codes. This practice ensures that the minimal data set does not contain patient identifiable information.

In order to obtain calculation baselines for the data analysis, historic data from the various participating organisations were collected and analysed. This case study benefited from one year's data from GPs, including GP Out-of-Office-Hours services, as well as eight years of hospital data. This yielded means and standard deviations for various codes.

A secure web-based decision support tool was developed for the purpose of this study by inFact Ltd. and was named ICARES (Integrated Crisis Alert and Response System). The software tool receives the MDS from the various participating organisations every night. Special web services have been written to interface, in a non-intrusive way, with the disparate electronic patient records. ICARES then maps all the diagnostic codes received onto the corresponding three sets of syndromes mentioned, and presents the analysed data in an easy to understand dashboard with a risk dial for each disease to the local unit of infectious disease control.

ICARES aggregates the actual data harvested and compares these values with those for the nearest current time window historically. Calculations in ICARES are currently performed using this Cumulative sum (CUSUM) method for a moving seven-day period (16). To calculate the equivalent historic period, the previous seven-day period is taken into consideration, adjusted for holidays.

Table 1. Trigger diagnostic codes

DBC/DOT code (Hospital)[a]	Representing syndrome/diagnosis
Respiratory tract infection	
INT401	Pneumonia
INT402	Interstitial pneumonia
INT409	Other respiratory tract infections
LON1401	Pneumonia
LON1405	Acute (trachea)bronchitis
KIN3104	Upper respiratory tract infection
KIN3202[b]	Asthma/bronchial hyperreactivity
KIN3207	Laryngotracheobronchitis
KIN3208	Lower respiratory tract infection
KIN3210	RSV bronchiolitis
Infectious hepatitis	
INT463	Viral hepatitis (not B or C)
INT944	Hepatitis B or C
MDL701	Hepatitis
MDL705	Hepatitis B or C with antiviral therapy
MDL718	Acute liver failure
KIN3312	Hepatitis
Meningitis/encephalitis	
INT441	Meningitis/encephalitis/brain abscess
NEU0101	Bacterial Meningitis
NEU0102	Non-bacterial meningitis
NEU0111	Encephalitis
KIN3511	Meningitis/encephalitis
ICPC (General Practice)	**Representing syndrome/diagnosis**
Respiratory tract infection	
R74	Acute upper respiratory tract infection
R77	Acute laryngitis/tracheitis
R78	Acute bronchitis/bronchiolitis
R80	Influenza
R81	Pneumonia
Infectious hepatitis	
D13	Icterus
D72	Infectious hepatitis
Meningitis/encephalitis	
N70	Poliomyelitis/(entero)viral infection CNS
N71	Meningitis/encephalitis

a. DBC/DOT codes from internal medicine, pulmonology, pediatrics, neurology and gastroenterology are used.

b. This code is only used in children under the age of 5 since asthma/bronchial hyperreactivity, at this age, is most often triggered by a respiratory tract infection.

The above information is synthesised in a risk dial with traffic light colors immediately recognisable as green to signify a normal setting, orange when a warning threshold has been reached corresponding to an incident ratio between 0.75 and 1.40 and red for an incident ratio of more than 1.40. The rates can only be calculated for the GP population since it is only in the GP practices that the number of patients, the denominator, is known. For hospital and Out-of-Hours General Practitioner services, colors are determined by rates of the 7-day numbers observed divided by the historic 7-day numbers. Thresholds are the same as those for incident ratio.

These colors on the dashboard provide a crude indication of current numbers versus historic numbers. If colors turn red, more profound investigation is warranted to define whether further action is needed. These action limits are visualized in the graphs and defined by three standard deviations above average.

Should the ICARES action limit be exceeded, i.e. indicating that a possible cluster is detected for that given institution, the local unit of infectious disease control will use this as a trigger for further investigation. After assessment of geographic information and raw data, they will consult the treating physicians to find out more about the specific diagnosis and patient characteristics of the possible cluster. Up-to-date information continues to be available on the dashboard in order to follow the cluster as it evolves over time. If a specific, microbiologically confirmed diagnosis is not available at the time when the trigger appears, diagnostic protocols for possible outbreaks have been put in place to deal with this. Parts of these protocols are adapted from current national guidelines (17).

The dashboard is an easy to use quick scan for possible clusters. If colors and numbers are within normal range, no further action is necessary and the dashboard can be reopened the next day. This visual quick scan of the dashboard is done daily by the local unit for infectious disease control in the Leiden-the Hague area and by the research team and takes less than one minute.

All alerts will be evaluated whether it have been real clusters or not. Reasons for false positive alerts will be documented as well as the use of additional, public health care initiated, diagnostic tests.

RESULTS

ICARES, the automated, real-time tool for the detection of clusters of infectious diseases has been tested on three disease entities since October 2013: respiratory tract infection, infectious hepatitis and meningoencephalitis.

After a run-in period of three months, the project started with one teaching hospital participating (catchment area approximately 200,000 inhabitants) and four GP practices with 33,117 patients (18). During the first 24 months, four Out-of-Hours General Practitioner services (catchment area approximately 500,000 inhabitants) and ten more GP practices joined, contributing to a total number of 78,924 GP patients (19;20).

GP coverage in the complete Leiden-The Hague study area was 6%. Since most of the health care facilities were located in the Leiden part of the study area, GP coverage in the Leiden region was 11%. Coverage of Out-of-Hours GP services in the Leiden region was 67%, hospital coverage was 27%.

On a daily basis, the local unit of infectious disease control and the research team checked the risk dials on the ICARES dashboard.

In the first two years of ICARES, eight signals of possible clusters were detected. Two of these alerts appeared to be a real cluster. Characteristics are outlined in table 2.

Table 2. Alerts during the first two years of ICARES

Alert	Syndrome (Health care institution)	Additional public health diagnostics	True cluster	Comment
1	Respiratory tract infection (GP)	No	No	Different causative agents and coding imperfections
2	Infectious hepatitis (GP)	Yes	No	Non-infectious hepatitis
3	Meningoencephalitis (Hospital)	No	Yes	Enterovirus encephalitis
4	Meningoencephalitis (Hospital)	No	No	Two unrelated cases of Listeria in Katwijk
5	Infectious hepatitis (GP)	No	No	Coding imperfections
6	Respiratory tract infection (Hospital and GP)	No	Yes	Long lasting influenza season with high peak incidence
7	Meningoencephalitis (Hospital)	No	No	Coding imperfections/double coding
8	Meningoencephalitis (GP)	No	No	Non-acute illness

Alert 3 was detected from August 8 2014 onwards (Figure 1). Eight cases of meningoencephalitis were reported within one week in the hospital (Figures 1 and 2). Prompt analysis ultimately revealed that three cases with Enterovirus encephalitis belonged to the same cluster. Two of these three were household contacts. The third case was from a different four-digit postal district.

The other five notifications from the cluster of meningoencephalitis were double coded or had another cause than Enterovirus. Daily evaluation of this cluster revealed a sharp decline in incidence after one week.

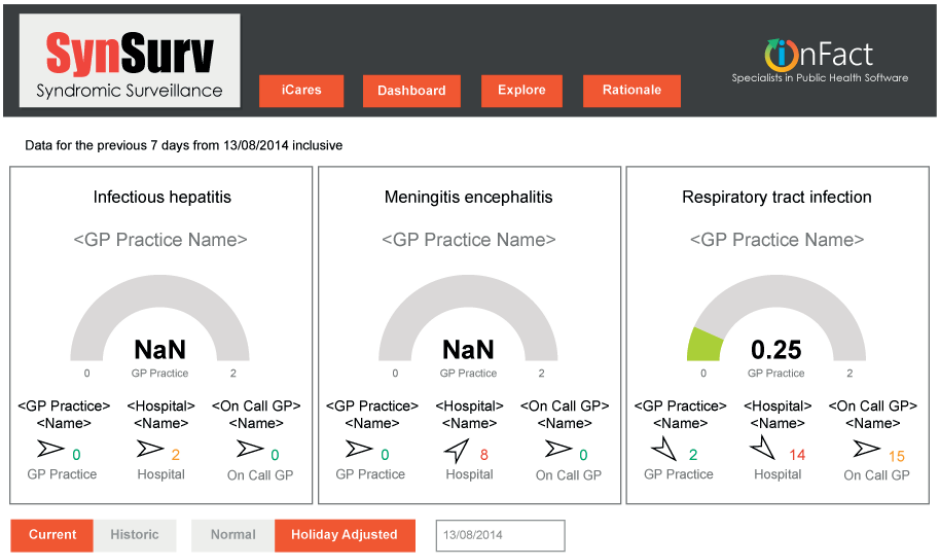

Figure 1. Dashboard on 13 August 2014 during meningoencephalitis outbreak
Dial numbers are incident ratios: the ratio between the observed previous 7 days incident rate with the equivalent historic incident rate. Rates are calculated as the numbers of incidents per 100,000 as based upon the GP practice's population data.
The dial color is set as green for an incident ratio of less than 0.75, orange for between 0.75 and 1.40 and red for greater than 1.40. Dials are limited to GP practices as these are the only ones where population data is available.
Colored numbers are absolute incident counts for the last 7 days for a given institution. The institution that is displayed, is the one with the largest incident ratio. This is the ratio between observed and historic using rate values if available, otherwise absolute counts. The color is determined in a similar manner to the dial color.
Trend arrows are determined from the ratio between the current week's (previous 7 days) observed incident rate (or observed absolute incident count if rate not available) and the same value as calculated for the previous week. The trend arrow reflects current week versus previous week.
A rising trend is shown for ratios greater than 1.1, stable for between 0.9 and 1.1, and falling for less than 0.9.
NaN = Not a Number. NaN is displayed when the equivalent historic 7 day period has zero cases. A ratio would result in a divide by zero error.

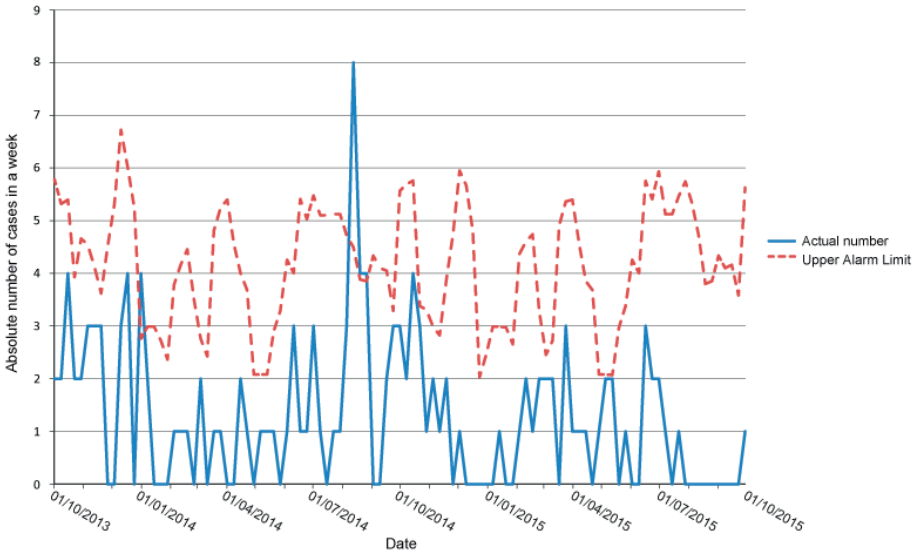

Figure 2. Hospital cases of meningoencephalitis 1/10/2013-1/10/2015

The peak in meningoencephalitis cases occurred during the Enterovirus season, which was also detected, retrospectively, by the virologic surveillance program in the Netherlands (21).

Alert 6 consisted of influenza cases in March-May 2015 (Figure 3). It was part of the 2014-2015 influenza season which was remarkably long lasting and had a higher peak incidence compared to previous influenza seasons.

Figure 4 represents hepatitis cases in the hospital. Numbers during study period did not exceed the upper alarm limit.

Two alerts were not analysed. From March 6 2014 onwards, a small peak of respiratory tract infections was detected (Figure 3). This alert coincided with a late, minor peak in Influenza-like illness, detected by national surveillance system. It was therefore not analyzed further.

On December 26 2013, the threshold for meningoencephalitis was exceeded (Figure 2). Discussion by the research team concluded that this could not be a real cluster, partly because of the low absolute numbers. Further evaluation was abandoned.

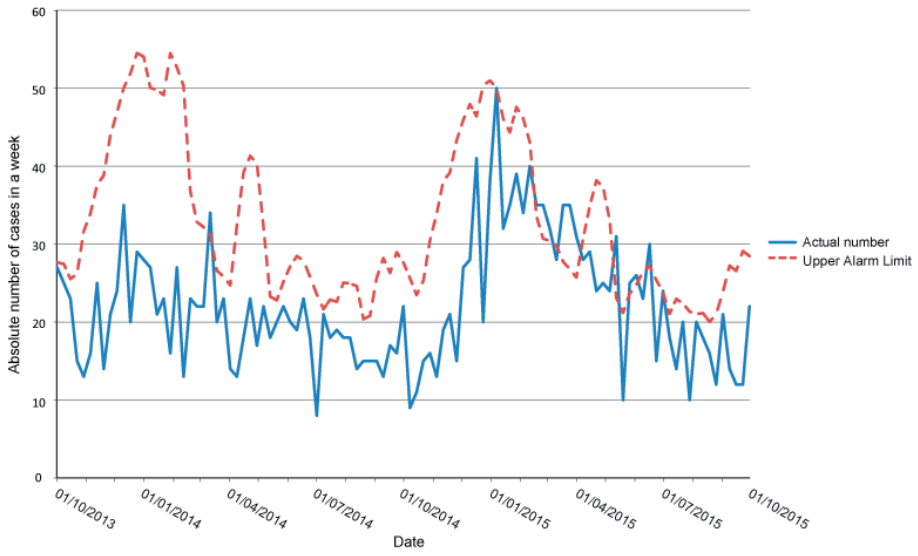

Figure 3. Hospital cases of respiratory tract infections 1/10/2013-1/10/2015

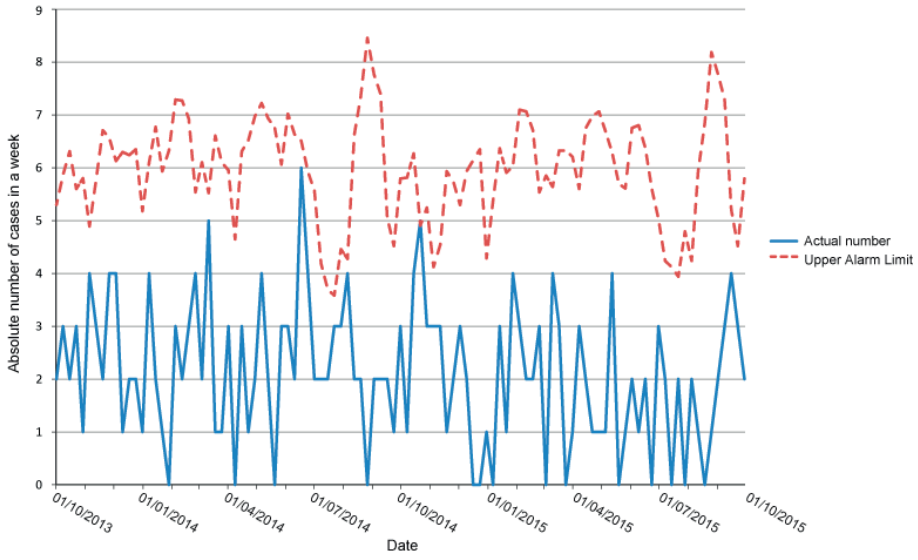

Figure 4. Hospital cases of hepatitis 1/10/2013-1/10/2015

DISCUSSION

We developed and tested ICARES as an automated, real-time tool for the detection of clusters of infectious diseases. In a small pilot region, ICARES detected differences in incidence in the three groups of diseases in real time (24-hour window) during the first two years of the project. Alert 3 and alert 6 demonstrate the ability of ICARES to detect and to monitor clusters of infectious diseases in real time.

Important strengths of ICARES are the robust diagnosis data with the minimal data set, the real-time collection and easily interpretable presentation of disease data, the historic comparison specific for each health care provider, the absence of administrative burden for medical professionals and the flexibility of the system.

Disease data should be very specific and we therefore opted in our project for definition by a medical doctor. In the Dutch health care system, doctors enter a diagnostic code in their medical record routinely. This diagnostic code most likely has a higher reliability than data used by other detection tools as Google Flu Trends and Triple S, using non-specific health indicators and proxy measures to define a syndrome (22). In our case study, the exceedingly long lasting flu season of 2014/2015 was notified and no significant alert was generated for the mild 2013/2014 flu season. On top of that, ICARES will represent the health care consumption in possible outbreaks since all patients in ICARES did visit a medical doctor.

Another strength of ICARES is the minimal data set. Details relating to geographic mapping or age cohort are important for source detection in the early phases of a possible outbreak. The minimal data set is non-patient specific and fully respects data privacy laws. But, if required, individual hospital-patient data can be traced by the treating physician since an encrypted patient identification number can be decrypted by the principal investigator in the hospital. At GP level, the treating GP can share information by finding the cases in a possible cluster via a query in their own GP information system. Diagnostics to evaluate the cluster (and the individual patient's illness) can be advised to treating physicians by public health care professionals. This was done during the second alert.

Daily, new data from health care providers are compared with their own historic numbers. Without significant changes in coding custom or patient population, this entails that the percentage of double coded patients or travelers would be the same in both historic group and current patients making false positive clusters for these reasons less likely.

Data acquisition and presentation on a dashboard are done daily. This contains the real-time character of ICARES enabling public health authorities to analyse clusters at an earlier stage. Other comparable systems, such as the Electronic Surveillance System for the Early Notification of Community-Based Epidemics (ESSENCE), show the difficulty in detecting an outbreak soon enough to start up control measures (23). So far, the limited amount of small clusters detected with ICARES is insufficient to evaluate its real-time character and to determine its ability to slow the spread of infection.

As shown in the third alert with a cluster of Enterovirus encephalitis, updates on the evolution of the cluster are made available on a daily basis enabling public health care authorities to inform policy makers and public adequately.

On the other hand, when numbers of infectious diseases are not above alarm threshold, a quick scan of the dashboard is usually enough to reassure public health care authorities.

The codes used for ICARES make it possible to capture clusters of a wide range of diseases via the three selected syndromes. Even new emerging infectious diseases presenting as one of these syndromes can be detected via ICARES. To implement ICARES fully, other syndromes will be added in the future. Also, in case of newly arising possible disease associations, any other disease entity might be selected for this type of surveillance.

An important reason is that ICARES algorithm is not based on a static threshold before triggering an alert. Seasonal variations in the incidence of syndromes warrant adjusting the baseline values of syndromes. The ICARES algorithm with adjusting baseline values for seasonal variations in the incidence of syndromes, gives rise to a moving threshold for cluster detection. The pragmatic and mature SPC-based (Statistical Process Control) algorithm used in ICARES can readily be used in most generalized case studies. Various challenges arising from shortcomings of other methods have been explored by various authors (24-28). CUSUM charts seem to adapt better to this type of analysis as they help improve the consideration of seasonal patterns as mentioned by Fricker et al (29).

This case study has several limitations as well.

Signal-to-noise ratio was questionable during this case study with two real clusters versus six false positive alerts. Positive predictive value is therefore 0.25. Although we are not aware of any missed clusters, we cannot calculate sensitivity.

Imperfections in coding for a new patient with a non-specific syndrome may constitute reasons for low signal-to-noise ratio. This may result in false positive alerts. This is illus-

trated by the alert 1, 5 and 7. Other reasons for false positive alerts might be provoked by other factors contributing to a syndrome resembling an infectious disease. A sudden increase in respiratory symptoms can be attributed to a contagious viral infection but also, e.g., to a high pollen count.

The relatively small number of health care facilities and, with that, the limited regional coverage during this first two years of ICARES may give rise to false positive and false negative alerts.

The historic data from our GPs only cover a one-year period and are therefore not robust. Eight-year historic hospital data might be too long as changes in care and population might make the oldest data irrelevant for upcoming cluster definition. Further work is therefore required to determine the appropriate length of history.

Currently, GP data is aggregated according to the underlying patient population data. This is not possible when considering hospitals and Out-of-Hours GP services as the exact catchment area is not known. As regional coverage broadens, assessment of this catchment area will also improve and incidence rates can be calculated for all health care facilities based on the total population in the (public health) district. As more health care facilities join the ICARES project, improved mathematical modelling to define alarm thresholds will be necessary.

Alerts are visible for public health care authorities within 24 hours after the treating physician routinely enters the trigger code. General Practitioners enter the ICPC code during the first consultation, DBC/DOT codes in hospital should be entered at first patient presentation. However, DBC/DOT codes can be changed when initial diagnosis changes and whether medical doctors abide by instant coding, is unknown. This could hamper real-time detection of clusters.

ICARES is a new and unique surveillance tool in the Netherlands to detect clusters of diseases in real time. Current local detection of small clusters depends on notification by medical doctors or laboratories as is defined in the Dutch Public Health Law (Wet Publieke Gezondheid), based on the International Health Regulations (IHR) (11). Nationwide, weekly updates of virologic results are published (21) and weekly updates about patients visiting their GP with influenza-like illness are reported (30). Automated tools for real-time detection of clusters are lacking. Systems for detection of acute hepatitis or meningoencephalitis are lacking as well.

Therefore, ICARES can improve outbreak detection in the Netherlands when used as a complement rather than a substitute for human involvement in interpreting cluster detection.

Diagnostic protocols in possible clusters have not been tested sufficiently during this project. It would be interesting to explore more disease syndromes, like food-borne diseases. This might improve its use for public health care authorities.

Further implementation of ICARES will enable cost benefit analysis. At this stage, maintenance costs are less than €10.000,- per year; daily efforts of local units of infectious disease control are minimal in case no thresholds are being exceeded. Besides time expenditure of existing staff, the development and primary piloting costs did not surpass €100,000.-.

Benefits will depend on the appearance of any clusters of infectious disease and the contribution of ICARES as a complement of surveillance tools in order to curb the outbreak.

To cite an outbreak that would have benefitted from an automated surveillance system, the current Zika epidemic in South America is an example. We could survey the illness as well as complications like microcephaly and Guillain Barre syndrome by adding diagnostic codes to ICARES.

As the project evolved, more institutions have expressed their willingness to participate. At the time of writing of this paper (22 November 2016) four hospitals, four Out-of-Hours General Practitioner services and 25 GP practices (87,380 patients) submit their consultation data daily. For GP patients, this leads to a coverage of approximately 12 % in the Leiden region. There is still some way to go to improve regional coverage and robustness of data.

CONCLUSIONS

ICARES was able to detect and to monitor local clusters of infectious diseases automatically and in real-time. Therefore it could be a complement to current surveillance tools in the Netherlands and other countries with highly digitalized health care administrations.

ACKNOWLEDGEMENTS

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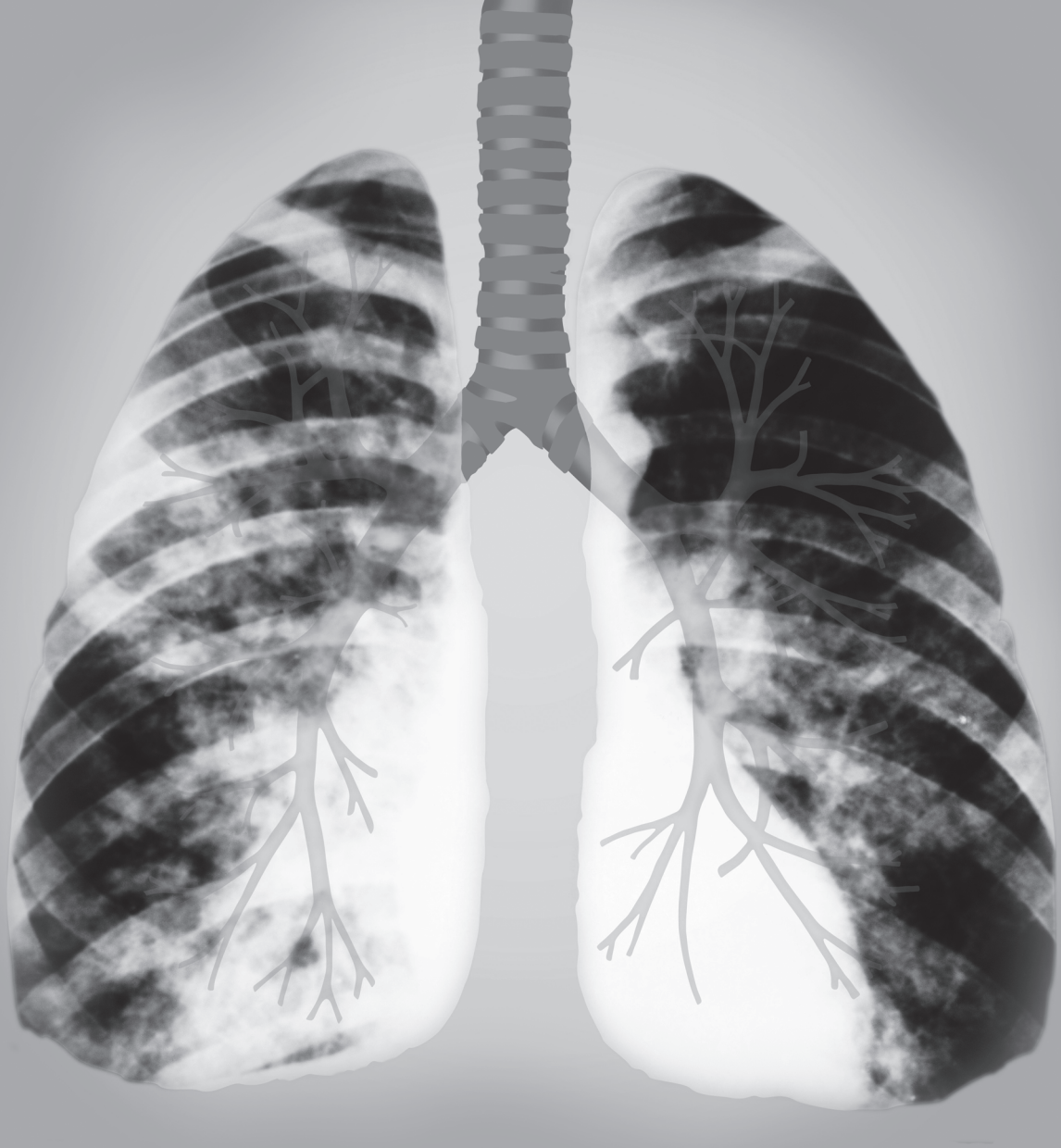

3

Acute respiratory infections in secondary care versus influenza-like illness in primary care in the Netherlands: hospital incidence peaks first.

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Submitted

ABSTRACT

Background

Surveillance of acute respiratory infections (ARI) in the Netherlands and other European countries is based mostly on primary care data, with little insight into the severe spectrum of the disease. We analyzed time-trends for ARI in secondary care, influenza-like illness (ILI) in primary care, and crude mortality, to assess the potential value of hospital data for surveillance.

Methods

We calculated the incidence of ARI in secondary care (Leiden University Medical Center), ILI in primary care (NIVEL Primary Care data base), and crude mortality (Statistics Netherlands) using three historical databases (2008-2016).

Results

Over eight years, the seasonal incidence peaks of ARI in secondary care occurred earlier than ILI incidence peaks, except during the influenza pandemic season of 2009/2010 and the post-pandemic season of 2010/2011. In the six seasons in which the ARI peak preceded the ILI peak, the median time-lag was eight weeks. The crude mortality peak lagged a median five weeks behind the ARI peak in all eight seasons.

Conclusions

In most seasons, the incidence peaks for ARI in secondary care preceded the peaks for ILI in primary care with a considerable time-lag. This is crucial information for preparedness and emergency control. Adding microbiological test results to these incidence data would be of great value in explaining the whole spectrum of ILI in primary care, ARI in secondary care, and mortality.

BACKGROUND

Most European countries have a well-established weekly near-real-time surveillance system for influenza-like illness (ILI) or acute respiratory infections (ARI) in primary care. In contrast, real-time surveillance data is rarely available on severe acute respiratory infections (SARI), i.e. those requiring hospital admission. The limited available historic and real-time data on severe respiratory infections, such as pneumonia as a complication of influenza, became apparent especially during the 2009 influenza A(H1N1) pandemic. In response, the World Health Organization (WHO) and European Centre for Disease Prevention and Control (ECDC) recommended the establishment of national SARI-surveillance systems to gain insight into the severity of epidemics and enable earlier detection of potential epidemics and pandemics.[1-3]

Surveillance is a vital tool to monitor shifts in the occurrence and burden of infections and diseases in the population, which is necessary for prevention and control.[4,5] In the Netherlands, weekly surveillance of ILI by sentinel general practitioners (GPs) was established in 1970 and virological test results were added in 1992, providing robust longitudinal data on incidence of ILI and influenza virus infection in the general practice population. The Dutch mortality monitoring system provides data on the total number of deaths from all causes, stratified by age group and region, with a weekly analysis of excess mortality.[6] It is a near-real-time surveillance system, but the weekly mortality data are not disease-specific.

SARI-surveillance has been the missing link in the existing respiratory infections surveillance systems in the Netherlands. The Dutch Hospital Data (DHD), a national register collecting the medical diagnoses of patients admitted to a Dutch hospital, provides data on hospital admission for SARI.[7] However it is available with a one-year time-lag and therefore not suitable for real-time surveillance. In 2015, a pilot study by the National Institute for Public Health and the Environment (RIVM) started in two hospitals, Leiden University Medical Center (LUMC) and Jeroen Bosch Hospital (JBH), with the main objective to set up SARI surveillance.[8,9] To assess the value of routinely collected data on respiratory infections in hospitals, it is essential to explore how it relates to data from already existing surveillance systems. Therefore, using historical data derived from the passive surveillance system at LUMC, we conducted an observational study on hospital consultations for ARI in the period 2008-2016, with two objectives:

1| validating the potential of routinely collected data for respiratory infection surveillance in hospitals
2| comparing time-trends for ARI in secondary care, ILI in primary care, and crude mortality monitoring data

METHODS

ARI in secondary care database

Data on patients with an ARI in secondary care during the period between week 40 of 2008 and week 20 of 2016 were provided by the LUMC, a tertiary university teaching hospital in Leiden, South Holland, with 585 beds and a catchment population of 323,269 persons.[10] The catchment population was calculated by dividing the total number of hospitalisations due to respiratory tract infection (RTI) by the total hospitalisations due to RTI in the Netherlands and multiplying this proportion by the total Dutch population size. The data required for the calculation of the catchment population was provided by the National Register of hospital discharge diagnosis (Dutch Hospital Data) (Appendix 1, Figure 4). A selection of International Statistical Classification of Diseases and Related Health Problems (ICD-10) codes related to RTI (J00-J22, A15, A16, A48.1, A70 and A78) was determined for the LUMC for the years 2014, 2015 and 2016. Taking into account the non-normal distribution of the catchment population over the available years, we used the median value for our incidence calculations.

Patients with ARI were defined as those consulting the LUMC emergency department (ED) or outpatient clinic who were registered with diagnostic codes corresponding to a RTI. These codes were based on the Dutch financial coding system (DBC/DOT), applied by the national Dutch Healthcare Authority (NZa) and used by all health care facilities in the Netherlands.[9] Depending on ARI severity, these patients were admitted to an intensive care unit (ICU) or regular ward or discharged for treatment at home. Patients discharged without admission do not fulfil the WHO SARI case definition[11], but we were unable to distinguish outpatients from admitted patients. Therefore, we used 'ARI in secondary care' as a proxy for SARI. The database included consultation date, gender, age category, and ward of admission (ICU/non-ICU)[9], but not microbiological data.

ILI in primary care database

Data from the Sentinel Practices of NIVEL Primary Care Database were used to calculate the incidence of ILI in primary care from week 40 of 2008 to week 20 of 2016.[12] The participating GPs (n=40) report on the weekly number of patients consulting them for ILI, which is defined as 1) sudden onset of symptoms, 2) fever, and 3) at least one of the following symptoms: cough, rhinorrhoea, sore throat, frontal headache, retrosternal pain or myalgia. The population covered by this sentinel network is approximately 0.8% (137,000 persons) of the Dutch population (17.2 million persons) and is representative for age, gender, regional distribution and population density.[13]

Crude mortality monitoring database

Deaths are reported to municipalities and then reported to Statistics Netherlands.[14] During the 2009 influenza pandemic, RIVM and Statistics Netherlands initiated a weekly monitoring system for crude mortality. It monitors the total reported number of deaths from all causes, stratified by age group and region. The presence of excess mortality is verified and reported weekly.[6] For our observational study, all-cause mortality data were collected from Statistics Netherlands for the province of South Holland with over 3.6 million persons (Appendix 1, Figure 4) in the period from week 1 of 2009 though week 20 in 2016.[15] It was not feasible to obtain crude mortality data specifically for the LUMC catchment area, because such data can only be extracted by province from the Statistics Netherlands database.

Statistical analysis

Data are presented for both the 'respiratory year' and 'respiratory season', defined respectively as the period from week 40 through week 39 of the following year and the period from week 40 through week 20 the following year. Data for 2015/2016 is limited to the respiratory season (week 40 of 2015 through week 20 of 2016). The incidence for ARI in secondary care was calculated as the number of patients consulting the hospital per week, divided by the total number of persons in the LUMC catchment population, and expressed per 10,000 persons. To calculate ARI incidence in secondary care as stratified by age groups (0-4, 5-59, and ≥ 60 years old), it was assumed that the age distribution of the total Dutch population in 2008-2016 was similar to the LUMC catchment population. However, it should be noted that the age categories used by Statistics Netherlands differ slightly from those in the LUMC and NIVEL databases (0-5, 5-65, and ≥ 65 years old).[15]

The ILI incidence in primary care was calculated as the number of ILI patients consulting the GP per week, divided by the total number of patients enrolled in participating sentinel GP practices, and expressed per 10,000 persons. The crude mortality in South Holland was calculated as the number of deceased patients, divided by the total number of persons of South-Holland and expressed per 10,000 persons. It is important to note that crude mortality was used only for comparing trends, as it reflected a larger population than the LUMC catchment population. Therefore, the magnitude of all-cause mortality per week was not relevant to this study.

Descriptive statistics were used to compare trends in ARI in secondary care, ILI in primary care, and crude mortality, including three-week moving average incidences, cumulative incidence, and peak incidence. The peak incidence per season for ARI, ILI, and crude mortality was defined as the highest incidence in a season. Data are presented for all ages in total and for the three defined age groups separately. The cumulative incidence

calculations were limited to seven respiratory years (2008/2009-2014/2015). The time-lag between peak ARI and ILI was defined as the number of weeks between the ARI incidence peak in secondary care and ILI incidence peak in primary care. The time-lag between peak ARI in secondary care and all-cause mortality was defined as the number of weeks between the incidence peak for ARI in secondary care at LUMC and the peak of crude mortality in South Holland. Median and interquartile range (IQR) are used to describe these time-lags. Statistical analysis was performed using SPSS (version 22) and Excel (version 2010).

RESULTS

Hospital and primary care consultations

Three-week moving incidence averages of ARI in secondary care and ILI in primary care showed clear peaks during the respiratory season. On visual inspection of the time series, elevations of ARI in secondary care appear broader than for ILI (Figure 1).

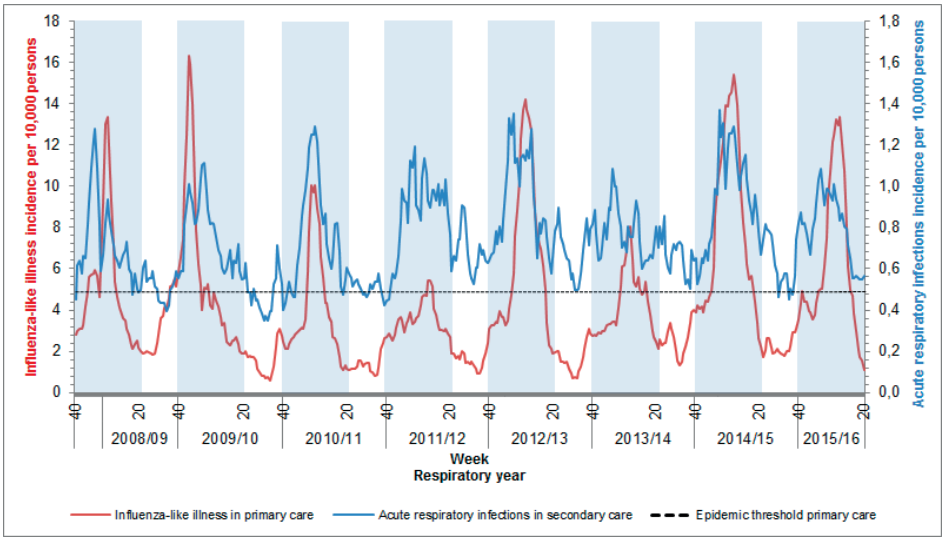

Figure 1. Three-week moving average incidence of acute respiratory infection in secondary care and influenza-like illness in primary care (2008-2016).
The epidemic threshold is 5.1 cases per 10,000 persons and is based primary care data.[16] Blue shading depicts the respiratory season (week 40 through week 20 the following year).

High ILI incidence was confined to the respiratory season (e.g. week 40 through week 20 the following year), whereas ARI incidence in secondary care showed a more diverse pattern, with clear peaks more frequent in winter but not entirely restricted to the respiratory season. The highest peak in weekly incidence for ARI in secondary care was

2.2 cases/10,000 persons (week 1 of 2015), and peak ILI incidence was 19.1 cases/10,000 persons (week 46 of 2009) (Appendix 2, Table 1, and Appendix 3, Table 2). The ARI peaks in secondary care generally occurred earlier than the ILI peaks in primary care, except during the influenza pandemic season of 2009/2010 and the post-pandemic season of 2010/2011. Overall, the median time-lag between ARI and ILI peaks was six and a half weeks (IQR 0 - 9 weeks). During the six seasons in which ARI peaked before ILI, the median time-lag was eight weeks (IQR 6 - 9 weeks). In the respiratory years of 2013/14 and 2015/16, the ARI peak in secondary care was reached earlier than the start of the influenza epidemic, based on ILI and virus diagnostic data from primary care in the Netherlands. Mortality in the province of South Holland as well as ARI in secondary care show winter peaks in the respiratory season. However, crude mortality elevations appear broader with less well-defined peaks than ARI elevations (Figure 2).

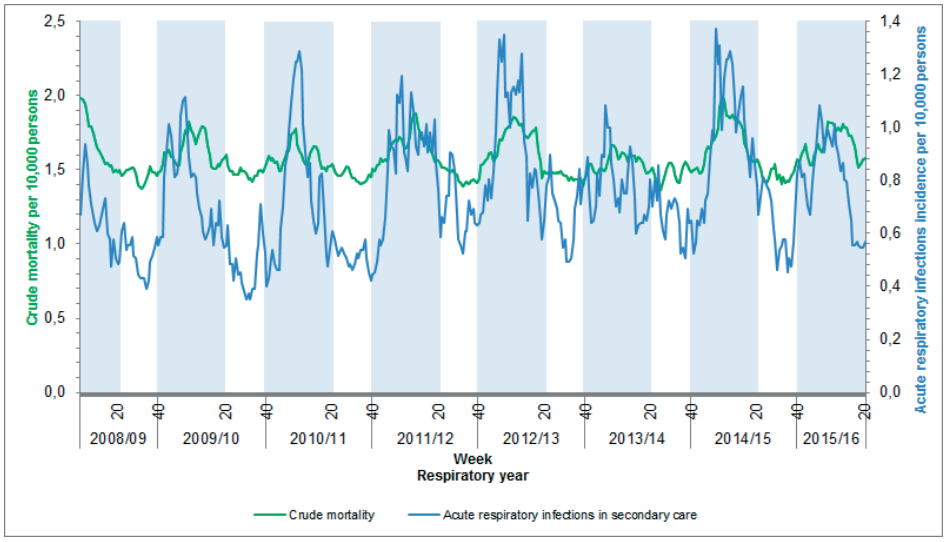

Figure 2. Three-week moving average incidence of acute respiratory infections in secondary care and crude mortality (2008-2016).
Blue shading depicts the respiratory season (week 40 through week 20 the following year).

Mortality almost exclusively occurred among patients 65 years and older. Overall, the crude mortality peak lagged a median 5 weeks behind the ARI peak (IQR 3 - 7 weeks).

Three respiratory seasons (2009/2010, 2011/2012 and 2014/2015) are presented below in more detail to demonstrate the value of routinely collected data on respiratory infections in hospitals.

Respiratory year 2009/2010

During the 2009 influenza pandemic period, ILI incidence in primary care peaked early in the respiratory season (week 46 of 2009), which was not the case for ARI in secondary care (week 52 of 2009). The peak for ARI in secondary care was lowest of all eight respiratory seasons (1.2 cases/10,000 persons). During the pandemic, the highest peaks for ARI in secondary care and ILI in primary care were seen in the 0-4-year olds (Appendix 3, Table 2, and Appendix 4, Table 3). In addition, the pandemic season showed a moderate cumulative incidence for ARI in secondary care (35 cases/10,000 persons), which was within the range of respiratory seasons 2008/2009 and 2010/2011. Compared to the other six respiratory years, the cumulative ILI incidence in 2009/2010 was also in the middle range (Appendix 5 Table 4).

Respiratory year 2011/2012

In the respiratory year 2011/20212, the ILI peak in primary care was low (7.4 cases/10,000 persons), but the peak for ARI in secondary care was considered moderate (1.6 cases/10,000 persons) compared to other eight respiratory years (Appendix 2, Table 1, and Appendix 3, Table 2). The cumulative incidence for ARI in secondary care was the second highest, while ILI cumulative incidence was the lowest of all seven respiratory years (Appendix 5, Table 4).

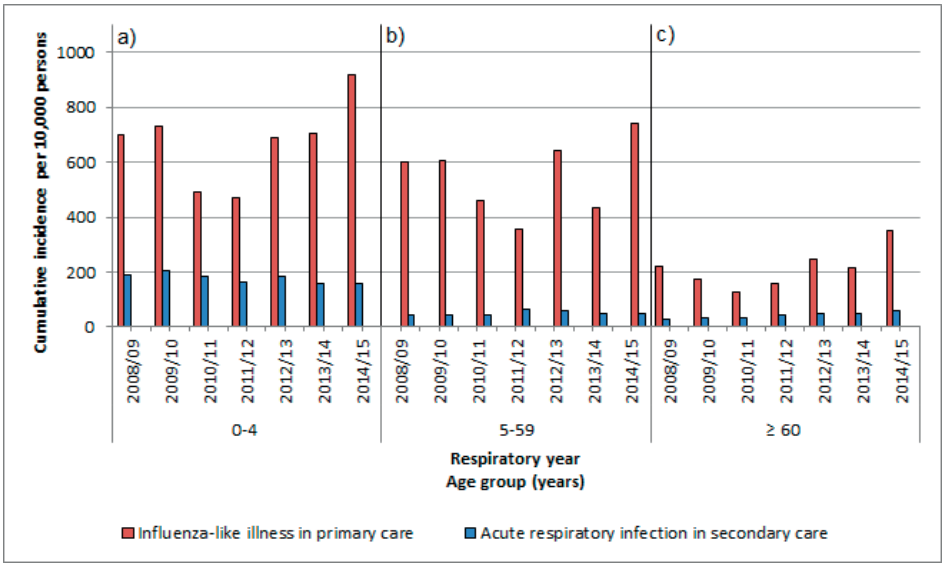

Figure 3. Cumulative incidence of acute respiratory infections in secondary care and influenza-like illness in primary care per age category (2008-2015).
Panel charts a, b and c present the cumulative incidence per age groups (0-4, 5-59, ≥ 60 years old) and respiratory year. The respiratory year 2015/2016 is not included, because data were complete to week 20.

Respiratory year 2014/2015

In the respiratory year 2014/15, a high peak was found for ILI in primary care (16.1 cases/10,000 persons) and ARI in secondary care (2.2 cases/10,000 persons). The highest peak in both primary and secondary care was found among 0-4-year olds, followed by ≥60-year olds (Appendix 3, Table 2 and Appendix 4, Table 3). The cumulative incidence for ILI in 2014/2015 was the highest since 2008/2009 (310 cases/10,000 persons), but cumulative incidence for ARI in secondary care was the lowest since 2008/2009 (31 cases/10,000 persons). All three age groups in primary care showed highest cumulative incidence in this year, while in secondary care this was the case only for ≥60-year olds (Figure 3).

DISCUSSION

This observational study demonstrates that routinely collected data can be used for describing trends of ARI in secondary care and may be suitable for near-real-time SARI-surveillance. We show that ARI incidence in secondary care peaked earlier than ILI incidence in primary care in six of the eight respiratory seasons, with a median time-lag of six and a half weeks. Similar trends were seen in crude mortality, primarily attributable to patients of 65 years and older, and ARI in secondary care.

ARI in secondary care versus ILI in primary care

Our principal finding that ARI in secondary care peaks before ILI in primary care in most respiratory seasons could be explained by high-risk patient groups. We hypothesised that these high-risk groups are elderly patients with comorbidities. As in many European countries, the Dutch population is ageing, and elderly patients with comorbidities increasingly live at home.[17,18] This frail, high-risk patient group is associated with an increased demand for hospital admissions.[19-21] In most seasons, this demand could be reflected in an earlier incidence peak for ARI in secondary care compared to the incidence peak for ILI in primary care. Only for the pandemic and post-pandemic seasons did we find an inverted time-lag, which is hard to explain without additional data on co-morbidities and microbiological test results. However, a disproportionately higher ARI incidence in the younger age versus older age groups is likely to play a role.[22,23]

The finding that ARI incidence in secondary care peaks before ILI in primary care in most respiratory seasons is important for SARI surveillance in terms of preparedness and emergency response.[24,25] Timeliness is critical for detecting outbreaks and taking required public health action to reduce their size, ultimately leading to lower morbidity and mortality [24,26]. Our result confirms the need for SARI surveillance data in the timely detection of future outbreaks and indicates that we cannot depend solely on primary care data.

Our results are consistent with another Dutch study in which respiratory ICU admissions[27] were compared with ILI incidence in primary care from 2007-2015.[28] Its data indicate that in six of the nine seasons studied, increase in respiratory ICU admissions preceded ILI trends with a median time-lag of one week. In contrast to our results, a German study by Buda et al. found that the trend of SARI peaks closely matched the peaks for respiratory infections in primary care in the influenza seasons 2012-2016.[29] Comparison with our study is difficult, because of large differences in methodology and health care systems.

ARI in secondary care versus crude mortality

Comparing ARI incidence in secondary care with crude mortality showed a similar trend, with peaks in winter over a period of eight respiratory years. The incidence peaks for crude mortality in the province of South Holland are probably associated with ARI peaks in secondary care in the LUMC catchment area, but mortality cannot be completely attributed to ARI because disease-specific data were not available to this study. The seasonality of crude mortality has been clearly documented and is primarily caused by increase in deaths in the elderly during winter.[30,31] Van Asten et al. stated that winter peaks of all-cause mortality are often largely attributed to influenza and sometimes cold snaps, but other pathogens, such as respiratory syncytial virus, parainfluenza, and norovirus, may also play a substantial role in the mortality of the elderly.[32]

Historical data on ARI in secondary care

Our results suggest that historical data on ARI in secondary care may be of value for early detection of outbreaks and for providing insight into the severity of epidemics, if used in a near-real-time surveillance system. In particular, the seasons 2009/2010, 2011/2012, and 2014/2015 illustrate their value for SARI surveillance. During the influenza A(H1N1) pandemic season, the cumulative ARI and ILI incidence indicated a relatively moderate season in hospitals and primary care, with the 0-4-year old age group most affected. This aligns with other studies and confirms the moderate impact of the influenza A(H1N1) pandemic.[22,23,33] The 2011/2012 season is of interest, because of a rather severe respiratory year in hospitals even while, based on primary care data, the criteria for an influenza epidemic were not met. The discrepancy went unnoticed at the time, because there was no real-time surveillance of ARI in secondary care. During the influenza A(H3N2)-dominant 2014/2015 season in the Netherlands, the longest influenza epidemic was recorded since the start of surveillance in 1970 and occurred against the background of an influenza vaccine mismatch.[34] Our data show high incidence peaks in both primary and secondary care, especially for patients ≥60 years of age. Such peaks often coincide with a high demand on bed capacity and increased need for qualified medical staff due to sickness absenteeism in hospitals.[35,36] If these data had been available on a weekly basis in 2014/2015, hospitals

might have been better prepared for the high number of patients by timely upscaling of bed capacity, using cohort isolation, and recruiting additional medical personnel.

Limitations

Several limitations should be taken into account when interpreting these findings. First, the absence of microbiological diagnostics results is an important barrier to interpreting incidence differences between ILI in primary care and ARI in secondary care. Data on microbiological test results would be needed to explain the whole spectrum of respiratory infections and to better understand the time-lag between ILI, ARI, and mortality per season. For example, the influenza-related SARI could be more accurately defined and make comparisons with ILI more biologically plausible. Together with data on medical history, such as co-morbidities and place of residence (e.g. long-term care facility versus home), it could clarify which patient group is primarily reflected in the peak of ARI incidence in secondary care. In the setting of SARI surveillance, detection of causative pathogens is crucial in mitigating the effect of disease outbreaks by taking timely health care interventions.[37-39]

A second limitation is that we used retrospective data to describe trends for ARI and ILI. Robust 'real-time' SARI-surveillance data are not yet available in the Netherlands. Thirdly, incidence calculations for ARI in secondary care were based on one hospital in the western part of the Netherlands. Although the catchment population of this hospital is large, inclusion of more hospitals with a nationally representative distribution would have increased representativeness and generalisability of the study results. Fourthly, this study used 'acute respiratory infections in secondary care' as a proxy for SARI patients, because no distinction could be made between patients admitted to hospital, reviewed at the outpatient clinic, or discharged home. This could have led to overestimation of incidence calculations.

CONCLUSIONS

This observational study shows that data on ARI in secondary care are of added value for early detection of outbreaks and providing insight into the severity of epidemics, if used in a near-real-time surveillance system. The principal finding is that in most respiratory seasons, the peak of ARI incidence in secondary care preceded the peak of ILI incidence in primary care. This is crucial information for preparedness and emergency control. Adding microbiological test results to these incidence data would be of great value in explaining the whole spectrum of ILI in primary care, ARI in secondary care, and mortality.

ACKNOWLEDGEMENTS

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APPENDIX 1

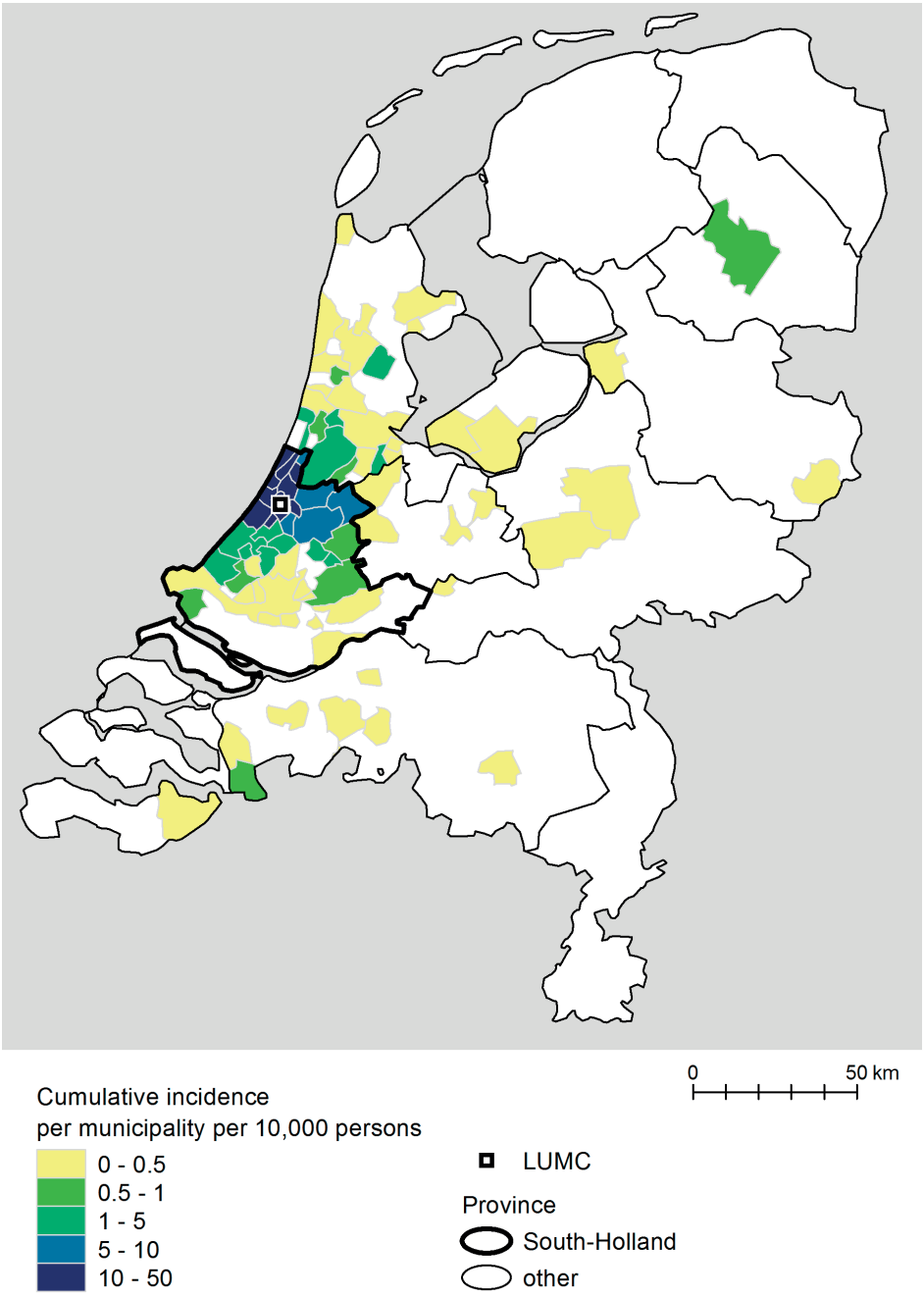


Figure 4 Cumulative incidence of respiratory tract infections per municipality per 10,000 persons in the catchment population of Leiden University Medical Center.

APPENDIX 2

Table 1 Incidence peak, peak week and time-lag for acute respiratory infections in secondary care, influenza-like illness in primary care, and crude mortality in the period 2008-2016.

Respiratory year	Dataset[†]	Peak (week number)	Time-lag relative to ARI (weeks)	Peak incidence[‡]
2008/2009	ARI	50		1.39
	ILI	3	5	14.14
	MOR	3	5	
2009/2010	ARI	52		1.21
	ILI	46	-6	19.07
	MOR	3	4	
2010/2011	ARI	5		1.39
	ILI	3	-2	11.34
	MOR	1	-4	
2011/2012	ARI	1		1.64
	ILI	10	9	7.42
	MOR	8	7	
2012/2013	ARI	51	6	1.55
	ILI	5		16.23
	MOR	5	6	
2013/2014	ARI	49		1.24
	ILI	7	10	8.98
	MOR	2	5	
2014/2015	ARI	1		2.23
	ILI	8	7	16.12
	MOR	3	2	
2015/2016	ARI	51		1.30
	ILI	7	9	14.81
	MOR	8	10	

[†]ARI: acute respiratory infections in secondary care [‡]incidence per 10,000 persons
ILI: influenza-like illness in primary care MOR: crude mortality

APPENDIX 3

Table 2. Incidence peak, peak week, and age group for acute respiratory infections in secondary care in the period 2008-2016.

Respiratory year	Age group (years)	Peak (week number)	Peak incidence†
2008/2009	0-4	50	12.58
	5-59	19	0.55
	≥ 60	49	1.32
	Total	*50*	*1.13*
2009/2010	0-4	51	9.73
	5-59	45	0.72
	≥ 60	36	1.38
	Total	*52*	*0.98*
2010/2011	0-4	3	10.83
	5-59	15	0.55
	≥ 60	5	1.80
	Total	*5*	*1.13*
2011/2012	0-4	49	7.22
	5-59	1	0.80
	≥ 60	7	2.32
	Total	*1*	*1.33*
2012/2013	0-4	51	11.84
	5-59	10	0.84
	≥ 60	10	1.95
	Total	*51*	*1.25*
2013/2014	0-4	52	7.82
	5-59	10	0.67
	≥ 60	23	2.02
	Total	*49*	*1.00*
2014/2015	0-4	1	9.04
	5-59	1	0.67
	≥ 60	1	3.54
	Total	*1*	*1.80*
2015/2016	0-4	51	10.46
	5-59	2	0.64
	≥ 60	2	1.54
	Total	*51*	*1.05*

†incidence per 10,000 persons

APPENDIX 4

Table 3. Incidence peak, peak week, and age group for influenza-like illness in primary care in 2008-2016.

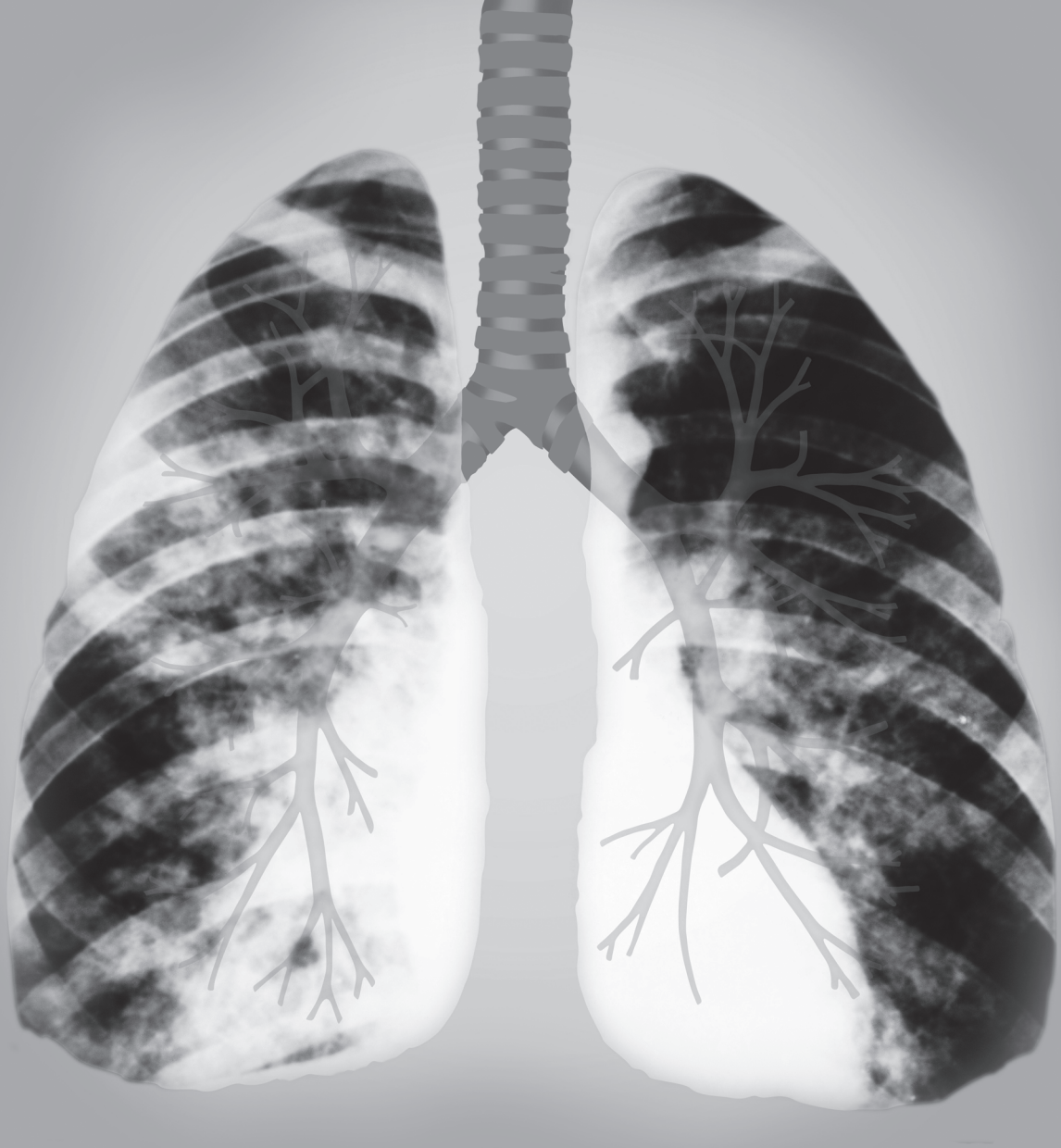
Respiratory year	Age group (years)	Peak (week number)	Peak incidence[†]
2008/2009	0-4	3	44.31
	5-59	4	11.79
	≥ 60	3	19.57
	Total	*3*	*14.14*
2009/2010	0-4	46	62.89
	5-59	46	19.70
	≥ 60	1	8.72
	Total	*46*	*19.07*
2010/2011	0-4	3	37.31
	5-59	3	11.17
	≥ 60	1	8.18
	Total	*3*	*11.34*
2011/2012	0-4	51	26.42
	5-59	10	5.93
	≥ 60	10	8.72
	Total	*10*	*7.42*
2012/2013	0-4	5	52.24
	5-59	5	15.07
	≥ 60	8	15.08
	Total	*5*	*16.23*
2013/2014	0-4	7	35.86
	5-59	7	7.51
	≥ 60	11	9.74
	Total	*7*	*8.98*
2014/2015	0-4	7	59.06
	5-59	8	12.65
	≥ 60	8	21.03
	Total	*8*	*16.12*
2015/2016	0-4	5	46.61
	5-59	7	13.86
	≥ 60	9	14.92
	Total	*7*	*14.81*

[†]incidence per 10,000 persons

APPENDIX 5

Table 4. Cumulative incidence of acute respiratory infections in secondary care versus influenza-like illness in primary care in the respiratory years 2008/2009-2014/15

Respiratory year	Cumulative incidence acute respiratory infection secondary care[†]	Cumulative incidence influenza-like illness primary care[†]
2008/2009	34	232
2009/2010	35	221
2010/2011	35	161
2011/2012	42	148
2012/2013	43	248
2013/2014	39	192
2014/2015	31	310
Total	*259*	*1513*



4

Clinical factors, C-reactive protein point of care test and chest X-ray in patients with pneumonia: a survey in primary care.

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ABSTRACT

Background

In patients with an acute lower respiratory tract infection, the decision to prescribe antibiotics is sometimes difficult. C-reactive protein point of care test and chest X-ray are available as additional diagnostic tests, but the usefulness in clinical practice is unknown. To assess the proportion of Dutch general practitioners that use additional diagnostics in patients with an acute lower respiratory tract infection and whether clinical factors and C-reactive protein point of care test affect the behaviour in requesting chest X-rays.

Methods

In 2014, a questionnaire was sent to a random sample of 900 Dutch general practitioners. Outcome parameters are the use of C-reactive protein and chest X-ray, the percentage of GPs who guide their decision in requesting chest X-rays by CRP testing and the expectation regarding presence or absence of pneumonia. In addition, distribution of considerations for requesting chest X-rays were assessed.

Results

Two hundred fifty-five completed questionnaires (29%) were returned. More than half (54%) use the C-reactive protein test, these GPs tend to use less chest X-rays (p=0.07). GPs overestimate the chance that pneumonia would be present on the radiograph and 70% consider the detection or exclusion of abnormalities other than pneumonia as the main reasons for requesting a chest X-ray.

Conclusions

GPs report that CRP results affect their behaviour regarding the request of a chest x ray in patients with lower respiratory tract infection and therefore research is needed to substantiate the use of these diagnostic tools for this purpose.

INTRODUCTION

In patients that present with an acute lower respiratory tract infection, the decision whether or not to prescribe antibiotics is sometimes difficult, especially in moderately ill patients [1, 2]. Antibiotics are used more restrictively by Dutch general practitioners (GP) than by their colleagues in other European countries [3]. Nevertheless, there are also large regional differences within the Netherlands [4]. These differences are an expression of the complexity of the consideration of whether or not to prescribe an antibiotic. In general, one can state that patients with acute bronchitis do not need antimicrobial treatment while patients with pneumonia do [5,6]. Unfortunately, for the diagnosis of pneumonia, the use of anamnesis and physical examination alone provide insufficient support [7-9].

Two types of additional (diagnostic) tests for acute lower respiratory tract infection can be used in general practice: the C-reactive protein point of care test (CRP POCT) and the chest X-ray. A low CRP (< 20 mg/l) can exclude pneumonia with reasonable certainty, irrespective of clinical findings, while an elevated CRP (> 100 mg/l) greatly increases the chance of pneumonia warranting antibiotic treatment [8,10]. A recent meta-analysis ascertained that even when clinical variables are taken into account, the CRP test can help to confirm or exclude pneumonia [11]. Different guidelines (e.g. the British and the Dutch guideline) therefore, indicated the use of the CRP test in moderately ill patients [1,12]. Studies that evaluated whether the CRP POCT reduced the number of antibiotic prescriptions showed variable results [13,14].

A chest X-ray can be used to detect pneumonia, but the use of this examination in all individuals in whom a pneumonia is suspected, is not recommended. The chest X-ray is currently only recommended in the Dutch guideline to investigate the cause of lack of recovery, uncertainty about the diagnosis or treatment, or when a condition other than pneumonia is suspected as an explanation for the symptoms [1]. The British guideline does not mention chest X-ray as a diagnostic tool in patients with suspected pneumonia or exacerbations of asthma and COPD. Every year GPs request about 31 chest radiographs per 1000 person-years [15]. Research into the effectiveness of requesting chest X-rays by the GP for certain subgroups of patients with an acute lower respiratory tract infection is lacking. The objective of this study was to assess the use of chest X-ray and the CRP POCT in patients with an acute respiratory tract infection in Dutch primary care. We asked the GPs about their estimates and experiences with this complex situation where evidence for a specific strategy is lacking.

METHODS

Study design and setting

Between May and September 2014 a questionnaire-based cross-sectional study was performed in the Netherlands. The registry from the *Netherlands Institute for Healthcare Research* (NIVEL) contains address information of all GPs in the Netherlands. A random sample of 900 addresses was drawn. The questionnaire (see below) was sent in May 2014 by mail to these family practice addresses.

Construction of the questionnaire

The two main investigators (GHG and RJP) held an exploratory focus group discussion with various GPs in the Leiden region, the Netherlands. In this discussion, open questions were asked about the way in which the GPs use additional diagnostic tests in patients with acute lower respiratory tract infection and in what way the results of the tests affect their treatment policy [16]. An acute lower respiratory tract infection was defined as complaints for less than three weeks.

With the results, a list with open and closed questions was generated and distributed among 15 GPs in the Leiden region via the newsletter of the Leiden Primary Care Research Network. The answers and feedback received via this route contributed to the final quantitative questionnaire.

Quantitative questionnaire

The questionnaire first asks about the number of years of work experience, the number of hours a week that the GP works at the general practice, and an estimate of the number of chest X-ray request in a year for patients with acute lower respiratory tract infection.

Main outcomes are the use of CRP POCT, the percentage of GPs who guide their decision in requesting chest X-rays by CRP testing and the expectation regarding presence of pneumonia on chest X-ray. In addition, indications for use of CRP POCT, clinical parameters and distribution of reasons for requesting chest X-rays (in GPs with and without CRP test available), which other pathology the GP wants to exclude and diagnostic and therapeutic consequences when pneumonia is present or absent were assessed.

The various characteristics and consequences could be scored on five-point Likert scales, with answers varying from “(almost) never”/”Very unimportant” to “(almost) always”/”Very important”. The complete questionnaire is available in the Supplementary Material.

Analysis

The returned questionnaires were anonymized. Descriptive analyses and comparison of proportion with Chi Square test were performed with SPSS (IBM, version 23).

RESULTS

Study population

Twenty-three questionnaires were returned due to outdated address details. In total, after one reminder letter, 255 of the 877 (29%) questionnaires were returned completed in September 2014. The respondents reported a median work experience of 14 years, (interquartile range, IQR, 9 - 22 years) and a median work week of 36 hours (IQR 30 - 41.5 hours) at the general practice.

Chest X-ray

Median reported number of chest X-rays per year for patients with an acute lower respiratory tract infection was 10 (IQR 4-12). The 24 respondents (9%) that never requested a chest X-ray for this indication, could not answer the remaining questions. Median work experience and work week in the respondents who never request a chest X-ray did not differ from respondents who did request chest X-rays.

Table 1 and 2 provide an overview of the reports of GPs regarding considerations and objectives to request a chest X-ray. The majority (70% of all GPs) consider the detection or exclusion of abnormalities other than pneumonia as one of the main reasons for requesting a chest X-ray. The exclusion of malignancy, heart failure, sarcoidosis, and tuberculosis are mentioned repeatedly. If the chest X-ray has been requested to exclude other pathology, the GP will state this in 90% of the cases on the X-ray application form. Factors that play an important role in the decision to request a chest X-ray are mainly age, smoking, and the duration of the complaints.

The expectation of 217 GPs (14 GPs did not answer this question and 24 never request a chest X-ray) to detect a lung infiltrate on the chest X-ray was less than 10% in 13% of GPs, between 10 and 20% in 19% of GPs, and more than 50% in 68% of GPs. If an infiltrate suspect for pneumonia is present, 227 of the 230 GPs (99%; 1 GP did not answer this question and 24 GPs never request a chest X-ray) often, to almost always, prescribe an antibiotic. In the absence of a pneumonia, 4% of GPs often to almost always, prescribe an antibiotic (Figure 1).

Table 1. Questionnaire response from general practitioners: Clinical factors in the consideration to request a chest X-ray in patients with an acute lower respiratory tract infection (n=226*).

Clinical factors in the consideration to request a chest X-ray	Rating	
	Important (%)	Neutral or unimportant (%)
Smoking	191 (85)	35 (15)
Duration of the complaints	186 (82)	40 (18)
Age	179 (79)	47 (21)
Presence of fever	98 (43)	128 (57)
Duration of fever	95 (42)	131 (58)
Response to previous antibiotics	92 (41)	134 (59)
Producing sputum, and sputum color	28 (12)	198 (88)

*29 respondents never requested chest X-rays and/or did not give an answer to this question.

Table 2. Questionnaire response from general practitioners: Reasons to request a chest X-ray in patients with an acute lower respiratory tract infection (n=228*).

Reasons to request a chest X-ray	Number of times indicated to be the most important (%**)
Detection or exclusion of other lung abnormalities, such as a lung tumor	159 (69.7)
Confirm the diagnosis of pneumonia	87 (38.2)
Exclude the diagnosis of pneumonia	76 (33.3)
Reassuring the patient	22 (9.6)
Uncertainty about further policy	21 (9.2)
As a guide to decide on antibiotic prescription	18 (7.9)
Conditions that GPs want to exclude	**Number of times indicated (%), N=190***
Lung cancer	160 (84.2)
Heart failure	46 (24.2)
Sarcoidosis	36 (18.9)
Tuberculosis	24 (12.6)
Pneumothorax	15 (6.9)
Other**	48 (25.2)

*27 respondents never requested chest X-rays and/or did not give an answer to this question.
**Percentages add up to >100% because some respondents gave more than one reason the same score.
*** some GPs who did not state the exclusion of other lung abnormalities as the most important reason also answered this question; in addition, several answers could be filled in.
**** other disorders included foreign body, pulmonary embolism, and systemic lupus erythematosus and were each mentioned by <5% of all respondents.

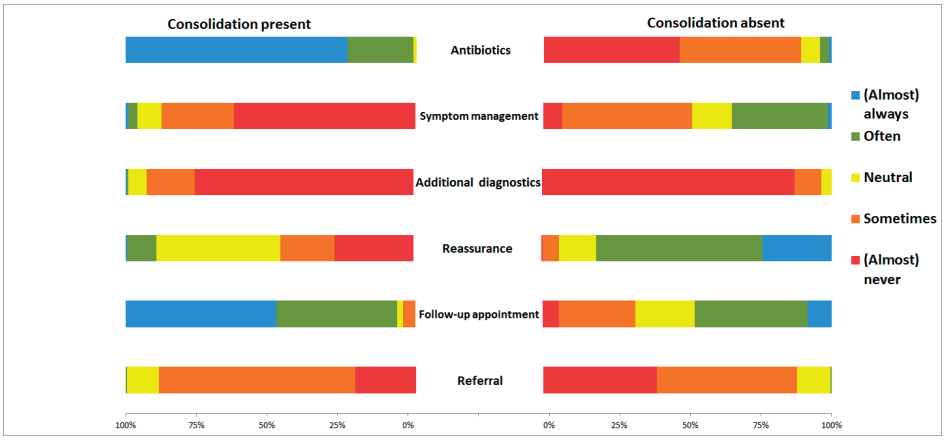

Figure 1. Questionnaire response from general practitioners: Policy following the chest X-ray in patients with an acute lower respiratory tract infection (n=230*).
Bi-directional bar chart. On the left the policy followed in case a pneumonia was detected on the chest X-ray, on the right the policy followed in case no pneumonia was detected on the chest X-ray. In the middle, description of the policy.
* 24 respondents never request a chest X-ray and one did not answer this question.

CRP point of care test

The CRP POCT is used by more than half of GPs (54%). A large proportion of them, also use the test to evaluate suspected infections other than pneumonia (Table 3), e.g. diverticulitis, urinary tract infection, or an unknown "other" infection. Eighty percent of all GPs reported that they foresee that CRP POCT can replace chest X-ray as a diagnostic test partially or completely. GPs with CRP test available are more confident than those that do not have this test available (86% versus 71%, p<0.01).

Table 3. Questionnaire response from general practitioners: Use and indications for use of the CRP point of care test (n=246*).

	Number (%)
Respondents that use the CRP point of care test in the general practice	**134 (54)**
Use only if there is a suspicion of respiratory tract infection	35 (26)
Use in case of suspected respiratory tract or other infection	83 (62)
Hardly ever use the test	16 (12)
In many cases, the CRP point of care test plays a role in the consideration to request a chest X-ray**	75 (56)
Respondents that do not use the CRP point of care test in the general practice	**112 (46)**
Would like to purchase the test in the future	85 (76)
Would not like to purchase the test in the future	27 (24)

* Nine GPs did not answer this question
** Respondents that indicated that this "often" or "(almost) always" plays a role.

Difference between GPs with and without CRP test

GPs with CRP POCT available reported to request less chest X-rays than their colleagues without CRP POCT available (median 6, IQR 3-10 versus median 10, IQR 5-14 respectively; p=0.07).

Expectation regarding presence of pneumonia did not differ between GPs with or without CRP POCT available (p=0.67).

Presence and colour of sputum was reported to be more important when considering chest X-ray by GPs without than those with CRP POCT available (Figure S1 in the supplementary material).

Guidance whether or not to prescribe antibiotics is reported as reason for requesting chest X-ray less frequently in GPs with CRP than in GPs without CRP. Other reasons were not different (see Figure S2 in the supplementary material).

GPs who do not use CRP POCT reported more frequently than those who do use CRP POCT to start symptom management in case pneumonia is confirmed (neutral to almost always 15% versus 9%; p=0.05) or ruled out with chest X-ray (neutral to almost always 57% versus 41%; p<0.01). All other policy items did not differ significantly between GP groups.

DISCUSSION

Main findings

This study shows that in 255 Dutch GPs the use of additional diagnostic tools for the suspicion of acute lower respiratory tract infection was diverse. GPs reported to estimate the probability of having a pneumonia as high among the patients for whom they request a chest X-ray. Nearly 70% of GPs request the photo mainly to exclude other pathology. More than half of the GPs had the CRP POCT available in 2014 and the majority used this test to determine whether or not to request a chest X-ray. GPs using CRP POCTs reported to request less chest X-rays than GPs who did not use this test. These latter GPs reported to use chest X-ray more often to guide the decision to prescribe antibiotics. Many GPs also used the CRP POCT for other purposes.

Strengths and limitations

The strengths of this study are the random sample of GPs in the Netherlands and the considerable number of 255 completed surveys that were available for analysis. The inventory based on focus group interview and pilot questionnaires during the pilot study means that

the diversity of ideas, experiences, and behaviors in the target group were well explored. The fact that both GPs with and without a CRP POCT, as well as GPs that vary from never to frequently requesting chest X-rays have responded, makes that the sample has, in any case, included all extremes of diagnostic policy.

A limitation of the study is the potential occurrence of sampling bias. The 'selection' of respondents could be different from that of the GPs who did not respond. Although the absolute number of questionnaires analyzed is considerable, the response rate of 29% is not high. A review by Creavin *et al.* showed a mean response rate of 61% [17]. However, response rate in recent surveys among Dutch GPs is substantially lower [17-19]. Respondents could be more interested in this topic than non-responders and thereby more aware of guidelines and evidence, resulting in more prudent use of diagnostic tools. The years of work experience and the number of working hours of the respondents correspond to the national average, 14.9 years and 31.2 hours per week respectively [20]. Moreover, McFarlane *et al.* demonstrated that higher response rates in a survey of physicians are not associated with lower selection bias [21].

Nonetheless, potential difference in characteristics between GPs who filled in the questionnaire and the ones that did not respond, might still be present. However, the study provides a useful insight into the considerations of the Dutch GP about additional diagnostic tools for acute lower respiratory tract infections.

The short questionnaire brings about that not every possible consideration has been asked. For example, it is not clear in what type of patient the CRP POCT is actually used, if CRP kinetics are taken into consideration and how GPs interpret the results. A previous study showed that most GPs use the CRP POCT in patients who are moderately ill when it is not immediately obvious whether or not the patient needs an antibiotic. In the same study, it was found that the CRP POCT is sometimes used too frequently, even in situations where this test should have no consequences for the policy [22].

This is a survey-based study about opinions and perceptions, which do not necessarily reflect the real management and prescription habits. The survey was completed in 2014. It is possible that with an increase in use, the interpretation of the results will also change slightly.

Interpretation

The expectation about the likelihood to detect a lung infiltrate on the X-ray is high. Two-thirds expect an infiltrate in more than 20% of patients. This estimate does not match

the findings in several primary care studies, where a pneumonia on the chest X-ray was detected in only 5 to 13% [8,11,23].

The chest X-ray is the gold standard for the detection or exclusion of pneumonia, while clinical features, including a low CRP value, can safely exclude pneumonia [11,12]. The added value of the chest X-ray in the detection or exclusion of pneumonia is therefore mainly present in the group of patients with a high probability of the presence of an infiltrate. This mainly concerns patients with clinical characteristics fitting with pneumonia that have a high CRP value. We hypothesize that GPs may request too much chest X-rays because they overestimate the likelihood of pneumonia. With better pre-test (pre-chest X-ray) assessment, for example by using CRP, they could rule out pneumonia more often without chest X-ray. On the other hand, GPs incorrectly withhold some patients from a chest X-ray because they do not adequately determine the group of patients with a high pre-test (pre-chest X-ray) probability, partially because only 54% in our study used CRP test. In addition, given the discrepancy between the pre-test assessment and the actual percentage of pneumonia present on lung images, pneumonia can often be excluded with a chest X-ray. In the latter case, antibiotics are prescribed less frequently.

The lack of evidence is the reason that the chest X-ray is currently not clearly defined in the standard of the Dutch Society of GPs or the British guidelines for the detection or exclusion of pneumonia [1,12]. However, this study shows that GPs already use the results of the CRP test in their decision to request a chest X-ray and/or that they foresee that the CRP test can replace the chest X-ray as a diagnostic tool.

Often the detection or exclusion of a condition other than pneumonia is indicated as the main reason to request a chest X-ray. In a European cohort of nearly 3,000 patients with acute cough who underwent a chest X-ray, a clinically relevant abnormality -other than pneumonia- was found in 3% [24]. Therefore, the chance that a GP will find such aberrations is small. A malignancy can be missed on the chest X-ray, especially if at that time an infiltrate is present in the same area. It is then preferable to repeat the chest X-ray after the pneumonia has been treated [25].

Exact information about availability and use of CRP POCT in European countries is not known. Oppong et al. reported that CRP POCT was available in 12 of 14 primary care networks in 13 European countries [26]. There were marked differences in the availability of CRP test between Spain and Denmark [27] and between CRP use in Belgium (3%), the UK (15%) and the Netherlands (48%) in 2012-2013 [28]. The use of CRP has increased in Scandinavian countries between 2004 and 2013 [29].

When comparing Danish primary care versus Spanish primary care, chest X-rays are used more frequently to confirm pneumonia in Spain [27].

Implications

With the frequent use of the CRP POCT to aid in the decision to request a chest radiograph, there appears to be a need for research into a diagnostic algorithm, that would incorporate clinical characteristics and a CRP result, to determine in which patient a chest X-ray has added value.

This study also shows that GPs using the CRP POCT often use this test for other infections than pneumonia. The use of the CRP test is only recommended for patients with acute lower respiratory tract infections or diverticulitis. For both disorders, the use of the CRP test has many limitations [1,30]. Restraint in the use of this test is therefore required until new research proves that either the CRP POCT has added value for other indications, or that the CRP test can replace a chest radiograph.

CONCLUSION

GPs widely use the CRP POCT and often base their decision to request a chest X-ray on the outcome. They overestimate the chance of finding a pneumonia in these patients. Clinical variables in combination with the CRP POCT, could help the GP to request chest radiographs more selectively for patients with acute lower respiratory tract infection. Research is however first needed to substantiate the use of these diagnostic tools for this purpose.

ACKNOWLEDGEMENTS

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SUPPLEMENTARY MATERIAL

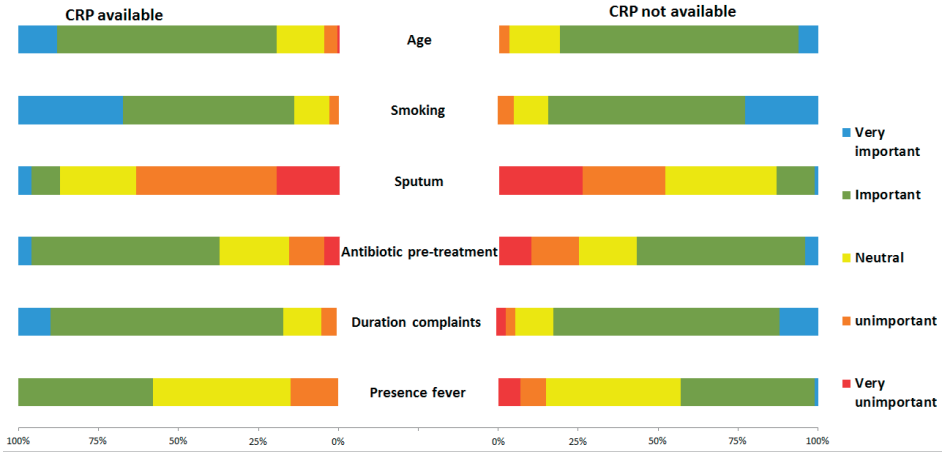


Figure S1. Bidirectional bar chart: questionnaire response of general practitioners with and without CRP test available, regarding the considerations to request a chest X-ray in patients with an acute lower respiratory tract infection (n=226*).

* 29 respondents never requested a chest X ray and/or did not give an answer to this question.

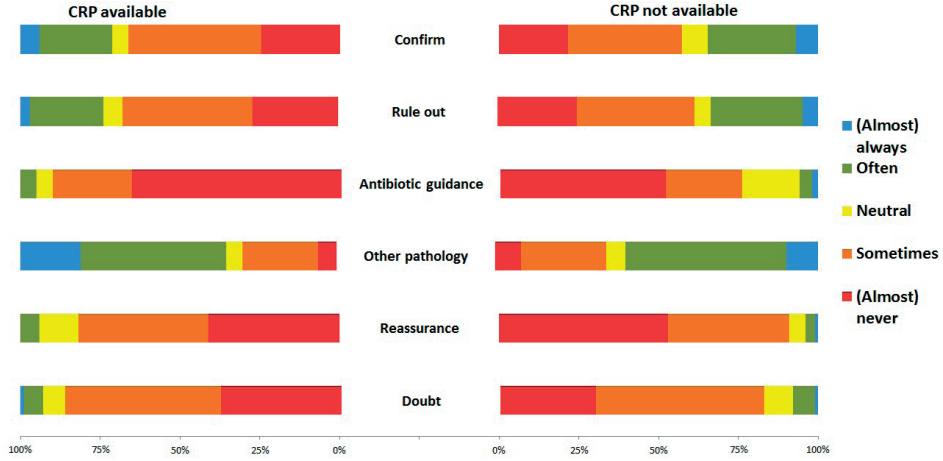
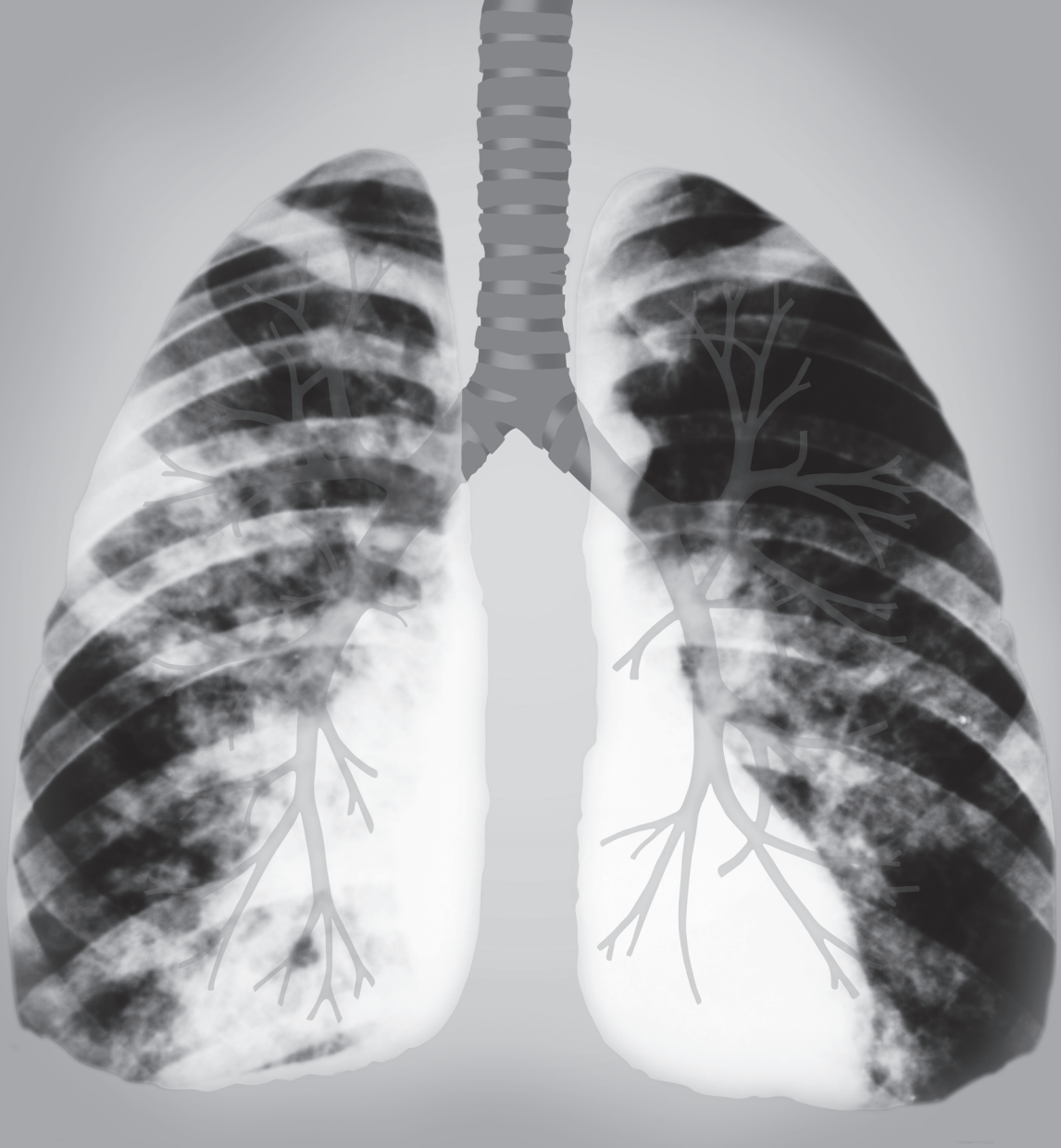


Figure S2. Bidirectional bar chart: questionnaire response of general practitioners with and without CRP test available, about reasons to request a chest X-ray in patients with an acute respiratory tract infection (n=228*).

* 27 respondents never requested a chest X ray and/or did not give an answer to this question.



5

Prediction model for pneumonia in primary care patients with an acute respiratory tract infection: role of symptoms, signs, and biomarkers.

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ABSTRACT

Background

Diagnosing pneumonia can be challenging in general practice but is essential to distinguish from other respiratory tract infections because of treatment choice and outcome prediction. We determined predictive signs, symptoms and biomarkers for the presence of pneumonia in patients with acute respiratory tract infection in primary care.

Methods

From March 2012 until May 2016 we did a prospective observational cohort study in three radiology departments in the Leiden-The Hague area, The Netherlands. From adult patients we collected clinical characteristics and biomarkers, chest X ray results and outcome. To assess the predictive value of C-reactive protein (CRP), procalcitonin and midregional pro-adrenomedullin for pneumonia, univariate and multivariate binary logistic regression were used to determine risk factors and to develop a prediction model.

Results

249 patients were included of whom 30 (12%) displayed a consolidation on chest X ray. Absence of runny nose and whether or not a patient felt ill were independent predictors for pneumonia. CRP predicts pneumonia better than the other biomarkers but adding CRP to the clinical model did not improve classification (-4%); however, CRP helped guidance of the decision which patients should be given antibiotics.

Conclusions

Adding CRP measurements to a clinical model in selected patients with an acute respiratory infection does not improve prediction of pneumonia, but does help in giving guidance on which patients to treat with antibiotics. Our findings put the use of biomarkers and chest X ray in diagnosing pneumonia and for treatment decisions into some perspective for general practitioners

BACKGROUND

Diagnosing pneumonia in general practice can be challenging. The recognition of pneumonia among other manifestations of respiratory tract infection (RTI) is important since pneumonia – according to the GP's guideline – requires antimicrobial treatment, has a worse prognosis than other RTIs and requires follow up. Pneumonia comprises (typical and atypical) bacterial and viral infection; the latter is not expected to benefit from antibacterial treatment. On the contrary, acute bronchitis and upper respiratory tract infections are most often of viral origin, and have an excellent prognosis and expectant strategy is generally appropriate (1-3). To differentiate pneumonia from other respiratory tract infections, clues to determine this diagnosis are needed. Unfortunately, anamnesis and physical examination lack sensitivity and specificity to diagnose pneumonia (4). Severely ill patients are more likely to have pneumonia, with a high pre-chance of bacterial origin, and should be treated with antibiotics while patients with uncomplicated respiratory tract infection are less ill and have no benefit from being treated with antibiotics. C-reactive protein (CRP) can help to confirm or rule out pneumonia, taking clinical signs and symptoms into account (5). In particular for moderately ill patients, different guidelines (e.g. the Dutch and the British guideline) point to the use of the CRP test. A low CRP (< 20 mg/l) can rule out pneumonia with reasonable certainty, irrespective of clinical signs and symptoms, while an elevated CRP level (> 100 mg/l) increases the chance of pneumonia and indicates a potential benefit from antibiotic treatment (6, 7). With CRP levels between 20 and 100 mg/l, decision to initiate antibiotics is left to the clinical picture and assessment of risk factors for a worse outcome (8, 9). The impact of this strategy on antibiotic prescription rate showed variable results (10).

Among other biomarkers for inflammation, procalcitonin (PCT) had limited added value in the diagnosis of pneumonia in this setting and studies on the prognostic value of the adrenomedullin precursor, mid-regional pro-adrenomedullin (MR-proADM), are currently lacking (7).

The reference 'golden' standard for establishing pneumonia is the chest X ray. A chest X ray in outpatients, however, does not improve outcome (11, 12) and therefore this is not routinely recommended in patients attending their general practitioner (GP) with suspicion of a community-acquired pneumonia. Different general practice guidelines do not provide clear guidance when to order a chest X ray in specific patients with acute respiratory infections (9, 13). Despite that, in 22% of patients with a suspected lower respiratory tract infection chest X ray is requested (14).

A survey among 255 Dutch GPs in 2014 learned that there is an urgent clinical need for an algorithm to define which patients with an acute respiratory tract infection benefit most from a diagnostic chest X ray (15). In the Netherlands, general practitioners ordered 31 chest X rays per 1,000 persons per year in 2000 (16). A large proportion of these are intended for patients with acute respiratory tract infections.

Herein, we evaluate a cohort of patients with an acute respiratory tract infection who had been referred by their GP for a chest X ray, to determine predictive factors for the presence of pneumonia.

METHODS

From March 2012 until May 2016 we did a prospective observational cohort study in three radiology departments in different hospitals in the western part of the Netherlands. Local ethical committee approved the study (protocol no. P08.065) and all participants provided written informed consent.

We included adult patients with an acute respiratory tract infection, referred to the radiology department by their general practitioner for a chest X ray to determine the presence of pneumonia. We confined the study to those patients with complaints for less than three weeks, as we intended to study the value in 'acute respiratory tract infection'.

Within an hour before or after chest X ray, clinical data were recorded via an interview and vital signs were measured. Diagnostic tests to find the causal agent of respiratory tract infection were taken: blood cultures were drawn, nasopharyngeal swabs for respiratory viruses and Mycoplasma, Chlamydia and Legionella spp. were collected, a sputum culture (to identify bacterial respiratory pathogens) was taken from persons who coughed up sputum. Blood samples were taken for biomarker testing. At inclusion, EDTA plasma was collected to determine CRP, PCT and MR-proADM. CRP is measured with a turbidimetric reaction detecting antigen-antibody complex (Roche Modular P800) (catalogue number 12000951/12000953/04956923190).

PCT is measured with Brahms Kryptor using an immunoassay with TRACE (Time Resolved Amplified Cryptate Emission) technology (catalogue number 82591/82592/825050).

MR-proADM is measured with Brahms Kryptor with an automated immunofluorescence assay using TRACE technology (catalogue number 82991/82992/829050).

Chest X-ray was made by GP's request and was not part of the study protocol. Both postero-anterior and lateral view were obtained. Radiology reports were made by certified radiologists with no knowledge of the current study. For an individual patient, one radiologist made a written report, with a clear conclusion, as part of regular patient care. These reports, intended for the GPs, were used to determine whether or not a consolidation was present.

We did not intervene with the GP's treatment strategy.

After 30 days, a follow up contact via telephone call was made. In this standardized telephone interview, clinical symptoms were evaluated, any antibiotic usage documented, and resolution of symptoms and newly diagnosed disease entities noted.

Our primary end point was the presence of a consolidation on chest X ray, i.e. pneumonia. In the past, several models with clinical signs and symptoms with or without biomarkers (CRP, PCT and MR-proADM) have been used to predict pneumonia (5, 7). With these models we compared the ability of biomarkers to correctly improve a prediction versus the situation where biomarkers are not available.

For prediction of pneumonia we used three predefined diagnostic risk groups. We defined a low risk group with a probability of pneumonia less than 2.5%, an intermediate risk group with a probability of pneumonia between 2.5 and 20% and a high risk group with a probability of pneumonia above 20%. We have chosen these cut-off values of the risk groups as these roughly represent daily decision making in general practice. With these cut-off values safe clinical decision making is possible in daily practice. Comparable criteria have been used in the GRACE cohort (7).

Predictors for pneumonia were selected using multivariate regression models. With equations derived from the multivariate regression models without and with biomarkers, we could identify patients in low, intermediate and high risk groups of pneumonia.

As only low and high risk of pneumonia would have clear consequences for GP management, i.e. withholding or prescribing antibiotic treatment respectively, we pose that change to a higher risk group in cases with pneumonia and to a lower risk group in cases without pneumonia would reflect useful reclassification which could improve decision making.

To calculate the overall reclassification improvement, we subtracted patients who were reclassified incorrectly from those who reclassified correctly and divide this number by the total number of study patients.

Secondary outcome measures were the presence of bacterial or viral agents and the antibiotic courses used in patients with and without pneumonia and in patients with or without bacterial infection. CRP, PCT and MR-proADM values were evaluated for their predictive ability for pneumonia, 30-day mortality and need for secondary care. We evaluated antibiotic courses in patients with treatable disease, i.e. consolidation or bacterial pathogen detected.

We also used our data to evaluate the findings of the GRACE cohort. Our findings were entered in the multivariate model of the GRACE cohort to assess the value of biomarkers to improve prediction by calculating the overall reclassification improvement.

Will their strategy to predict consolidation on chest X-ray (i.e. pneumonia) apply in our cohort? The results of this evaluation are described in the supplementary material.

Statistics

We used descriptive statistics to describe baseline characteristics. Descriptive analysis included means with confidence intervals or medians and interquartile ranges, as appropriate.

To assess the predictive value of CRP, PCT and MR-proADM for pneumonia, area-under-the-curve (AUC) of receiver operating characteristics (ROC) curves were calculated. This analysis determined which biomarker will be used in the regression model.

Univariate and multivariate binary logistic regression will be used to evaluate clinical parameters and biomarkers (CRP, PCT and MR-proADM) as predictors for pneumonia. The multivariate prediction model of our cohort consists of variables which are clinically relevant or have a P value less than 0.1 in univariate analysis.

Cut off points for CRP and PCT as they have been used in the GRACE algorithm will be used. For MR-proADM two cut off points will be used. The first MR-proADM cut off point is 0.646 nmol/l. This was the optimal cut off point to discriminate patients with low risk community acquired pneumonia (PSI I-III) from patients with high risk CAP (PSI IV and V) with sensitivity of 92% and specificity of 55% (17).

The second MR-proADM cut off point is 1.00 nmol/l. In patients with febrile urinary tract infections, this is the optimal cut off to predict 30 day mortality (18).

RESULTS

Between March 2012 and March 2016 we included 249 patients via alternating radiology departments from 2 teaching hospitals and 1 regional hospital in the western part of the Netherlands. The patients were included during all seasons of the year. Baseline characteristics of the cohort are described in Table 1 and in the supplementary material (Table S1).

Table 1. Baseline characteristics of the cohort

Total number of patients	249
Female (%)	127 (51.0)
Median age in years (IQR)	56 (43-67)
Duration of complaints:	
- Less than a week (%)	45 (18.1)
- Between one and two weeks (%)	104 (41.8)
- Between two and three weeks (%)	97 (39.0)
Comorbidity (%)	196 (78.7)
Hospital admission in previous year (%)	32 (12.9)
Received influenza vaccination (%)	108 (43.4)
Antibiotic usage previous 3 months (%)	
- None	121 (48.6)
- One course	95 (38.2)
- More than one course	33 (13.3)
Antibiotic courses (%)	
- Amoxicillin	48 (29.6)
- Amoxicillin with clavulanic acid	7 (4.3)
- Penicillin	5 (3.1)
- Doxycycline	38 (23.5)
- Macrolide	14 (8.6)
- Quinolone	3 (1,9)
- Other	4 (2.5)
- Unknown antibiotic	43 (26.5)
Smoking (previous or current) (%)	141 (56,6)
Median CRB-65 score* (IQR)	0 (0-1)

IQR=interquartile range

* CRB-65 severity score predicting 30 day mortality with higher score implicating higher 30 day mortality. C= new onset confusion, R= respiratory rate ≥30/minute, B= Blood pressure (Systolic < 90 mm Hg or Diastolic ≤ 60 mm Hg), 65= Age ≥65

Detection of pneumonia on chest X ray

In 30 (12%) of patients, a pneumonia was detected on chest X ray.

Detection of respiratory pathogen as cause of infection

In our study, in 41% of patients a viral infection was established, in 1% a pneumococcal infection, in 2% a *Haemophilus influenzae* infection. In two patients *Mycoplasma pneumoniae* (in sputum) was detected, in three patients (two sputum samples and one nasopharyngeal swab) *Chlamydia pneumoniae* and in three sputum samples *Legionella spp.* was detected (*Legionella pneumophila* PCR was negative in these patients). Respectively one (3.7%), three (11.1%) and two (7.4%) had a consolidation on chest X ray (see table S2 in the supplementary appendix). In one of the eight patients with an atypical pathogen (i.e. *Legionella spp.*), both *S. pneumoniae* and rhinovirus were detected.

Antibiotic prescriptions

A total number of 104 antibiotics were prescribed for 83 patients (Table S1 supplementary material). Of all patients with consolidation or bacterial pathogen detected (treatable disease), 19/33 (58%) have received one or more antibiotic courses after chest X ray. Of 199 patients without treatable disease, 64 (32%) have received antibiotic treatment.

Thirty-six patients (14%) were referred to the hospital (24 outpatient clinic and 12 were admitted), none of the patients died within 30 days after chest X ray. Neither CRP nor PCT nor MR-proADM could predict the need for hospital care within 30 days after chest X ray (Figure S1 ROC curve biomarkers and need for hospital care after chest X ray).

In two patients, abnormalities besides consolidation were detected. During follow up, the first appeared to be a calcified benign nodus and the second appeared to be atelectasis due to a mucus plug. No malignancies were detected. More outcome details are available in table S1 in the supplementary appendix.

Prediction for pneumonia

Univariate analysis of clinical risk factors for pneumonia is described in Table S3 (supplementary appendix). Antibiotic use in the previous three months or influenza vaccination was not a risk factor for pneumonia in our cohort. We drafted three age cohorts with the same number of patients in each cohort (eight patients aged 64 were present, these were all categorised in the eldest group).

Results of multivariate analysis with signs and symptoms are described in Table 2. Absence of runny nose and whether or not a patient felt ill were independent predictors for pneumonia in our clinical risk model. Calibration of this model was good with a Hosmer-Lemeshow test of 4.53 (df=7, P=0.72); Nagelkerke R square 0.29.

Table 2. Multivariate analysis of clinical variables in prediction model for pneumonia in 249 patients presenting at radiology department with acute respiratory tract infection in primary care.

Diagnostic variable	Multivariable OR (95%CI)	P value
Age cohort (18-47 years is reference category)		0.28
• 48-63	2.17 (0.65-7.26)	
• ≥64	0.49 (0.03-7.44)	
Runny nose absent	3.00 (1.23-7.33)	0.02
Feel ill	14.89 (3.27-67.91)	0.00
Current smoker	0.34 (0.09-1.39)	0.13
Oxygen saturation	0.88 (0.67-1.16)	0.38
CRB-65 score* (0 is reference category)		0.35
• 1	6.77 (0.51-89.78)	

* CRB-65 severity score predicting 30 day mortality with higher score implicating higher 30 day mortality. C= new onset confusion, R= respiratory rate ≥30/minute, B= Blood pressure (Systolic < 90 mm Hg or Diastolic ≤ 60 mm Hg), 65= Age ≥65
No values for CRB65 score of 2 since only 2 patients were present in that category.

With variables in multivariate analysis with P<0.10, we made the prediction equation (see table S4A in the supplementary appendix) for the presence of a consolidation on chest X ray, using clinical signs and symptoms only:

1/(1+exp−(−4.492+1,142×absence of runny nose (0 or 1)+2.550×feel ill (0 or 1))

Biomarker for guidance of the presence of pneumonia

In table 3 multivariate analysis of clinical variables and biomarkers predicting pneumonia are described. Calibration of this model was good with a Hosmer-Lemeshow test of 10.09 (df=8, P=0.26); Nagelkerke R square 0.36.

In univariate analysis, CRP predicts pneumonia better than PCT and MR-proADM do (supplementary material table S3 and Figure S2. ROC curve biomarkers and pneumonia on chest X ray). Therefore, only CRP is present in the multivariate model. We have used the cut-off point of 30 mg/l to make the results comparable with the GRACE findings.

With variables in multivariate analysis which are clinically relevant or have P<0.10 (we did not use current smoker since this represents more likely the type of patients for which chest X ray was deemed necessary), we made the prediction equation (see table S4B in the supplementary appendix) for the presence of a consolidation on chest X ray, using clinical signs and symptoms and CRP (>30mg/l):

1/(1+exp−(−4.797+1.230×absence of runny nose (0 or 1)+ 2.378×feel ill (0 or 1)+ 1.572xCRP>30mg/l (0 or 1))

Table 3. Multivariate analysis of clinical variables and biomarkers in prediction model for pneumonia in 249 patients presenting at radiology department with acute respiratory tract infection in primary care.

Diagnostic variable	Multivariable OR (95%CI)	P value
Age cohort (18-47 years is reference category)		0.54
• 48-63	1.74 (0.48-6.30)	
• ≥64	0.61 (0.05-8.07)	
Runny nose absent	3.12 (1.22-8.00)	0.02
Feel ill	13.33 (2.80-63.40)	0.00
Current smoker	0.27 (0.06-1.19)	0.08
Oxygen saturation	0.94 (0.72-1.24)	0.68
CRB-65 score* (0 is reference category)		0.41
• 1	5.29 (0.46-61.15)	
CRP > 30 mg/l	4.66 (1.73-12.55)	0.00

* CRB-65 severity score predicting 30 day mortality with higher score implicating higher 30 day mortality. C= new onset confusion, R= respiratory rate ≥30/minute, B= Blood pressure (Systolic < 90 mm Hg or Diastolic ≤ 60 mm Hg), 65= Age ≥65
No values for CRB-65 score of 2 since only 2 patients were present in that category.

In table 4, the reclassification with CRP added to the model is described. The improvement in classification can now be calculated. Of all patients with pneumonia, 8 are reclassified to higher risk group and 0 to lower risk groups. Reclassification improvement in patients with pneumonia is 8/30= 26.7%.

In patients without pneumonia reclassification improvement is (0-17)/212= -8.0%. From the total cohort, 8 have been reclassified correctly and 17 have been reclassified incorrectly. Therefore, the overall reclassification improvement is -9/242= -3.7% with adding CRP to the model.

Twenty-three patients (16%) in the intermediate risk group with signs and symptoms only, were reclassified into high risk group when adding CRP to the model. Eight of these (35%) had pneumonia. None were reclassified into low risk group.

Using our own model with CRP, consolidation was present in none in the low risk group, 6.4% in the intermediate risk group and 32.4% in the high risk group.

Table 4. Reclassification table using results from multivariate analysis

Risk according to sign and symptoms without CRP	Risk according to signs and symptoms plus CRP>30mg/l							
	Patients with pneumonia				Patients without pneumonia			
	<2,5%	**2,5%-20%**	**>20%**	**Total**	**<2,5%**	**2,5%-20%**	**>20%**	**Total**
<2,5%	0	0	0	0	49	2	0	51
2,5%-20%	0	8	8	16	0	115	15	130
>20%	0	0	14	14	0	0	31	31
Total	0	8	22	30	49	117	46	212

In 1 patient clinical variable is missing; in 6 patients CRP value is missing.

DISCUSSION

In patients referred by their general practitioner for a chest X-ray in the course of an acute respiratory tract infection, one in eight (12%) showed a consolidation on the chest X ray, i.e., was diagnosed with community acquired pneumonia. Biomarkers like CRP, PCT and MR-proADM do not help discriminate between presence or absence of an infiltrate on chest X-ray over that of a model with clinical characteristics only, but CRP did help to guide the physician on treatment decisions.

In all low risk patients (21% of study population) a pneumonia is absent and therefore, the chest X ray has very little added value.

Our study has several strong and weak points. Strengths of our study are the fairly complete patient data including 30 day follow up and the extensive microbiological testing. During the study project, we found that none of the patients had positive blood cultures; therefore, because of futility, we stopped collecting blood cultures after the first 92 blood cultures proved negative.

The findings in our study underscore the importance of collecting some basic patient data in daily primary care. For instance, the question about the patient feeling ill proved to be the best independent predictor for the presence of pneumonia in our cohort. This is in line with other reports (19, 20).

Latest report about aetiology in CAP in the Netherlands stems from 2004; in that report, 10% of the patients was infected with an atypical pathogen (21).

Since 2011, the Dutch guideline 'Acute cough' prescribes to start with amoxicillin antibiotic treatment instead of doxycycline in patients with presumptive pneumonia (9). Apparently,

this change in empiric treatment has not resulted in an increased prevalence of atypical pathogens in those who present with persistent cough despite amoxicillin therapy. In our cohort the prevalence of atypical pathogens was 4% only and in many cases it remained uncertain whether these pathogens were the cause of infection or represent asymptomatic carriage (22). Interestingly, 20% of patients with consolidation on X-ray in our study had microbiological proven *Legionella spp.*, *Mycoplasma pneumoniae* or *Chlamydia pneumoniae*.

We used nasopharyngeal swabs for virus and atypical pathogen detection. Although sputum samples have a higher detection rate than nasopharyngeal swabs, adequate sputum samples were only available in a minority of patients and they were used to culture bacterial pathogens (23). With more adequate sputum samples available and with both culture and molecular testing on these sputum samples, diagnostic yield might have been increased. In addition, prolonged illness or prolonged coughing is a frequent symptom after clearance of the causative agent in respiratory tract infection (24, 25). Presumably, in a proportion of patients the causative agent has already been cleared while symptoms are still present.

Collection of patient data, diagnostic sampling and chest X ray were all within one hour. Therefore, all our results reflect the same stage of disease.

Another strength is the value of our cohort to evaluate the GRACE findings in a different cohort of patients (see supplementary material).

Although, there are several weaknesses in our study that need explanation. Since we did not include the patients at the general practice, we do not have the results of physical examination of the GP (crackles and diminished vesicular breathing). In the GRACE study these variables were important in predicting pneumonia.

We chose to include patients with a chest X ray since this examination is considered the gold standard for the presence or absence of pneumonia. Therefore, we have included patients at radiology departments. In the Netherlands, general practitioners do not have their own radiology facilities at their practice. Primary care patients should visit a hospital for a chest X ray making this diagnostic a demanding procedure for patients. Our study includes a selected proportion of patients with an acute respiratory tract infection. In these study patients, GPs felt the patient might benefit from a chest X ray as it would confirm or refute a pneumonia or other lung pathology. This is a small fraction of the total number of patients visiting their GP with and acute respiratory tract infection (15). The patients who were not referred for chest X ray were diagnosed and treated according to the Dutch

guideline 'Acute Cough', and GPs estimated that these patients would not benefit from a chest X ray, as these did not present a diagnostic dilemma (9).

This selection results in a study population of patients who have not responded to GP's empirical therapy, patient for whom doubt about diagnosis or treatment is present or – as assessed by the GP – have a high chance of showing other relevant pulmonary abnormalities. Almost 80% of the study patients had co-morbidity, more than 50% has used antibiotics in the previous 3 months and 81% of patients had complaints for more than one week. The majority of patients did not show a pneumonia (219/249) and had only mild disease given their median CRB-65 score of 0 (IQR 0-1). The results of this study are therefore generalisable to this specific patient population. The finding that current smoking is negatively associated with the presence of pneumonia, suggests that GPs have lower threshold to order chest X ray in smoking than in non-smoking patients (Table S3).

Although at first site it seems counterintuitive that patients aged 48-63 years are at increased risk for having pneumonia and older patients have relative low risk (Table 3), it is highly likely that older patients are referred for chest X ray earlier than younger patients.

Although we included patients year-round and the GRACE study included patients during winter months, the percentage of patients having pneumonia in our cohort (12%) is considerably higher (12 versus 5%). This is to be expected since these patients were selected by GPs assessment to be at risk for CAP or another serious lung disorder (26). The proportion of patients with pneumonia in our cohort corresponds with the numbers found in other cohorts (6, 27).

The GRACE model was developed to help GPs in the decision regarding the diagnosis of patients with acute cough. Our study includes a selected proportion of patients with acute cough and therefore(15) our cohort is enriched with patients with pneumonia (12%) compared to the GRACE cohort (5%). In addition, only a minority of consolidations has disappeared on chest X ray in the first three weeks after start of treatment of pneumonia (28, 29). Therefore, we suppose that the consolidations present at start of complaints, would still be visible on chest X ray during our study.

Overall reclassification improvement in the GRACE model was 29% (7). In this mildly ill cohort, the reclassification improvement was mainly due to reclassifying patients from intermediate risk to the low risk group.

On the contrary, in our study cohort of more severely ill patients, most benefit was present in reclassifying intermediate risk patients to the high risk group. On the basis of a CRP

measurement, 23 of 146 patients (16%) in the intermediate risk group should be reclassified into the high risk group. Because this group is enriched for persons with pneumonia, it would likely benefit from antibiotic treatment. However, CRP did not help in reclassification of intermediates into the low risk group.

Reclassification from the intermediate risk group into the low or high risk group, by adding CRP level to the diagnostic process but without a chest X ray, would be relevant in daily practice. Also classification into low or high risk group would have direct impact on treatment decision, respectively withhold or initiate antibiotic treatment, and these results could have implications for future decision making in general practice. In our model using CRP (Table 4), 117/242 (48%) is classified in either the low or the high risk group. Thus, for these patients, an antibiotic treatment decision can be made without a chest X ray. These findings need to be validated in a new cohort.

We have chosen to use overall reclassification improvement instead of net reclassification improvement as it was used in the GRACE analysis (7). The net reclassification counts the percentages of two groups (with and without pneumonia), with complete different numbers of patients (30 patients with pneumonia versus 212 without). This leads to overrepresentation of the percentage from the smallest group of patients. The overall reclassification improvement values every patient in the same way, with or without pneumonia.

The overall reclassification improvement with CRP added to the model, did not help to discriminate between presence or absence of an infiltrate on chest X-ray over that of a model with clinical characteristics only (-3.7%). CRP did help to guide the physician on treatment decisions since 23 patients (Table 4) were reclassified into the high risk group that – according to guidelines – warrant antibiotic treatment. Eight of these 23 reclassified patients (35%) had pneumonia.

Using our model for antibiotic treatment decision in patients for whom chest X ray is considered during acute respiratory tract infection, clinical signs and symptoms alone can identify patients at low risk for pneumonia (who should not be treated) and patients a high risk for pneumonia who probably benefit from antibiotic treatment. Patients who are at intermediate risk (2,5-20%) for having pneumonia, using clinical signs and symptoms only, would benefit from CRP testing to identify the patients who have a high risk of pneumonia. Of 146 intermediate risk patients, 23 (16%) would be reclassified in the high risk group when adding CRP in the decision model (Table 4).

In general the different kinetics such as a short half-life, especially for MR-proADM, make markers like PCT and MR-proADM of less value than CRP when it comes to diagnose and

treat pneumonia in general practice. Also, MR-proADM is released from endothelium in response to systemic inflammation and is a marker of severity of pneumonia (30). Our cohort of primary care patients, however, displayed little systemic inflammation and this may have deemed MR-proADM less clinically relevant. Studies on MR-proADM as a biomarker in respiratory tract infections in primary care are scarce (31). Our findings of CRP and procalcitonin are in accordance with other studies (7, 32).

CONCLUSIONS

Our model would preclude the need for a diagnostic chest X rays in 21% of GP patients with an acute respiratory tract infection (the low risk group). CRP predicts pneumonia better than the other biomarkers but adding CRP to the clinical model did not improve classification (-4%); however, CRP helped guidance of the decision which patients should be given antibiotics. Our findings put the use of biomarkers and chest X ray in diagnosing pneumonia and for treatment decisions into some perspective for general practitioners.

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SUPPLEMENTARY MATERIAL

Table S1. Characteristics of the cohort

Baseline characteristics	
Total number of patients	249
Female (%)	127 (51.0)
Median age in years (Interquartile range)	56 (43-67)
Duration of complaints:	
Less than a week (%)	45 (18.1)
Between one and two weeks (%)	104 (41.8)
Between two and three weeks (%)	97 (39.0)
Throat pain (%)	86 (34.5)
Coryza/runny nose (%)	159 (63.9)
Cough (%)	223 (89.6)
Sputum (%)	185 (74.3)
Dyspnoea (%)	189 (75.9)
Fever or feverishness (%)	173 (69.5)
Days since the last episode of fever(%):	
0, today fever	21 (8.5)
1, yesterday fever	21 (8.5)
2 days ago fever	10 (4.0)
More than 2 days ago fever	71 (28.6)
Not applicable or don't know	125 (50.4)
Myalgia (%)	101 (40.6)
Headache (%)	125 (50.2)
Joint pain (%)	55 (22.1)
Feel ill (%)	155 (62.2)
Comorbidity (%)	196 (78.7)
Hospital admission in previous year (%)	32 (12.9)
Most recent hospital discharge:	
0-3 months ago	10
4-6 months ago	5
6-12 months ago	17
Visited foreign country in previous 3 months (%)	82 (32.9)
Received invitation for influenza vaccination (%)	155 (62.2)
Received influenza vaccination (%)	108 (43.4)
Antibiotic usage previous 3 months (%)	
None	121 (48.6)
One course	95 (38.2)
More than one course	33 (13.3)
Antibiotic courses (%)	

Table S1. Characteristics of the cohort (continued)

Baseline characteristics	
Amoxicillin	48 (29.6)
Amoxicillin with clavulanic acid	7 (4.3)
Penicillin	5 (3.1)
Doxycycline	38 (23.5)
Macrolide	14 (8.6)
Quinolone	3 (1,9)
Other	4 (2.5)
Unknown antibiotic	43 (26.5)
ADL* support (%)	7 (2.8)
Pregnant or breastfeeding (%)	0 (0)
Smoking (previous or current) (%)	141 (56.6)
Median packyears of those who have current or previous smoking (interquartile range)	19 (8-30)
Race or ethnic group (%)	
White/Caucasian	221 (88.8)
Asian	20 (8.0)
North African	1 (0.4)
Black	4 (1.6)
Plan after chest X ray	
It will be determined after chest X ray result is available for GP	188 (75.5)
Start with antibiotics	42 (16.9)
Start with medical treatment other than antibiotic	2 (0.8)
There is no plan	17 (6.8)
Outcome	
30 day mortality	0 (0)
Outcome day 30	
Complete recovery	147 (59.0)
Missing	17 (6.8)
Total duration of complaints	
<1 week	5 (2.0)
1-<2 weeks	29 (11.6)
2-<3 weeks	46 (18.5)
≥3 weeks	152 (61.0)
Missing	17 (6.8)
Duration of fever	
0	117 (47.0)
1-3	44 (17.7)

Table S1. Characteristics of the cohort (continued)

Baseline characteristics	
4-7	38 (15.3)
8-14	10 (4.0)
>14 days	9 (3.6)
Unknown	15 (6.0)
Missing	16 (6.4)
Still coughing	
Yes	79 (31.7)
No	150 (60.2)
Missing	20 (8.0)
Duration of coughing	
0 days	19 (7.6)
1-7 days	14 (5.6)
8-14 days	31 (12.4)
15-21 days	34 (13.7)
>21 days	132 (53.0)
Unknown	3 (1.2)
Missing	16 (6.4)
Antibiotic treatment after chest X ray	
No	149 (59.8)
Yes, 1 course	64 (25.7)
Yes, > 1 course	19 (7.6)
Missing	17 (6.8)
If antibiotic used, which one	
Total number of treatments	104
Amoxicillin	25
Amoxicillin/clavulanic acid	3
Feneticillin	4
Doxycycline	23
Macrolide	11
Quinolone	4
Unknown	34
Side effects	
Total number	30
Allergic	2
Diarrhoea	12
Nausea/vomiting	6
Yeast infection	3
Other	7
Other treatment besides antibiotics	

Table S1. Characteristics of the cohort (continued)

Baseline characteristics	
No	129 (51.8)
Oral steroids	21 (8.4)
Lung inhalers	57 (22.9)
Oseltamivir	2 (0.8)
Codeine	7 (2.8)
Other	14 (5.6)
Referral to hospital	
No	196 (78.7)
Outpatient clinic	24 (9.6)
Admission to ward	12 (4.8)
Length of stay	
1-3 days	6
4-7 days	2
8-14 days	2
> 14days	2

* Activities of Daily Living

Table S2. Outcome details

Number of patients	**All patients (249)**	**With consolidation on chest X ray (30)**	**Without consolidation (219)**
Mean age (years)	55.5 (95% CI 53.5-57.4)	61.4 (95% CI 55.9-66.8)	54.7 (95%CI 52.6-56.8)
Female	127 (51.0%)	13/30 (43.3%)	114/219 (52.1%)
BMI (median)	26.0 (IQR 23.5-29.6)	25.3 (IQR 22.9-30.8)	26.0 (IQR 23.5-29.6)
Smoking (history)	141 (57.8%)	19/30 (63.3%)	122/214 (57.0%)
Median respiratory rate (median)	15 (13-18)	16 (14-20)	15 (13-18)
CRB-65 score 0:1:2	170:71:2	15:14:0	149:58:2
Influenza vaccination	108 (43.4%)	15 (50.0%)	93 (42.5%)
Antibiotic pre-treatment	128 (51.4%)	19 (63.3%)	109 (49.8%)
Diagnostic results			
Any viral agents	97/238	9/30 (30.0%)	88/208 (38.6%)
Influenza A	15/237	0/30	15/207 (6.3%)
Influenza B	5/237	0/30	5/207 (2.4%)
RSV	3/237	0/30	3/207 (1.4%)
Parainfluenza 1-4	10/237	0/30	10/207 (4.8%)
Metapneumovirus	8/236	3/30 (10.0%)	5/206 (1.7%)
Rhinovirus	47/236	4/30 (13.3%)	43/206 (14.4%)
Coronavirus	7/237	2/30 (6.7%)	5/207 (2.4%)
Adenovirus	2/238	0/30	2/208 (1.0%)
Bocavirus	0/238	0/30	0/208
S. pneumoniae	2/249	2/30 (6.7%)	0/219
H. influenzae	5/249	1/30	4/219
H. parainfluenzae	1/249	1/30	0/219
K. pneumoniae	1/249	0	1/219
Legionella spp.	3/228	2/27	1/201
Mycoplasma pneumoniae	2/228	1/27	1/228
Chlamydia pneumoniae	3/228	3/27	0/228
After chest X ray			
Completely resolved (day 30)	147/232 (63.4)	13/26 (50.0)	134/206 (65.0)
Antibiotic use after chest X ray			
No	149/232 (64.2)	8/26 (30.8)	141/206(68.4)
1 course	64/232 (27.6)	13/26 (50.0)	51/206 (24.8)
> 1 course	19/232 (8.2)	5/26 (19.2)	14/206 (6.8)
One or more side effects from antibiotics	28/122 (23.0)	8/23 (34.8)	20/99 (20.2)
Lung cancer detected	0/249	0/30	0/219
Hospital referral			
No	196/232 (84.5)	18/26 (69.2)	178/232 (86.4)
Outpatient clinic	24/232 (10.3)	4/26 (15.4)	20/206 (9.7)
Admission to ward	12/232 (5.2)	4/26 (15.4)	8/206 (3.9)
30 day mortality	0/249	0/30	0/219

Table S3. Univariate analysis of diagnostic variables and pneumonia in 249 patients presenting at radiology department with acute respiratory tract infection in primary care.

Diagnostic variable	Missing	Total (n=249)	Pneumonia present (n=30)	Univariable OR (95%CI)	P value
Mean (SD) Age (years)	0 (0.0)	55 (16)	61 (15)	1.03 (1.00-1.06)	0.03
Age cohort	0				0.12
• 18-47		83	5		
• 48-63		80	11	2.49 (0.82-7.51)	0.11
• ≥64		86	14	3.03 (1.04-8.85)	0.04
Men	0 (0.0)	122 (49.0)	17 (57)	1.42 (0.66-3.06)	0.37
Current smoker	5 (2.0)	58 (24)	3 (10)	0.32 (0.09-1.10)	0.07
current smokers:					
• Median no. of pack years (IQR)	6 (10)	20 (10-30)	40 (16-43)	1.02 (0.98-1.07)	0.36
No. of weeks illness before chest X ray	3 (1.2)				
• < 1 week		45 (18)	9 (30)		
• 1-2 weeks		104 (42)	10 (33)	0.43 (0.16-1.13)	0.09
• >2 weeks and ≤3 weeks		97 (39)	11 (37)	0.51 (0.20-1.34)	0.17
Cough	0 (0.0)	223 (90)	25 (83)	0.53 (0.18-1.53)	0.24
Phlegm	1 (0.4)	185 (75)	19 (66)	0.61 (0.27-1.39)	0.24
Breathless	0 (0.0)	189 (76)	25 (83)	1.68 (0.61-4.59)	0.32
Runny nose absent	1 (0.4)	89 (36)	16 (53)	2.27 (1.05-4.91)	0.04
Fever	0 (0.0)	173 (70)	24 (80)	1.88 (0.74-4.80)	0.19
Chest pain	0 (0.0)	82 (33)	6 (20)	0.47 (0.18-1.20)	0.33
Throat pain	1 (0.4)	86 (35)	13 (43)	1.52 (0.70-3.30)	0.29
shivering	0 (0.0)	135 (54)	16 (53)	0.96 (0.45-2.06)	0.92
Muscle ache	0 (0.0)	101 (41)	13 (43)	1.14 (0.53-2.46)	0.74
Headache	0 (0.0)	125 (50)	17 (57)	1.34 (0.62-2.90)	0.45
joint pain	0 (0.0)	55 (22)	7 (23)	1.08 (0.44-2.68)	0.86
Feel ill	0 (0.0)	155 (62)	28 (93)	10.14 (2.36-43.65)	0.00
Confused	0 (0.0)	2 (1)	1 (3)	7.52 (0.46-123.46)	0.16
symptoms in friends and relatives	0 (0.0)	94 (38)	9 (30)	0.68 (0.30-1.55)	0.35
Birds at home	0 (0.0)	20 (8)	4 (13)	1.95 (0.61-6.28)	0.26
Hotel in previous month	0 (0.0)	54 (22)	4 (13)	0.52 (0.17-1.56)	0.24
Sauna in previous month	0 (0.0)	21 (8)	2 (7)	0.75 (0.17-3.40)	0.71
Received influenza vaccination	0 (0.0)	108 (43)	15 (50)	1.36 (0.63-2.91)	0.44
Hospital admission in previous year	0 (0.0)	32 (13)	3 (10)	0.73 (0.21-2.55)	0.62
Antibiotic use in previous 3 months	0 (0.0)	128 (51)	19 (63)	1.74 (0.79-3.84)	0.17
Any comorbidity (pulmonary, cardiac, diabetes mellitus)	0 (0.0)	76 (31)	11 (37)	1.37 (0.62-3.04)	0.44
median syst blood pressure (IQR)	2 (0.8)	130 (120-140)	128 (118-142)	1.00 (0.97-1.02)	0.79
Median Diastolic blood pressure (IQR)	2 (0.8)	82 (78-90)	80 (74-90)	0.98 (0.94-1.02)	0.34

Table S3. Univariate analysis of diagnostic variables and pneumonia in 249 patients presenting at radiology department with acute respiratory tract infection in primary care. (continued)

Diagnostic variable	Missing	Total (n=249)	Pneumonia present (n=30)	Univariable OR (95%CI)	P value
Tachycardia >100 beats/min	0 (0.0)	4 (2)	1 (3)	2.48 (0.25-24.67)	0.44
Temperature >37.8 0C	13 (5.2)	8 (3)	2 (7)	2.71 (0.52-14.14)	0.24
Tachypnoea (>24 breaths/min)	10 (4.0)	7 (3)	1 (3)	1.21 (0.14-10.46)	0.86
Median Oxygen saturation (IQR)	6 (2.4)	98 (97-98)	97 (96-98)	0.78 (0.62-0.97)	0.03
Median CRB-65 score (IQR)	11 (4.4)	0 (0-1)	0 (0-1)	2.05 (0.98-4.29)	0.06
Blood test results					
CRP (mg/l)					
Median (IQR)	6 (2)	5.4 (1.0-14.8)	24.1 (5.2-81.5)	1.33 (1.18-1.50)§	0.00
>20	6 (2)	48 (20)	16 (53)	6.46 (2.88-14.53)	0.00
>30	6 (2)	40 (17)	14 (47)	6.29 (2.75-14.38)	0.00
>50	6 (2)	22 (9)	8 (27)	5.17 (1.95-13.69)	0.00
>100	6 (2)	8 (3)	5 (17)	14.00 (3.16-62.13)	0.00
Procalcitonin (µg/l):					
Median (IQR)	6 (2)	0.05 (0.03-0.07)	0.07 (0.03-0.12)	1.34 (0.97-1.83)¶	0.07
>0.25	6 (2)	4 (2)	2 (7)	7.54 (1.02-55.64)	0.05
>0.50	6 (2)	2 (1)	1 (3)	7.31 (0.45-120.08)	0.16
Midregional proadrenomedullin (MR-proADM)					
Median (IQR)	8 (6)	0.58 (0.47-0.76)	0.67 (0.53-0.82)	2.87 (0.73-11.20)	0.13
>0.646	8 (6)	96 (40)	17 (57)	2.19 (1.01-4.74)	0.05
>1,00	8 (6)	16 (7)	4 (13)	2.55 (0.77-8.50)	0.13

§ Per 10 mg/l increase

¶ Per 0.1 µg/l increase

Table S4A. Variables used to make the equation for the model with signs and symptoms only.

Diagnostic variable	Multivariable OR (95%CI)	P value	B
Runny nose absent	3.13 (1.39-7.06)	0.01	1.14
Feel ill	12.81 (2.92-56.28)	0.00	2.55
Intercept	-4.492		

Table S4B. Variables used to make the equation for the model with signs and symptoms and CRP

Diagnostic variable	Multivariable OR (95%CI)	P value	B
Runny nose absent	3.42 (1.44-8.13)	0.01	1.230
Feel ill	10.78 (2.38-48.89)	0.00	2.378
CRP > 30 mg/l	4.82 (1.99-11.66)	0.00	1.572
Intercept	-4.797		

We did not use current smoker since this represents more likely the type of patients for which chest X ray was deemed necessary.

The GRACE analysis in the current study cohort

In a recent evaluation of the GRACE study, in which 5% of 2820 patients with acute cough had pneumonia on chest X ray, the addition of CRP to the clinical prediction rule correctly reclassified 29% of patients (into low, intermediate or high probability of pneumonia). Mostly, patients were reclassified into a lower risk group. Procalcitonin did not add relevant diagnostic information (van Vugt SF, et al. BMJ. 2013;346:f2450.).

Here, we present the results of the evaluation of the GRACE derived-model in our cohort of patients. Our results were entered in the multivariate model of the GRACE cohort to assess the value of CRP to improve prediction by calculating the overall reclassification improvement.

The GRACE equation used results from GP physical examination. Since we do not have these data, we made three models using the GRACE algorithm. Firstly, we assume both crackles and diminished breathing sounds present in all patients, secondly, we assume both crackles and diminished breathing sounds absent in all patients and thirdly, we assume both crackles and diminished breathing sound present in the patients with consolidation and absent in the patients without.

Equations using clinical parameters without and with CRP have been used to classify patients in different predefined risk groups and calculate the overall reclassification improvement when adding CRP to the model.

Results

Using GRACE algorithm to assess overall classification improvement with adding CRP, assuming both crackles and diminished vesicular breathing absent, the overall reclassification improvement is 28.8%. Assuming both crackles and diminished vesicular breathing present, the overall reclassification improvement is 4.4%. Assuming both crackles and diminished vesicular breathing sounds present only in patients with pneumonia, the overall reclassification improvement is 21.8%. See GRACE reclassification and overall reclassification calculation (Tables S5A-C).

Discussion

We have used three scenarios to detect the possible differences in findings of the model. The range of overall reclassification improvement is wide (4.4 to 28.8%), but better than using our own model. Overall reclassification improvement in the GRACE model was 29%.

Using GRACE algorithm, reclassification into low or high risk group when adding CRP occurred in 6 to 35% of patients, depending on the scenario assumed. In addition, in two out

of three scenarios, the percentage of patients with pneumonia in the low risk group is too high. Therefore, our best case and worst case scenario do not perform well, making our model less valuable for evaluation of the GRACE prediction model.

Table S5A. GRACE reclassification: comparison of diagnostic risk for presence of pneumonia by GRACE diagnostic model with and without addition of measurement of CRP; Scenario assuming both crackles and diminished vesicular breathing absent.

Risk according to "symptoms and signs" model (without CRP)	Risk according to "symptoms and signs" model plus CRP continuous							
	Patients with pneumonia				Patients without pneumonia			
	<2.5%	2.5-20%	>20%	Total	<2.5%	2.5-20%	>20%	Total
<2.5%	0	1	0	1	35	4	0	*39*
2.5-20%	5	20	1	26	73	89	0	*162*
>20%	0	0	0	0	0	0	1	*1*
Total	5	21	1	27*	108	93	1	202*

* In 13 patients temperature is missing, 1 other clinical variable is missing (absence of runny nose). In 6 patients without pneumonia, CRP values are missing.

From the total cohort, 75 have been reclassified correctly and 9 have been reclassified incorrectly. Therefore, the overall result is 66/229=28.8% correctly reclassified patients with adding CRP to the model.

Table S5B. GRACE reclassification: comparison of diagnostic risk for presence of pneumonia by GRACE diagnostic model with and without addition of measurement of CRP; scenario assuming both crackles and diminished vesicular breathing present.

Risk according to "symptoms and signs" model (without CRP)	Risk according to "symptoms and signs" model plus CRP continuous							
	Patients with pneumonia				Patients without pneumonia			
	<2.5%	2.5-20%	>20%	Total	<2.5%	2.5-20%	>20%	Total
<2.5%	0	0	0	0	0	0	0	*0*
2.5-20%	0	5	6	11	0	123	7	*130*
>20%	0	0	16	16	0	11	61	*72*
Total	0	5	22	27	0	134	68	202*

* In 13 patients temperature is missing, 1 other clinical variable is missing (absence of runny nose). In 6 patients without pneumonia, CRP values are missing.

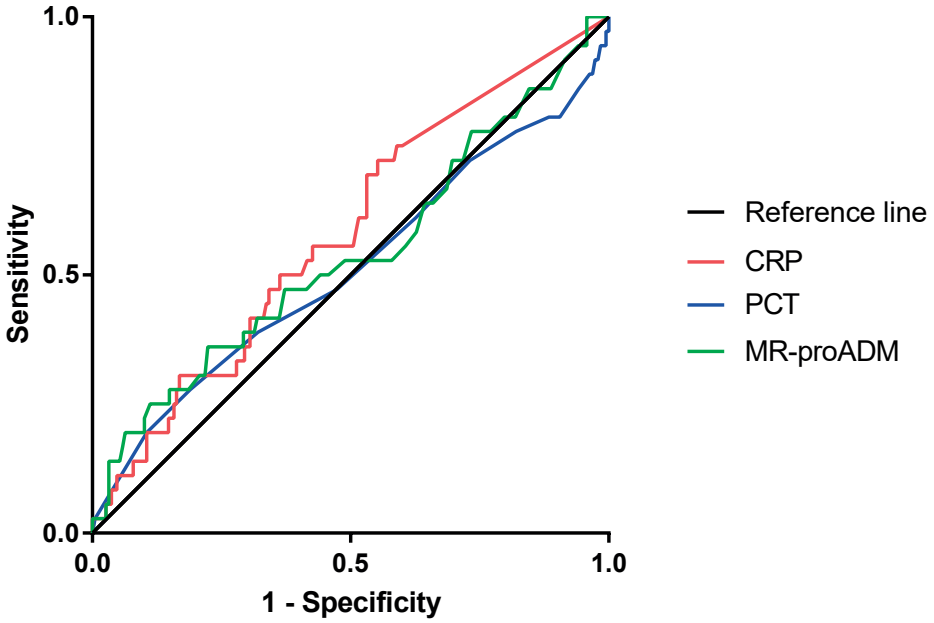
From the total cohort, 6+11=17 have been reclassified correctly and 7 have been reclassified incorrectly. Therefore, the overall result is 10/229=4.4% correctly reclassified patients with adding CRP to the model.

Table S5C. GRACE reclassification: comparison of diagnostic risk for presence of pneumonia by GRACE diagnostic model with and without addition of measurement of CRP; scenario assuming both crackles and diminished vesicular breathing present in patients with confirmed pneumonia.

Risk according to sign and symptoms without CRP	Risk according to signs and symptoms plus CRP>30mg/l							
	Patients with pneumonia				Patients without pneumonia			
	<2,5%	2,5%-20%	>20%	Total	<2,5%	2,5%-20%	>20%	Total
<2,5%	0	0	0	0	35	4	0	39
2,5%-20%	5	6	0	11	73	89	0	162
>20%	0	14	2	16	0	0	1	1
Total	5	20	2	27	108	93	1	202

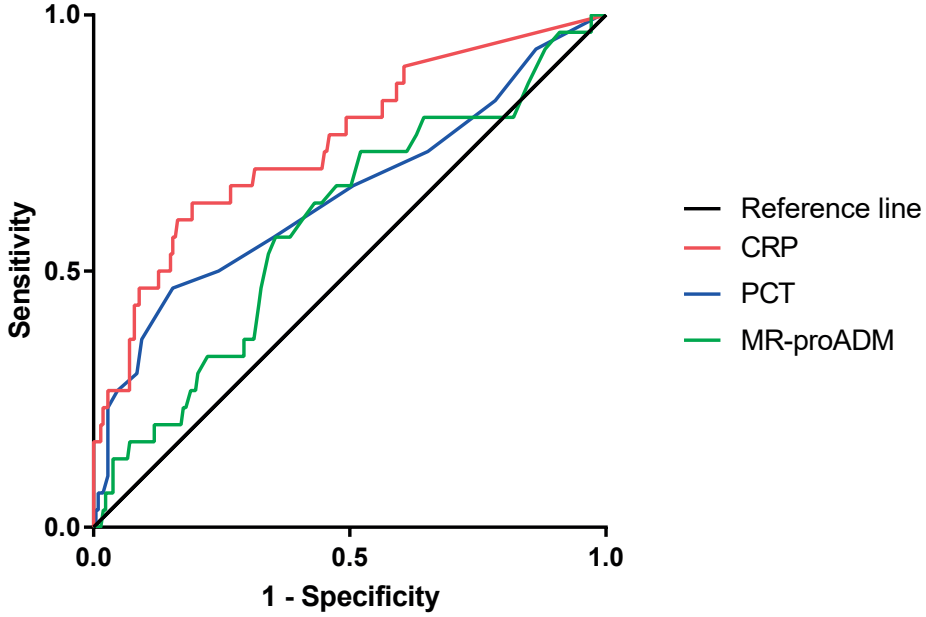
* In 13 patients temperature is missing, 1 other clinical variable is missing (absence of runny nose). In 6 patients without pneumonia, CRP values are missing.

From the total cohort, 73 have been reclassified correctly and 19+4=23 have been reclassified incorrectly. Therefore, the overall result is 50/229=21.8% correctly reclassified patients with adding CRP to the model.



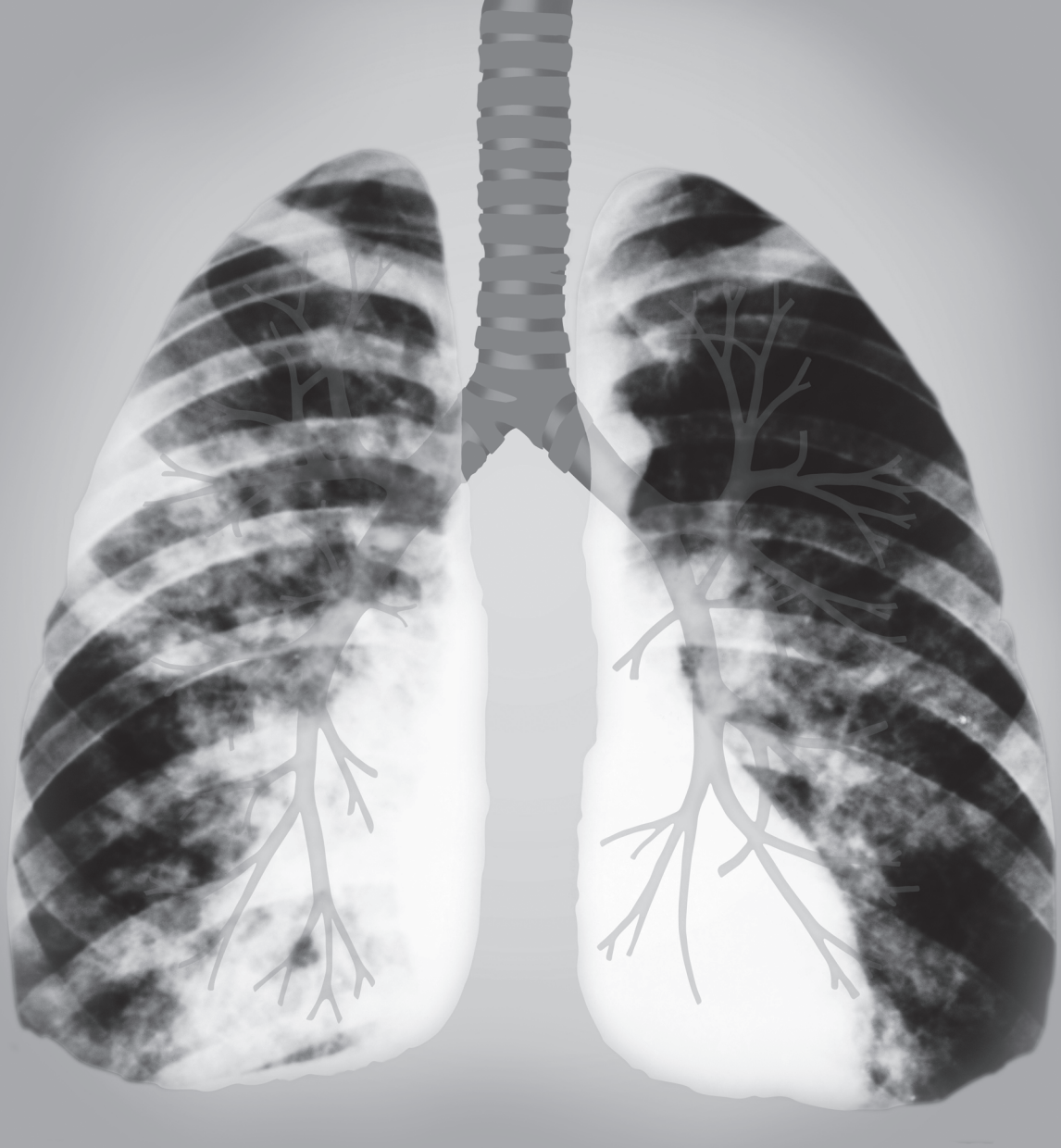
Biomarker	Area under the curve (95% confidence interval)
C-reactive protein	0.58 (0.48-0.68)
Procalcitonin	0.51 (0.40-0.63)
Midregional pro-adrenomedullin	0.54 (0.43-0.65)

Figure S1. ROC curve biomarkers and need for hospital care after chest X ray



Biomarker	Area under the curve (95% confidence interval)
C-reactive protein	0.75 (0.65-0.85)
Procalcitonin	0.65 (0.53-0.77)
Midregional pro-adrenomedullin	0.59 (0.49-0.70)

Figure S2. ROC curve biomarkers and consolidation on chest X ray



6

Influenza vaccination in patients with lung cancer receiving anti-programmed death receptor 1 immunotherapy does not induce immune-related adverse events.

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ABSTRACT

Background

Influenza vaccination is recommended in cancer patients to reduce influenza-related complications. Recently more immune related adverse events (irAE) were demonstrated in lung cancer patients who were vaccinated with the trivalent seasonal influenza vaccine during anti-PD1 immunotherapy. Confirmation of these findings is essential before recommendations on influenza vaccination may be revoked.

Methods

In this cohort study in lung cancer patients receiving nivolumab 3 mg/kg every two weeks during two influenza seasons (2015/16 – 2016/17) irAEs have been monitored. Incidence, timing and severity of irAEs were compared between vaccinated patients and non-vaccinated patients.

Findings

In a compassionate use program 127 lung cancer patients had been treated with at least one dose of nivolumab during two national influenza vaccination campaigns from September until December of 2015 and 2016. Forty-two patients had received the influenza vaccine and 85 patients were not vaccinated. Median follow up period was 118 days (IQR 106-119). Mean age was 64 years (range 46-83).

In vaccinated and non-vaccinated patients the incidence of irAEs was 26% and 22%, respectively, rate ratio 1.20 (95% CI 0.51 – 2.65). The incidence of serious irAEs was 7% and 4%, respectively, rate ratio 2.07 (95% CI 0.28 – 15.43). Influenza vaccination during nivolumab did not result in significant differences in rates of discontinuation, death, clinical deterioration or tumour response between groups.

Interpretation

Influenza vaccination in lung cancer patients receiving anti-PD-1 immunotherapy does not induce immune related adverse events in our cohort. With this result, influenza vaccination should not be deterred from this group of patients.

INTRODUCTION

Immunotherapy has become a standard novel treatment option for several malignancies and across all tumour stages. The immune system plays a critical role in fighting off cancer by detecting and controlling the proliferation of malignant cells.[1] CD8+ T cells are key players in the anti-tumour immune response and these cells have therefore been an important target for immunotherapeutic interventions. Immune checkpoints on activated T cells are inhibitory pathways that modulate the intensity and the extent of the immune response, preventing persistent immune activation and autoimmunity.[2] The anti-tumour response of the immune system can be enhanced by blocking these checkpoint inhibitors by use of antibodies against CTLA-4 (ipilimumab), programmed death receptor 1 (PD-1) (nivolumab and pembrolizumab) and its ligands (PD-L1 (atezolizumab, avelumab, durvalumab) and PD-L2.[2,3] Currently approved indications include melanoma, renal cell carcinoma, non-small cell lung cancer, urothelial carcinoma, head and neck cancer, Merkel cell carcinoma and Hodgkin's lymphoma, and new indications are under investigation.[4]

Anti-PD1 induced stimulation of the immune system can cause immune-related adverse events in 0.2-5.6% per organ system.[5] Immune-related adverse events (irAEs) are affecting the endocrine organs, skin, colon, liver, lungs, kidney and pancreas, but all other organs may be affected.[6] Although it is believed that the adverse events are a result of the disrupted immunologic homeostasis, the exact pathogenesis is still poorly understood.[7] Furthermore, flares of underlying autoimmune disease have been documented in patients receiving checkpoint inhibitors.[8]

Cancer patients are eligible for influenza vaccination due to their increased risk of developing complications when infected with seasonal influenza viruses and because influenza infections result in interruptions of cancer treatment.[9] Patient with lung cancer commonly have additional reduction of pulmonary function due to COPD and would benefit from influenza vaccination.[10] Additionally, symptoms caused by respiratory tract infections such as influenza infection can be similar in presentation to pulmonary immune-related adverse events, posing therapeutic dilemmas about continuation of immunotherapy or initiation of immunosuppressive agents to alleviate irAEs. Finally, since it was found that antibiotic use or change in microbioma may inhibit the clinical benefit of checkpoint inhibitors, interventions during immunotherapy should be applied that reduce the chance of febrile episodes leading to undesired administration of empiric antibiotics.[11] In order to reduce the possibility of influenza virus infection to cause a clinical deterioration in patients with multiple pulmonary co-morbidities and because of above mentioned considerations, seasonal influenza vaccination of patients treated with chemotherapy should be strongly advocated.[12]

It is not known whether administration of additional antigens to cancer patients receiving immunotherapy, for example due to vaccination, may result in a higher incidence of vaccine-related adverse events or (serious) irAEs. Recently, Läubli and colleagues demonstrated an unexpected high incidence of 52% of irAEs in a cohort of 23 patients undergoing treatment with PD-1/PD-L1 antibodies.[13] Influenza vaccination proved to be immunogenic during anti-PD1 immunotherapy, because no differences between patients and healthy controls in vaccine-induced antibody titers against the included influenza antigens were observed.

Confirmation of these findings in larger cohorts is required and should clarify whether the reported results in a small number of subjects should translate into a deferral or even a contra-indication of influenza vaccination, which is a universally recommended measure in cancer patients to decrease influenza-related complications. Therefore, we investigated the effect of the influenza vaccination on the incidence of irAEs in a uniform cohort of lung cancer patients undergoing checkpoint blockade treatment with antibodies against PD-1.

MATERIALS AND METHODS

We performed a cohort study comparing the incidence of irAEs and serious irAEs in the influenza vaccinated subgroup versus the unvaccinated subgroup. Ethical approval was obtained from the Medical Ethical Committee of the Antoni van Leeuwenhoek Hospital in Amsterdam, the Netherlands.

Study Population

Patients were identified in the nivolumab compassionate use program database of the Antoni van Leeuwenhoek hospital in Amsterdam. This database contains demographic data and prospectively collected clinical course and response data of patients with advanced lung cancer receiving intravenous administration of nivolumab 3 mg/kg every two weeks. Patients who had been administered at least one dose of nivolumab during the influenza vaccination seasons between September 1st and January 1st of 2015-16 or 2016-17 were enrolled. In the Netherlands, persons at risk from complications of influenza infections - all people aged 60 year or older and people with specified chronic diseases - are invited by their general practitioner for vaccination with a trivalent inactivated influenza vaccine free of charge between October and December.

The influenza vaccination status of the patients was obtained retrospectively via a short questionnaire sent to the general practitioners of those patients. Patients were included in the vaccinated group if they received an influenza vaccination after they started the

nivolumab treatment or if they were vaccinated at most 30 days before receiving the first dose of nivolumab. All other patients, including those who had been vaccinated more than 30 days before receiving the first dose of nivolumab, were included in the non-vaccinated group.

Assessment

Demographic data, medical history, tumour stage at the start of treatment, adverse events, the grade of the adverse events and the tumour response after vaccination were evaluated. From date of vaccination, the follow-up period was until March 1st of the following year. Non-vaccinated patients were included as controls with an identical follow-up period to determine the incidence of irAEs: the median date of influenza vaccination in the other group until March 1st of the following year. Adverse events were classified in irAEs and non-immune-related adverse events (non-irAE) by two investigators, unaware of the vaccination status, according to the classification criteria described by J.B.A.G. Haanen and colleagues.[14] The incidence of irAEs in influenza vaccinated group versus unvaccinated group is the primary outcome. Secondary outcome measures were grading of the adverse events, incidence of serious irAEs and non-irAEs and tumour effect. Grading of the adverse events was done according to the Common Terminology Criteria for Adverse Events, version 4.0. Possible effects on the tumour response were assessed with the use of the Response Evaluation Criteria in Solid Tumours.[15] For this assessment, the first available CT-scan after the vaccination date, or median vaccination date in the control group, was compared to the latest CT-scan before this date.

Statistical analysis

Descriptive statistics of baseline demographic and clinical characteristics were composed both for the vaccinated and for the unvaccinated group. Variables were checked for normality and means or medians were calculated accordingly. Differences in the average age and average time until irAEs occurred between both groups were analysed by independent sample t-tests. Treatment duration was compared using Mann-Whitney U tests and ordered logistic regression was used to compare the treatment response of the two groups.

Differences in the rate of adverse events, irAEs and serious irAEs, differences in sex and differences in treatment discontinuity between the two study groups were analysed by Fisher exact tests and Chi square tests. SPSS version 23 was used for statistical analyses.

RESULTS

The compassionate use program database included a total of 213 patients with non-small cell lung cancer who underwent treatment with nivolumab. Four patients contributed twice because they received nivolumab treatment during the inclusion window both in 2015 and in 2016. The total number of eligible cases was therefore 217. From this cohort, 131 cases had been administered at least one dose of nivolumab between September 1st and January 1st. From these 131 cases, 42 had been vaccinated: four before receiving the first dose of nivolumab (median 11, range 1 – 26 days), 33 during treatment with nivolumab and five after receiving the final dose of nivolumab (median 26, range 20 – 61 days). For four cases the vaccination states could not be retrieved (Figure 1.). The median vaccination date was November 2nd.

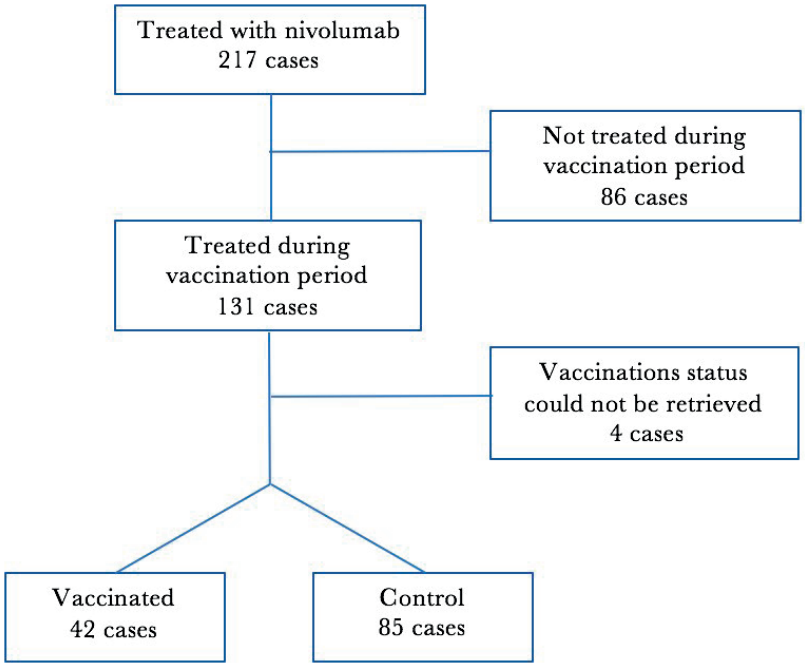

Figure 1. Flowchart of selection process.

The final analysis consisted therefore of 42 cases in the vaccinated group and 85 cases in the control group. Baseline characteristics are shown in table 1. The study population had a mean age of 63 years. The vaccinated group was marginally older than the unvaccinated group, but the difference reached significance. No significant differences were found in sex, treatment duration and treatment intensity between the vaccinated group and the control group.

Table 1. Baseline characteristics of the patients receiving immunotherapy, contrasting those who were vaccinated with trivalent inactivated influenza vaccine and those who were not vaccinated.

	Total n=127	Vaccinated n=42	Control n=85	p-value
Mean age (95% CI)[a]	62.6 (61.2 – 64.1)	64.8 (62.4 – 67.1)	61.6 (59.7 – 63.5)	0.04
Male	61 (48.0%)	23 (54.8%)	38 (44.7%)	0.29
Clinical stage				0.70
III	6 (4.7%)	1 (2.4%)	5 (5.9%)	
III/IV	2 (1.6%)	1 (2.4%)	1 (1.2%)	
IV	119 (93.7%)	40 (95.2%)	79 (92.9%)	
Median nr. of cycles nivolumab received at vaccination/start FU[b] (IQR)[c]	5.5 (3 – 12)[d]	4.5 (2 – 14)[e]	6 (3 – 12)[f]	0.66
Median treatment duration in days at vaccination /start FU[b] (IQR)[c]	78 (28 – 174)[d]	58.5 (18 – 211)[e]	81.5 (40 – 165)[f]	0.60
Median nr. of cycles nivolumab received at end FU[b] (IQR)[c]	9 (5 – 15)	9.5 (4 – 18)	9 (6 – 14)	0.71
Median treatment duration in days at end FU[b] (IQR)[c]	132 (70 – 222)	125 (39 – 281)	134 (72 – 213)	0.95

a 95% confidence interval
b Follow-up
c Interquartile range
d 106 cases eligible
e 38 cases eligible
f 68 cases eligible

The median follow-up duration did not differ significantly between both groups. (107 and 118 days in the vaccinated and control group, respectively)

In the vaccinated group, 11 irAEs were observed during follow up (incidence of 26%), whilst 19 irAEs were found in the control group (incidence of 22%). The vaccinated group was not at a significantly higher risk of developing irAEs, rate ratio 1.20 (95% confidence interval 0.51 – 2.65). The most commonly observed immune-related adverse events were toxicities of the endocrine organs (incidence of 8%), followed by pulmonary adverse events. Also the risk of serious irAEs (grade 3-5) was not significantly higher in the vaccinated group compared to the control group, rate ratio 2.07 (95% confidence interval 0.28 – 15.43). Three serious irAEs were found both in the vaccinated group (incidence of 7%) and in the control group (incidence of 4%) (Table 2).

A sub-analysis was done excluding the four cases that had been vaccinated in the 30 days before the first dose of nivolumab. Thirty-eight cases from the vaccinated group remained eligible. Also in this analysis, no significant increased risk was found for developing irAEs, rate ratio 1.20 (95% confidence interval 0.50 – 2.71) or serious irAEs, rate ratio 1.52 (95% confidence interval 0.13 – 13.25).

Table 2. Outcome parameters

	Total n=127 [13393 days][a]	Vaccinated n=42 [4367 days][a]	Control n=85 [9026 days][a]	Rate Ratio [CI-interval]
Adverse events	67 (53%)	22 (52%)	45 (53%)	1.01 [0.58 – 1.71]
irAEs (all grades)	30 (24%)	11 (26%)	19 (22%)	1.20 [0.51 – 2.65]
Endocrine		3 (7%)	7 (8%)	
Pulmonary		4 (10%)	4 (5%)	
Gastrointestinal		1 (2%)	2 (2%)	
Hepatic		1 (2%)	2 (2%)	
Arthritis		1 (2%)	1 (1%)	
Neurological		1 (2%)	0	
Skin		0	1 (1%)	
Other		0	2 (2%)	
Serious irAEs (grade 3-5)	6 (5%)	3 (7%)	3 (4%)	2.07 [0.28 – 15.43]
Gastrointestinal		1 (2%)	1 (1%)	
Hepatic		1 (2%)	1 (1%)	
Neurological		1 (2%)	0	
Skin		0	1 (1%)	
Outcome				
Death	26 (20%)	11 (26%)	15 (18%)	p = 0.26[b]
Discontinuation due to irAEs	6 (5%)	2 (5%)	4 (5%)	p = 1.00[b]
Discontinuation due to progression or clinical deterioration	62 (49%)	21 (50%)	41 (48%)	p = 0.85[b]

a Total person-time in follow-up
b P-value fisher exact test

A second sub-analysis was done including only those patients that had been vaccinated in between bi-weekly infusions with nivolumab (n=33). No significant increased risk was found for developing irAEs, rate ratio 1.33 (95% confidence interval 0.55 – 3.01) or serious irAEs, rate ratio 1.69 (95% confidence interval 0.14 – 14.72).

Furthermore, the total number of adverse events showed no significant differences between the vaccinated group and the controls, rate ratio 1.01 (95% confidence interval 0.58 – 1.72). No significant difference was observed in the time until irAEs occurred as well. In the vaccinated group, the mean time from the date of vaccination until a first irAE occurred was 47.6 days, whereas in the control group, the mean time until a first irAE occurred was 58.7 days (p=0.41, 95% CI -38.67 – 16.45).

No difference in treatment discontinuation as a result of irAEs was found between both groups. The total number of patients that had stopped their nivolumab therapy at the end

of the follow-up period (due to irAEs or clinical deterioration or progression) was similar in both groups (55% in the vaccinated groups versus 53% in the control group, p=0.85). Tumour response and mortality did not differ between both groups.

To control for the difference in age between the two study groups, a sub-analysis was performed including patients above the age of 50 only: 41 patients in the vaccinated group and 75 patients in the control group. Mean age was not significantly different between groups (p=0.22). In this subgroup, the vaccinated cases were not at higher risk for developing irAEs, rate ratio 1.36 (95% confidence interval 0.58 – 3.16) or serious irAEs, rate ratio 1.86 (95% confidence interval 0.25 – 13.86) compared to the controls.

DISCUSSION

Our study demonstrates that there is no significant difference in the likelihood of immune-related adverse events and serious immune-related adverse events between patients who have received influenza vaccination and in patients without. Furthermore, no significant differences in treatment outcome, discontinuation rates or tumour response were observed between the two groups.

The overall incidence of irAEs found in our study (24%) is comparable with the incidence of 26.5% found by El Osta et al. in a meta-analysis including 1259 patients treated with antibodies against PD-1.[16] Furthermore, the rate of serious irAEs found in our study (5%) is consistent with the 7% rate found in this meta-analysis. Our study experienced little drop-out with only four cases missing in the final analysis and the results of sub-analyses were consistent with our general findings. Further strengths of our study are a uniform cancer type and stage and a single drug being explored.

Our study had some limitations that are inherent to the design of the study. We did not collect anti-influenza antibodies or immunological markers to elucidate the markers associated with the development of irAEs. Due to the period of observation of five months at most, the occurrence of late sequelae could not be determined. However, due to the immunologic rationale underlying the potential increase in irAEs, it is expected that irAEs would occur within the usual time frame. Since registration of vaccine-related adverse events was missing, a potential increase in reactogenicity of the vaccine cannot be ruled out. Possible influenza infections during treatment were not monitored and therefore vaccine effectiveness cannot be established in our cohort. Vaccine effectiveness will likely be preserved, because the study by Läubli et al. did not observe significant differences between patients treated with checkpoint inhibitors and healthy controls in vaccine-induced

antibody titers against all tree viral antigens of the inactivated influenza vaccine.[13] Furthermore, the number of temporary interruptions of immunotherapeutic treatment because of influenza-related complications was not measured in this study. The vaccinated group was on average three years older than the control group, but in sub-analyses including only older patients similar outcomes regarding the risk of adverse events were found, strengthening our assumption that the small difference in age is unlikely to have influenced our results. Finally, we do not know the reason for not vaccinating the patients in the control group. Notwithstanding, we believe it is unlikely that the unknown reason would have affected the incidence of irAEs.

With the increased sample size of our study, we could not confirm the findings in the small study by Läubli and colleagues who reported a statistically significant increased rate of immune-related adverse events in patients who received an influenza vaccination whilst undergoing checkpoint blockade. However, our study does not provide a definitive answer, because it is underpowered to detect a very small, but significant increase in the risk of irAEs.

The immunological mechanisms potentially associated with an increased risk of irAEs when patients receiving checkpoint inhibitors are vaccinated with the non-adjuvanted inactivated influenza vaccine are not clear. Whether vaccines induce or aggravate autoimmunity has been debated at length without a definitive verdict, but accidental reports do hint at that association, potentially related to particular adjuvants, described as the autoimmune/inflammatory syndrome induced by adjuvants (ASIA syndrome).[17] However, the trivalent inactivated influenza vaccine does not contain adjuvants. Therefore, considering the uncertainties about the aetiology of autoimmune phenomena related to vaccination, whether pathophysiological pathways resulting in these vaccine-related adverse events coincide with those in irAEs related to checkpoint inhibitors, and which host factors predispose patients to the occurrence of irAEs, potential immunological causality needs to be clarified. As such, possible explanations remain speculative. Reassuringly, in our observational study no safety alarm about the combination of influenza vaccination and immunotherapy became apparent. Until more data is available from long-term prospective studies or the observation of an increase in the incidence of irAEs due to concurrent vaccination with other non-adjuvanted vaccines – such as the polysaccharide pneumococcal vaccine – or adjuvanted vaccines – such as the conjugated pneumococcal vaccine – during immunotherapy becomes apparent, influenza vaccine appears safe. As a consequence, seasonal influenza vaccination can still be advocated in cancer patients receiving anti-PD1 immunotherapy.

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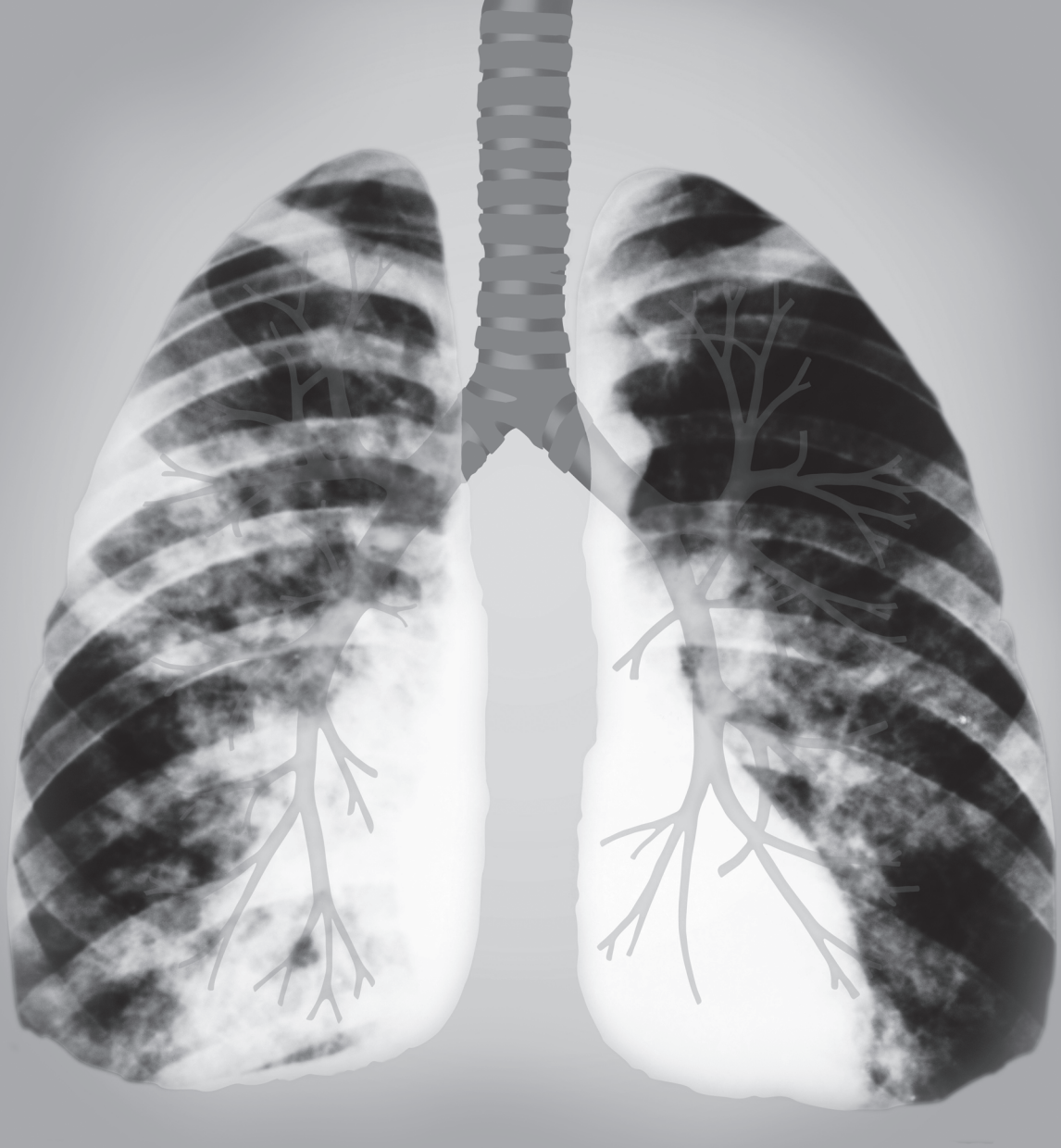

7

The severe flu season of 2017-2018: making a case for the vaccination of healthcare professionals.

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INTRODUCTIE

In de afgelopen winter duurde het griepseizoen 18 weken, twee keer zo lang als gebruikelijk. Ongeveer 340.000 personen consulteerden hun huisarts wegens een griepachtig ziektebeeld. In de periode tussen oktober 2017 en mei 2018 werden in heel Nederland naar schatting 900.000 mensen door het influenzavirus geveld. Veel ziekenhuizen waren overbelast door het hoge aanbod van patiënten. Wat lag aan die overbelasting ten grondslag? En is er een manier om dit te voorkomen?

Tijdens piekweken van de griepepidemie in februari en maart consulteerden in Nederland tegen de 170 per 100.000 mensen de huisarts wegens griepachtige klachten. Het betrof vooral kleine kinderen tot 4 jaar en ouderen (1). Meer dan 70% van de neuskeelwatten van de patiënten in deze piekweken bleek positief voor het influenzavirus. Veel patiënten moesten worden opgenomen vanwege complicaties van influenza, meestal longontsteking. Dit betrof naar schatting 16.000 personen. Ook overleden er tijdens de uitbraak naar schatting 9500 mensen, wat meer is dan gebruikelijk in het griepseizoen (1). Opmerkelijk was dat mensen vooral ziek werden van het griepvirus type B (Yamagata-lijn), dat niet eerder vanaf het begin van een griepseizoen zo overheerste als in het afgelopen jaar.

Ziekenhuizen overbelast

In de media verschenen berichten over zwaarbelaste huisartsen en ziekenhuizen die het zorgaanbod niet langer aankonden (2), met afdelingen Spoedeisende Hulp die de deuren tijdelijk sloten (3), of uitstel van geplande opnames en operaties (4). Ofschoon ziekenhuizen voor dergelijke situaties noodplannen hebben, stelden zorgverleners ook andere creatieve oplossingen voor om de druk op de zorg tijdens een intensief griepseizoen op te kunnen opvangen. Een voorbeeld daarvan is het instellen van een ‘zorghotel’ voor ouderen die het thuis niet langer kunnen bolwerken, maar die eigenlijk te goed zijn voor opname in het ziekenhuis (5).

Welke factoren spelen een rol?

Er is nog geen gedegen analyse van de factoren die verantwoordelijk zijn voor de hoge druk op de ziekenhuiszorgverlening tijdens een intensief griepseizoen zoals afgelopen winter. Waarschijnlijk spelen meerdere factoren een rol. In het LUMC kwamen we voorlopig tot de volgende drie: (a) het hoge aantal patiënten dat opgenomen moet worden als gevolg van griep, (b) het uitvallen van mantelzorgers die geveld zijn door de griep en (c) het uitvallen van zorgverleners die zelf griep krijgen.

Aanbod patiënten
Tijdens een griepepidemie stijgt het aantal patiënten dat opgenomen moet worden. De indicatie betreft dan niet alleen complicaties van de griep, zoals longontsteking, maar ook decompensatie van onderliggende ziekten, zoals diabetes mellitus en hartfalen, door griep.

Uitval mantelzorgers
Wanneer mantelzorgers van ouderen met griep zelf ook door griep geveld worden, kunnen zij niet langer bijspringen. Niet de ernst van de griep, maar de zorg die de oudere nodig heeft kan dan de reden worden voor ziekenhuisopname.

Hetzelfde geldt mutatis mutandis voor het terug naar huis plaatsen van opgenomen kwetsbare ouderen die herstellende zijn van griep, maar nog enige tijd extra hulp en thuiszorg nodig hebben. Als de mantelzorg is uitgevallen, blijven zij langer in het ziekenhuis dan medisch gezien strikt noodzakelijk is. Het fenomeen dat ouderen in een griepseizoen beslag leggen op ziekenhuisbedden zal toenemen, want Nederland vergrijst en steeds meer ouderen blijven met steun van mantelzorgers of thuiszorg langer zelfstandig wonen.

Uitval zorgverleners
Ook verpleegkundigen en artsen krijgen griep en vallen uit. Bij absentie van zorgverleners stellen ziekenhuizen hun zorgcapaciteit bij en gaan zij soms noodgedwongen over tot sluiting van de Spoedeisende Hulp en reductie van het aantal opnamebedden in een periode waarin de behoefte daaraan juist het hoogst is (zie info ‘Overbelasting: cijfers uit Leiden’). Het doorverwijzen van patiënten naar andere ziekenhuizen verhoogt bovendien de druk op het regionale netwerk.

Uitval zorgverleners voorkómen

Wordt het niet tijd om alvast één factor aan te pakken, namelijk de uitval van zorgverleners door griep? Vaccinatie tegen influenza biedt daar de mogelijkheid voor.

Influenzavaccinatie: wat is het doel?

Influenzavaccinatie dient meerdere doelen, in willekeurige volgorde: het eigen belang van de zorgverlener, namelijk het voorkómen van griep; een organisatiebelang, namelijk bijdragen aan de continuïteit van de ziekenhuiszorg (6); en een empathisch ideaal dat appelleert aan de professionele norm van zorgverleners, namelijk patiëntveiligheid, in dit geval het voorkómen van overdracht van het influenzavirus op kwetsbare patiënten. Daarmee doelen wij in de eerste plaats op immuungecompromitteerde patiënten, bij wie een vaccinatie geen bescherming biedt en infectie met influenzavirus tot ernstige morbiditeit en sterfte leidt (7).

Lage vaccinatiegraad

In Nederland is vaccinatie van zorgverleners tegen influenza bepaald niet vanzelfsprekend. Integendeel, in een onderzoek onder bijna de helft van de Nederlandse ziekenhuizen kwam het RIVM in 2014 tot een vaccinatiegraad onder zorgverleners van slechts 13% (8). Mogelijk is dit percentage de laatste jaren iets gestegen; zo ligt de vaccinatiegraad van zorgverleners in het LUMC de laatste jaren tussen de 23 en 39%. Nederland staat niet alleen in dit teleurstellend lage percentage. Onderzoek in Europese landen toonde een vaccinatiegraad onder zorgverleners van 6-29% (9). In de Verenigde Staten stelde het Virginia Mason Medical Center in Seattle in 2005 als eerste zorginstelling influenzavaccinatie voor haar verleners verplicht ('fittness-for-work'). Voor 31 van de ruim 5000 medewerkers werd op verschillende gronden een uitzondering gemaakt; een handvol medewerkers nam ontslag wegens dit ziekenhuisbeleid ten aanzien van influenzavaccinatie (10). Het ziekenhuis verloor later de rechtszaak hierover die was aangespannen door de Washington State Nurses Association, maar de vaccinatiegraad is toch zeer hoog gebleven. In veel Amerikaanse staten hebben ziekenhuizen dit voorbeeld gevolgd, en de gemiddelde vaccinatiegraad onder zorgverleners in de Verenigde Staten ligt nu boven de 80% (www.cdc.gov/flu/healthcareworkers.htm).

Waarom is de vaccinatiegraad zo laag?

Waarom maken zo weinig zorgverleners gebruik van de mogelijkheid tot een kosteloze influenzavaccinatie? Er zijn veel determinanten die bepalen of een medewerker in de zorg zich al dan niet tegen griep laat vaccineren. Uit kwalitatief onderzoek kwam naar voren dat zorgverleners diverse opvattingen over griep huldigen die maken dat ze afzien van vaccinatie (11-13).

Opvattingen als: 'ik ben nooit ziek', 'het risico dat ik griep krijg is laag', 'als ik al griep krijg stelt het weinig voor', 'handen wassen en een mondmasker bij griep is afdoende om overdracht tegen te gaan', enzovoort. En dan is er nog de opvatting dat verplichting tot influenzavaccinatie zoals die in de Verenigde Staten is uitgevoerd schade doet aan de persoonlijke autonomie, die door werkgevers gerespecteerd moet worden.

Persoonlijke autonomie?

Er zijn grenzen aan de vrijheid van zorgverleners om zich bij influenzavaccinatie te beroepen op persoonlijke autonomie. Die vrijheid raakt bijvoorbeeld aan patiëntveiligheid, aan de zorgplicht die een ziekenhuis heeft voor alle opgenomen patiënten, en aan een breed gedragen professionele norm van de beroepsgroep. Een kwetsbare patiënt mag verwachten dat hij of zij tijdens een griepseizoen alleen gevaccineerde zorgverleners tegenover zich vindt. Het beginsel van patiëntveiligheid ligt samengebald in 'primum non nocere'. Bij dit beginsel gaat het er niet om hoeveel nadeel een patiënt ondervindt wanneer de

arts iets doet of nalaat, maar het impliceert dat we élke maatregel horen te omarmen die tot grotere patiëntveiligheid leidt. In dat licht is het opmerkelijk is dat de verplichting om tegen hepatitis B te worden gevaccineerd –waarbij patiëntveiligheid ook een belangrijk onderdeel van de argumentatie was – zonder veel ophef is doorgevoerd.

Het influenzavaccin: optimisten en pessimisten

In de discussies over de wenselijkheid om zorgverleners te vaccineren tegen influenza komt ook steevast ter sprake wat de kwaliteit is van het bewijs dat influenzavaccinatie werkt, en hoe de balans is tussen werkzaamheid en schadelijkheid van het vaccin.

Het huidige influenzavaccin is allesbehalve perfect en de effectiviteit is beperkt in vergelijking met andere vaccins. Maar zelfs in het afgelopen jaar, waarin het circulerende influenzavirus type B (Yamagata-lijn) niet in het vaccin vertegenwoordigd was, voorkwam het vaccin in Nederland het optreden van griep bij 44% van de mensen die zich in 2017 hadden laten vaccineren (1). Dat het influenzavaccin gemiddeld grofweg de helft van de gevallen van griep voorkómt, is de afgelopen griepseizoenen het gebruikelijke beeld in Nederland. Ook uit de systematische Cochrane-reviews – met een laatste update in februari 2018 – blijkt dat influenzavaccinatie meer dan de helft van influenzainfecties voorkómt bij gezonde volwassenen (risicoratio: 0,41) en bij ouderen (risicoratio: 0,42) (14,15).

Als we de werkzaamheid van het influenzavaccin zo beschouwen, ziet de optimist het glas halfvol, en de pessimist het glas halfleeg. Vertegenwoordigers van deze laatste groep bekritiseerden de afgelopen jaren fel de brede toepassing van het vaccin, zoals in een redactioneel in het *Nederlands Tijdschrift voor Geneeskunde* en in het *Geneesmiddelenbulletin* (16,17). De insteek van de optimist is dat een halvering van het aantal patiënten met griep een welkom gegeven kan zijn, omdat griep veel voorkomt en optreedt in een relatief korte tijdsbestek. Voor- en tegenstanders zijn het er doorgaans wel over eens dat de vaccins op basis van geïnactiveerd influenzavirus veilig zijn. En al circuleert er op sociale media foutieve informatie over de veiligheid van vaccins (zie bijvoorbeeld www.theguardian.com/society/2018/aug/23/russian-trolls-spread-vaccine-misinformation-on-twitter?CMP=twt_gu), na ruim 60 jaar toepassing van het vaccin in landen op alle continenten is duidelijk dat ernstige complicaties uiterst zeldzaam zijn. De bijwerkingen blijven veelal beperkt tot een pijnlijke arm ter plaatse van de injectie. In Nederland bevestigt de rapportage van Bijwerkingencentrum Lareb dit beeld (18).

Kortom, het is biologisch plausibel dat influenzavaccinatie van zorgverleners leidt tot minder griep in deze groep, en indirect tot minder ziekteverzuim en overdracht naar anderen; bovendien is de vaccinatie veilig. Zo bezien is influenzavaccinatie van zorgverleners een

belangrijke pijler onder élk beleid dat gericht is op continuïteit van ziekenhuiszorg tijdens een intensief griepseizoen.

Nosocomiale griep

Patiënten kunnen in het ziekenhuis griep oplopen van zorgverleners die het influenzavirus onder de leden hebben. Deze nosocomiale griepinfecties worden opgemerkt wanneer ze leiden tot lokale uitbraken. Daarvan zijn er meerdere beschreven die sterfte van patiënten tot gevolg hadden, waaronder één in het LUMC (19-24). Daar overleden twee patiënten met een ernstige afweerstoornis op de afdeling Hematologie aan de gevolgen van respiratoir falen door de griep (21).

Zorgverleners dragen griep over aan patiënten

Dat zorgverleners door het influenzavirus geïnfecteerd worden staat vast. Waar het virus jaarlijks gemiddeld circa 5 tot soms wel 10% van de bevolking treft, is de incidentie onder zorgverleners die blootstaan aan patiënten met griep ruim 2 keer zo hoog (25).

Evenzo staat vast dat zorgverleners het influenzavirus kunnen overdragen aan collegae en patiënten. Natuurlijk hebben de meeste patiënten ook andere contacten tijdens een ziekenhuisopname, zoals familie of bezoekers, maar veel van de directe, intensieve contacten verlopen via zorgverleners. Hoewel griep in een griepseizoen een belangrijke reden is tot ziekteverzuim onder zorgverleners, blijkt dat lang niet alle zorgverleners met verschijnselen van griep zich ziek melden (25). Het influenzavirus wordt bovendien al overgedragen vóórdat degene die erdoor getroffen is, ziekteverschijnselen heeft.

Beschermende maatregelen om bij griepklachten overdracht naar collegae of patiënten tegen te gaan, zoals het dragen van een mondmasker, voldoen daarmee niet. Strikte naleving van de extra hygiënemaatregelen moet gedurende het gehele griepseizoen – het afgelopen seizoen duurde 18 weken – onverminderd toegepast worden, en zelfs die maatregelen blijken niet afdoende (26).

Duidelijk is dat vrijwel elk jaar individuele patiënten een influenzainfectie oplopen tijdens hun verblijf in het ziekenhuis. Om een voorbeeld te geven: in de griepseizoenen 2014/2015 en 2015/2016 waren 6 van de 157 PCR-bevestigde influenzainfecties onder patiënten in het LUMC met zekerheid nosocomiaal overgedragen. Het betrof onder andere een patiënt die enkele dagen vóór zijn griepinfectie een niertransplantatie had ondergaan en sterke immunosuppressiva gebruikte. Dergelijke patiënten met een door medicatie veroorzaakte lymfopenie kunnen het griepvirus slecht klaren en dragen het soms wekenlang met zich mee, wat langdurige verpleging in isolatie noodzakelijk maakt.

Helpt vaccinatie tegen nosocomiale overdracht?

Hoe zouden we de influenzavaccinatie onder zorgverleners kunnen bevorderen? Bij voorkeur zou je over gedegen onderzoeken willen beschikken die aantonen dat zo'n vaccinatieprogramma inderdaad ook nosocomiale overdracht door zorgverleners voorkómt. In een meta-analyse concludeerden medewerkers van de Centers for Disease Control and Prevention (CDC) dat influenzavaccinatie van zorgverleners bijdraagt aan patiëntveiligheid, omdat het in onderzoeken een belangrijke afname bewerkstelligde van het optreden van influenza-achtige ziektebeelden en sterfte onder opgenomen patiënten (27). De onderzoeken die opgenomen werden in deze meta-analyse zijn daarna op verschillende methodologische punten bekritiseerd en een update van een Cochrane-review over dit onderwerp concludeerde dat er momenteel geen krachtig bewijs is dat influenzavaccinatie van zorgverleners een vermindering geeft van de ziektelast bij opgenomen patiënten (28).

Hoewel een krachtig bewijs op dit punt dus ontbreekt, is er wél overtuigend bewijs dat influenzavaccinatie van specifieke groepen of gemeenschappen leidt tot zogenoemde kudde-immuniteit, die niet-gevaccineerde personen binnen die groep beschermt tegen griep (29-31). Dit 'proof of concept' maakt het alleszins aannemelijk dat ook vaccinatie van zorgverleners in ziekenhuizen en verpleeginstellingen de kwetsbare patiënten beschermt (32,33).

CONCLUSIE

Niet alleen het intensieve griepseizoen van afgelopen winter en de discussies over continuïteit van ziekenhuiszorg die dit seizoen opriep, maar ook de toegevoegde waarde van influenzavaccinatie aan patiëntveiligheid maken dat het hoog tijd is uitvoering te geven aan het Gezondheidsraadadvies uit 2007, te weten: om te komen tot een zo hoog mogelijke – liefst 100% – influenzavaccinatiegraad onder zorgverleners (34).

De opgave voor professionals is om samen met de ziekenhuis- en instellingsbestuurders én bedrijfsartsen het gesprek aan te gaan met twijfelende collegae, in ieder geval met alle zorgverleners die contact met patiënten hebben, en hen met de feiten en de juiste argumenten te overtuigen. Omdat zo'n discussie nog onvoldoende breed en indringend gevoerd is, is een vaccinatieplicht – zoals in de Verenigde Staten – op dit moment een te ingrijpend middel. Wel kan nagegaan worden of bij nieuw aan te stellen medewerkers de jaarlijkse influenzavaccinatie, naast de hepatitis B-vaccinatie, als norm verplicht te stellen is. Met influenzavaccinatie beschermen we onszelf én onze meest kwetsbare patiënten,

en dragen we bij aan de continuïteit van de ziekenhuiszorg, ook tijdens een intensief griepseizoen.

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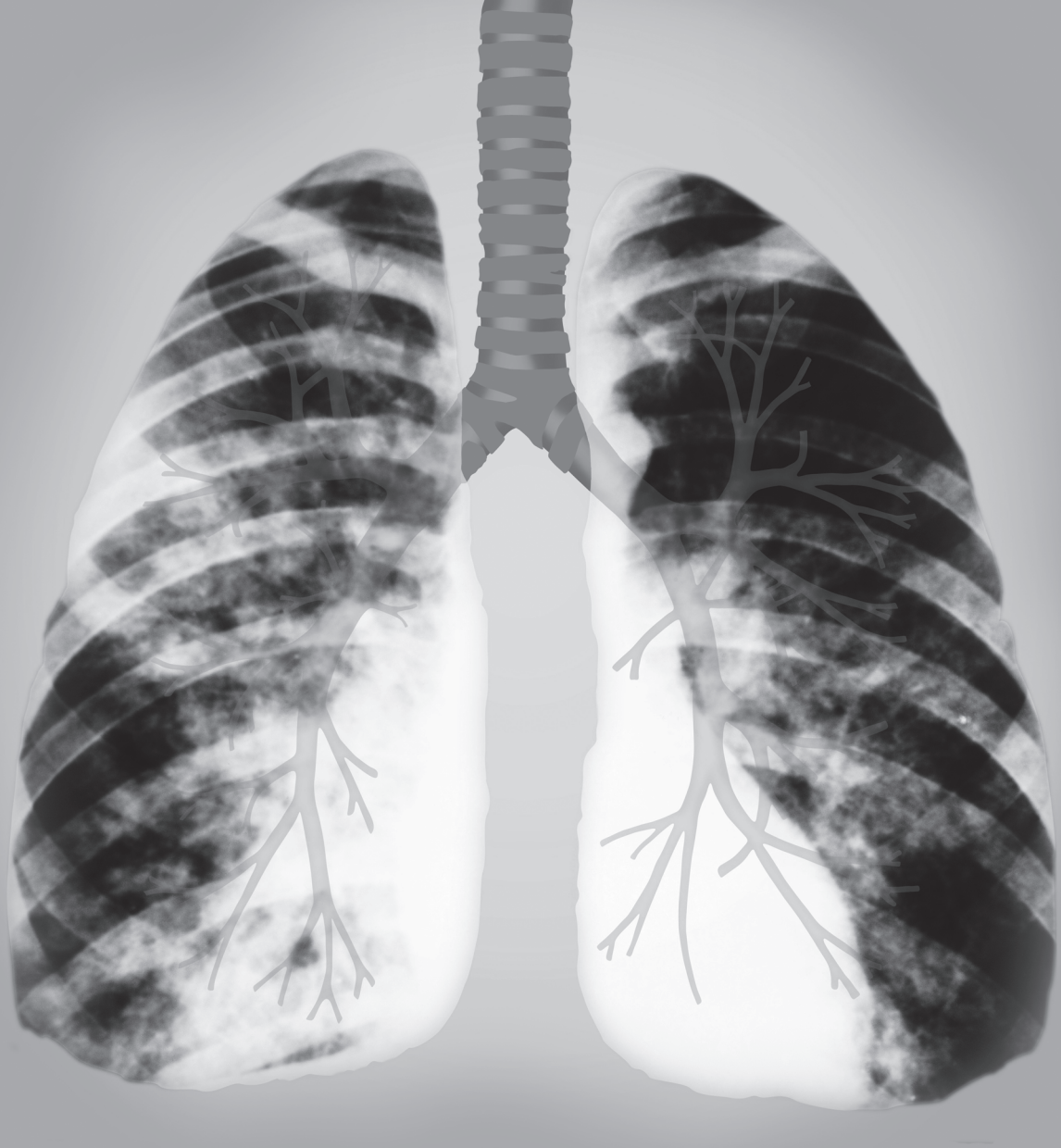
OVERBELASTING: CIJFERS UIT LEIDEN

In de Leidse regio kondigden Spoedeisende Hulpen en Eerste Harthulpen tussen mei 2017 en april 2018 in totaal 314 keer een tijdelijke presentatiestop af omdat de vraag naar zorg de aanwezige maximale opnamecapaciteit van het ziekenhuis overschreed.

Tijdens het griepseizoen gebeurde dit 10 keer extra per maand vergeleken met de periode buiten het griepseizoen, namelijk gemiddeld 37 presentatiestops per maand (overeenkomend met 102 uren sluiting) versus 26 presentatiestops per maand (overeenkomend met 84 uren sluiting). In de piekmaanden van het griepseizoen, februari en maart 2018, lag ook de piek van de presentatiestops. In het afgelopen griepseizoen kwamen 13% meer patiënten naar de Spoedeisende Hulp van het LUMC voor beschouwende specialismen als interne geneeskunde, longziekten en hartziekten dan buiten het seizoen. Het aantal acuut opgenomen patiënten in de weken van het griepseizoen lag voor de afdeling Interne Geneeskunde 8% hoger dan buiten het griepseizoen en voor de afdeling Longziekten tot 50% hoger.

Het aantal nieuwe gevallen van ziekteverzuim onder zorgverleners in de periode januari-maart 2018 lag per maand 66% hoger dan het halfjaar ervoor en erna: bijna 20% van het zorgpersoneel ‘aan bed’ meldde zich gedurende enkele dagen ziek. Buiten het griepseizoen was gemiddeld 1,45% fte van de medewerkers ziek (gerekend als percentage van het totaal aantal fte). Binnen het afgelopen griepseizoen was het ziekteverzuimpercentage in fte 2,52%, een toename van 74%. Kortom, de uitval van zorgverleners door griep en de hogere werkdruk voor het personeel dat nog wel aan het werk was viel samen met een extra hoge zorgvraag door patiënten met complicaties van griep. Dit verklaart dat soms overgegaan moest worden tot presentatiestops en beddensluiting.



8

Effectiveness of oseltamivir in reduction of complications and 30-day mortality in severe influenza infection.

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ABSTRACT

Objectives

The benefit of oseltamivir treatment in patients admitted with influenza virus infection and the design of studies addressing this issue, have been questioned extensively. Since the influenza disease burden is substantial and oseltamivir treatment is biologically plausible, we assessed the clinical benefit of oseltamivir treatment in adult patients admitted with severe seasonal influenza virus infection in daily practice with a propensity score model.

Methods

A multicenter, retrospective cohort study was conducted to compare the effectiveness of treatment with and without oseltamivir <48 hours after admission in patients admitted with laboratory-confirmed influenza virus infection in three large hospitals in the Netherlands. Propensity score matching was used to compare clinical relevant outcome variables.

Results

Thirty-day mortality, as well as the composite endpoint of 30-day mortality or intensive care unit admission >48h after admission, were reduced by 9% (p= 0.04) and 11% (p= 0.02) respectively. Length of hospital stay and in-hospital mortality rates all showed a trend towards reduction. The median duration between symptom onset and initiation of treatment was 3.0 days.

Conclusions

This study demonstrates that, in daily practice, patients admitted with influenza virus infection should be treated with oseltamivir, even if they have complaints for more than 48 hours.

INTRODUCTION

Patients with seasonal influenza virus infection can develop severe disease which requires hospitalization. In these patients, optimal treatment may reduce morbidity, mortality and associated costs substantially. In the United States, the cumulative influenza incidence of laboratory-confirmed influenza hospitalizations was 6.2 per 10,000 and 10.3 per 10,000 in the 2016/2017 and 2017/2018 flu seasons respectively (1). Unfortunately, these data are not available for Europe. In hospitalized patients, intensive care unit (ICU) admission rates and mortality rates are 15-34% and 4-12% (2-5). In 2013, the annual costs for patients hospitalized with influenza virus infection in the Germany were estimated to be 90 million Euros (6).

Neuraminidase inhibitors are the primary treatment option for patients with severe influenza infection. Evidence regarding clinical effectiveness of neuraminidase inhibitors is however inconsistent. No benefit was demonstrated in several studies (7-9) and the statistical methods of studies showing benefit, have been questioned extensively (10-14). In hospitalized patients, most treatment guidelines recommend the use of the neuraminidase inhibitor oseltamivir despite the lack of solid evidence (15, 16). Hence, compliance with these guidelines is poor (17). This may be due to this lack of evidence for the prevention of complications by oseltamivir treatment in hospitalized patients and the finding that a reduction in mortality is most evident in patients who start treatment within 48 hours after the onset of symptoms (18, 19). In clinical practice, the majority of patients who present to a hospital has had symptoms for more than 48 hours (18, 20, 21). In these cases, the benefit of late initiation of treatment (>48 hours after symptom onset) has been questioned. Furthermore, compliance to treatment guidelines may be poor due to the uncertainty about the diagnosis at initial hospital presentation. Once influenza is laboratory-confirmed, physicians are more inclined to prescribe oseltamivir (17, 22, 23). All these factors interfere with physicians' confidence in the benefits of oseltamivir treatment (24, 25). In addition, negative reporting about oseltamivir has further increased the uncertainty of oseltamivir´s potential benefit (26, 27).

Despite symptoms already being present for more than 48 hours, viral shedding is present in all patients admitted to the hospital with confirmed influenza virus infection, and prolonged viral replication was found in the majority of these patients (28-31). For these patients, oseltamivir treatment would be biologically plausible (32). Therefore, we investigated the effect of oseltamivir treatment in adult patients hospitalized for influenza virus infection in a healthcare system where the majority of patients come to the hospital after more than 48 hours of illness. To assess clinical effectiveness of oseltamivir, an observational cohort study using propensity score methods was performed.

PATIENTS AND METHODS

Design and study population

A multicenter, retrospective cohort study was conducted to estimate the effectiveness of oseltamivir in patients admitted with laboratory-confirmed influenza virus infection (33). Two university medical hospitals (Leiden University Medical Center, 585 beds, and University Medical Center Utrecht, 1100 beds) and one teaching hospital (Jeroen Bosch hospital, 575 beds) participated in the study.

All patients with laboratory-confirmed influenza from two or three consecutive influenza seasons between October 1st, 2013 and April 1st, 2016 were screened for eligibility. Lists with adult patients (≥ 18 years) with positive PCR test results for influenza A or B virus in respiratory samples (sputum, nasopharyngeal or throat swab, or bronchoalveolar lavage (BAL)) were obtained. Patients with influenza A or B virus-positive samples who were hospitalized within seven days before or after virologic confirmation were included. Patients with hospital-acquired influenza infection, i.e., if symptoms had started ≥72 hours after hospital admission, were excluded.

Data collection and study definitions

Data about demographic characteristics, start of symptoms, dates of hospital admission and discharge, influenza type (A or B), comorbidity, CURB-65 score (34), start and stop of oseltamivir treatment, and start of antibacterial treatment at hospital admission and intensive care unit (ICU) admission within 48 hours after admission were obtained from the electronic medical records. ICU admission < 48 hours after hospital admission was used as a marker of severity. Comorbidity was categorized into cardiovascular disease, chronic pulmonary disease, and immunodeficiency. Immunodeficiency was defined as either the presence of solid organ transplantation (SOT), hematological malignancy, or hematopoietic stem cell transplantation (HSCT), chronic use of immunosuppressive medication or chemotherapy in the past six months, or HIV with CD4+-T-lymphocyte counts ≤200 cells/µl.

We defined oseltamivir treatment started within 48 hours after hospital admission as adequate treatment (18, 21, 35-37). We compared this group of patients with the group who had not been treated with oseltamivir within 48 hours after admission. During the study period, oseltamivir was the only neuraminidase inhibitor used in the three hospitals. Dutch national guidelines did not recommend the use of oseltamivir for outpatients. Therefore, it was assumed that the patients did not receive oseltamivir before hospital admission.

Primary outcome parameters were: 30-day mortality, in-hospital mortality, length of hospital stay, and the composite endpoint of 30-day mortality and/or ICU admission >

48 hours after hospital admission. ICU admission > 48 hours after hospital admission is regarded as a complication influenza virus infection. We used this composite endpoint to assess the clinical benefit of oseltamivir.

For subgroup analysis, chest X-rays have been assessed for the presence or absence of a consolidation by independent radiologists. Consolidation is regarded as marker for ongoing viral replication and inflammatory response in the lower respiratory tract. In a secondary analysis, outcome parameters were assessed in the subgroup of patients with a consolidation on chest X-ray.

Statistical analyses

Continuous variables were reported depending on distribution as means with standard deviations or as medians with interquartile ranges (IQR), categorical variables were reported as numbers with percentages. Univariate analyses were performed to compare baseline variables between groups, using Fisher's Exact tests, Chi-squared tests, and Wilcoxon rank tests as appropriate.

By using the Propensity Score Matching (PSM) and Inversed Probability Weighting (IPW) the outcome parameters were compared between the group who received adequate treatment and the group who did not receive adequate treatment (see below).

Survival analysis was performed to assess the time to event in both groups. The log-rank test was used to compare the survival distributions. All statistical analyses were performed using STATA software version 14 (StataCorp, College Station, TX, USA).

Propensity score methods

Propensity score methods can be used to analyze observational data concerning a specific treatment outcome by defining which individuals have the same probability of receiving the intervention (here: adequate oseltamivir treatment) and by also accounting for the probability of a defined outcome. By assessing the outcome in relation to the intervention for patients with similar (i.e. matched) propensity scores, it is aimed to attain the results that reflect those of a randomized study (38).

In this study, propensity scores were generated using a multivariable logistic regression model based on confounding variables as identified by the univariate analyses. Variables that were associated ($p<0.20$) with the allocation of treatment and with the primary endpoint of 30-day mortality, and were plausible confounders, were selected for input in a logistic regression model to calculate the propensity scores. The matching algorithm used a nearest neighbor method in a 1:1 ratio without replacement and a caliper (maximum

probability distance) of 0.20. To balance baseline variables between groups of patients adequately treated with oseltamivir and those who were not, the model was calibrated to allow a maximum standardized difference of 0.1 (10%).

In the matched cohort, comparison of endpoints between groups was performed by assessment of the average treatment effect in the treated population (ATT) with Student's-t-test, Fishers' exact, or Wilcoxon signed rank test, as appropriate.

IPW was used as a sensitivity analysis, i.e. to assess the robustness of the results obtained by PSM.

Reporting and Ethics

The study was approved by each hospital's ethical review board and performed and reported according to the STROBE statement for observational studies and a checklist of proposed guidelines for the reporting of propensity score methods (39, 40). Research data were pseudonymized and securely stored, according to the General Data Protection Regulation (GDPR). All data generated or analyzed during this study are included in this article.

RESULTS

Characteristics of the complete cohort

Of 408 screened patients, 18 were excluded because they had hospital-acquired infection, missing data of onset of symptoms, or viral testing could not rule out hospital acquisition. In the final analysis, 390 patients admitted to the hospitals with laboratory-confirmed, community-acquired influenza virus infection, were included. Median age was 65 years (IQR 51-77), 42% was female. Comorbidity was present in 80% of patients, of these 60% had cardiovascular comorbidity, 42% had pulmonary comorbidity, and 46% was immunocompromised. A considerable number of 47 solid organ transplant recipients (12%) and 21 (5%) stem cell transplant recipients were included in the cohort.

One-hundred-thirty-eight (35%) patients received adequate treatment. The median duration between symptom onset and initiation of oseltamivir was 3.0 days (IQR 2.0-4.6; missing data in 13 patients).

Of the remaining 252 patients, 49 (19%) received oseltamivir > 48 hours after admission and 203 (81%) were not treated with oseltamivir. Overall, median length of hospital stay was 5.0 days (IQR 2.9-10.0). Seventy patients (18%) needed to be admitted to the ICU, 62 of them were admitted to the ICU within 48 hours after hospital admission. In-hospital mortality was 21/390 (5.4%), 30-day mortality was 30/390 (7.7%).

Table 1. Baseline characteristics before and after propensity score matching

	Cohort before matching					Cohort after matching				
	oseltamivir ≤48h		no oseltamivir ≤48h			oseltamivir ≤48h		no oseltamivir ≤48h		
	N#	%	N#	%	P*	N	%	N	%	P*
Total	**138**		**252**			**88**		**88**		
Gender					1					1
Male	80	58.0	146	57.9		51	58.0	51	58.0	
female	58	42.0	106	42.1		37	42.0	37	42.0	
Type of influenza					0.05					1
A	115	84.6	186	75.6		71	80.7	70	79.5	
B	21	15.4	60	24.4		17	19.3	18	20.5	
Presence of any comorbidity					0.04					0.7
No	23	16.7	53	21.0		15	17.0	18	20.5	
Yes	115	83.3	198	78.6		73	83.0	70	79.5	
Pre-existing cardiovascular disease					0.59					1
No	74	53.6	127	50.4		43	48.9	44	50.0	
Yes	64	46.4	125	49.6		45	51.1	44	50.0	
Pre-existing lung disease					0.15					0.63
No	98	71.0	160	63.5		60	68.2	56	63.6	
Yes	40	29.0	92	36.5		28	31.8	32	36.4	
Immunocompromised					0.00					0.76
No	61	44.2	185	73.7		50	56.8	47	53.4	
Yes	77	55.8	66	26.3		38	43.2	41	46.6	
Mean age in years	58.4		65.1		0.00	62.3		62.5		0.93
Elderly (>65 years old)					0.00					1
No	88	63.8	109	43.4		45	51.1	45	51.1	
Yes	50	36.2	143	56.7		43	48.9	43	48.9	
CURB-65 score					0.27					0.38
0	18	15.9	27	12.9		14	15.9	15	17.0	
1	35	31.0	56	26.7		25	28.4	23	26.1	
2	36	31.9	60	28.6		29	33.0	22	25.0	
3	18	15.9	54	25.7		15	17.0	24	27.3	
4	4	3.5	12	5.7		3	3.4	4	4.5	
5	2	1.8	1	0.5		2	2.3	0	0	
Admission to ICU ≤48h after presentation					0.00					0.21
No	101	73.2	227	90.1		69	78.4	71	80.7	
Yes	37	26.8	25	9.9		19	21.6	17	19.3	
Empiric antibiotics					0.01					0.85
No	20	14.6	65	25.9		13	14.8	11	12.5	
yes	117	85.4	185	74.1		75	85.2	77	87.5	

* Fisher´s exact test, or Chi-squared test if >2 rows

Numbers do not always add up to 390 since there are some missing data. In particular, CURB-65 scores are missing in 67 patients

Baseline characteristics differed between the patients who received adequate treatment (n=138) versus patients who did not (n=252). Younger patients, patients with comorbidity, or with concomitant antibiotics, and patients admitted to the ICU within 48 hours after admission were more likely to be treated with oseltamivir (**Table 1**).

Thirty-day mortality in influenza patients increased with higher CURB-65 scores at admission (**Table 2**).

Table 2. CURB-65 score and 30-day mortality

	30-day mortality
CURB-65 score	
0	0/45 (0)
1	2/91 (2.2)
2	8/96 (8.3)
3	12/72 (16.7)
4	4/16 (25.0)
5	1/3 (33.3)

CURB-65 severity score: C= new onset confusion, Urea >7mmol/l, R= respiratory rate ≥30/minute, B= Blood pressure (Systolic < 90 mm Hg or Diastolic ≤ 60 mm Hg), 65= Age ≥65 (34)

Propensity score matching

The propensity score model was built with nine variables from the multivariable logistic regression model (age, age>65, type of influenza, CURB-65 score, pre-existing lung disease, pre-existing cardiovascular disease, immunocompromised, empiric antibiotics, and ICU admission within 48 hours after hospital admission). The hospital of admission was not a confounder. After successful propensity score matching, 88 patients remained in both groups (**Table 1** and **Figure 1**).

Outcome with propensity score matching

Thirty-day mortality and the composite endpoint in the adequate treatment group were, respectively, 9.1% and 11.4% lower than in the group who did not receive oseltamivir within 48 hours after admission. The number needed to treat to prevent one ICU admission or death within 30 days is approximately nine. Both in-hospital mortality and length of hospital stay showed a trend towards reduction (**Table 3**). In patients who received adequate treatment, median duration of symptoms before start of treatment was 3.0 days (IQR 2.0-4.1 days).

Table 3. Outcome using propensity score matching in the group of influenza patients treated with oseltamivir within 48 hours after hospital admission versus the group without this treatment

Outcome variable	Untreated (%)	Treated (%)	Difference (%)	OR	95%CI	p
30-day mortality	12/88 (13.6)	4/88 (4.6)	-8/88 (9.1)	0.30	0.07-1.07	0.04
In-hospital mortality	9/88 (10.2)	3/88 (3.4)	-6/88 (6.8)	0.31	0.05-1.31	0.13
Composite endpoint	14/88 (15.9)	4/88 (4.6)	-10/88 (11.4)	0.25	0.06-0.86	0.02
Median length of hospital stay in days (IQR)	6 (2.8-11.0)	4 (2.6-8.0)	-	-	-	0.14

OR, odds ratio; CI, confidence interval; ICU, intensive care unit; IQR, interquartile range
Composite endpoint = 30-day mortality and/or ICU admission >48h after hospital admission

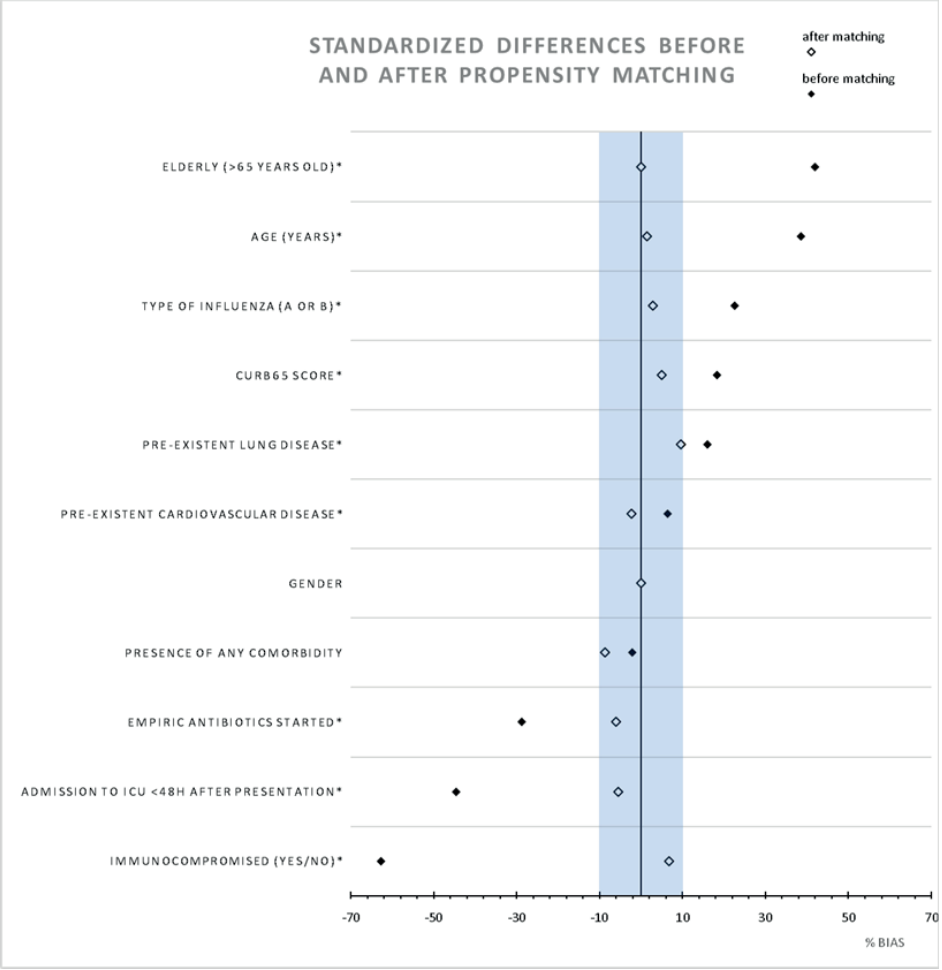

Figure 1. Standardized differences before and after propensity matching

Outcome with inversed probability weighting

The composite endpoint showed a reduction of 8% (p=0.05). This leads to a number needed to treat to prevent one ICU admission or death within 30 days of approximately 13. Thirty-day mortality, in-hospital mortality and median length of stay all showed a trend towards reduction (**Table 4**).

Survival analysis

Survival analyses are presented in Figure S1 and S2 in the supplementary data. Thirty-day mortality and the composite endpoint were better in the group who received adequate treatment. The first death occurred three days after hospital admission.

Table 4. Outcome with IPW in the group of influenza patients treated with oseltamivir within 48 hours after hospital admission versus the group without this treatment

Outcome variable	Coefficient	SE	95% CI	p-value
30-day mortality	-0.07	0.38	-0.14 - 0.00	0.06
In-hospital mortality	-0.04	0.03	-0.11 - 0.03	0.22
Composite endpoint	-0.08	0.04	-0.15 - 0.00	0.05
Median length of hospital stay in days	-1.38	-1.05	-3.44 - 0.67	0.19

SE, standard error; CI, confidence interval; ICU, intensive care unit
Composite endpoint = 30-day mortality and/or ICU admission >48h after hospital admission

Subgroup analysis in patients with consolidation on chest X-ray

Sixty patients (34%) in the matched cohort had a consolidation on the chest X-ray on the day of hospital admission. Half of the patients (n=30) received adequate treatment. Seven patients who did not receive this treatment (23%) died within 30 days or reached the composite endpoint versus two (7%) who did receive adequate treatment (p=0.07). In-hospital mortality was 17% (5/30) in patients who did not receive adequate treatment versus 3% (1/30) in the ones who did (p=0.09).

DISCUSSION

During three consecutive influenza seasons, the burden of patients admitted with community-acquired influenza virus infection in three hospitals was substantial: the median length of stay was five days, and 70 of 390 patients needed ICU admission. In the propensity score matched cohort (mean age of 62 years and substantial comorbidity), oseltamivir treatment within 48 hours after hospital admission reduced 30-day mortality as well as the composite endpoint of 30-day mortality and/or ICU admission >48h after hospital admission. Adequate treatment also showed a trend towards reduced length of hospital stay. The median duration between symptom onset and initiation of oseltamivir was 3.0 days.

Our study confirms the 30-day mortality benefit of adequate treatment which has been observed previously (41). Similarly, the meta-analysis by Muthuri et al. using PSM, showed a reduction of in-hospital mortality in influenza A (H1N1)pdm09 virus infected patients that were treated with oseltamivir, odds ratio 0.81 (18). The odds ratio for 30-day mortality in our cohort is 0.30.

There are important differences between the Muthuri cohort and our cohort that need consideration. Firstly, in the Muthuri cohort only 5% of patients was aged 65 or older and

only 6% were immunocompromised (18). This does not reflect the type of patients with seasonal influenza virus infection that presented to the hospital in more recent influenza seasons (42). Nowadays, mostly elderly patients are affected and become hospitalized by an influenza virus infection and/or secondary bacterial infection. In addition, increasing numbers of hospitalized patients are immunocompromised (1). Our cohort reflects this type of patients with 193/390 (49%) are over 65 years of age, and 143/389 (37%) are immunocompromised.

Secondly, the healthcare systems in the countries contributing to the meta-analysis of Muthuri are different from the Dutch healthcare system. In the Netherlands and other European countries, patients are usually referred to hospitals after consulting their general practitioner. This gatekeeper function of the general practitioner leads patients to come to the hospital later and potentially to start oseltamivir longer after onset of symptoms. However, in the study by Muthuri, the median time from start of symptoms to start of antiviral treatment was three days, similar to that time in our complete cohort (3.0 days, IQR 2.0-4.6).

In contrast to patients with uncomplicated influenza virus infection, hospitalized patients have prolonged influenza viral shedding (43-47). Therefore, the time window to start treatment (within 48 hours after symptom onset) seems irrelevant. In our cohort, with 87/125 (70%; 13 missing) of the treated had symptoms for more than two days, treatment with oseltamivir within 48 hours after hospital admission reduced 30-day mortality and the composite endpoint. This illustrates the biological plausibility of oseltamivir treatment effect during a larger time window in patients with prolonged viral replication, i.e., the ones that are hospitalized. This becomes more clear in the patients with chest X-ray-confirmed pneumonia. Although not significant due to the small size of the subgroup, the differences in 30-day mortality and composite endpoint between the treated and untreated groups are more striking than in the overall matched cohort. However, this also indicates that the difference in the matched cohort is not caused by an effect limited to the patients with consolidation. These results provide pragmatic guidance in the decision to start oseltamivir treatment in patients hospitalized with influenza virus infection.

The strength of our study is the multicenter design in a community with a well-developed primary care network. In the Netherlands, most patients with acute respiratory tract infections are treated by their general practitioner. The selection of patients who present to a hospital consists of patients with severe disease and patients who are vulnerable, especially through immunocompromised status. In daily practice, this is the most relevant patient group in which to assess the clinical effect of oseltamivir.

The analyses with both the PSM and IPW are consistent and with these statistical methods we maximally reduced the impact of selection bias. A similar study in 506 influenza patients in South Korea found completely different results (48), but did not use a propensity score model.

Hospital mortality as outcome parameter, used in the meta-analysis from Muthuri (18), has been questioned extensively because of the bias that discharged patients are more likely to be in a better condition than those who could not be discharged (competing risk for death) (14). Our 30-day mortality is, therefore, a more appropriate outcome parameter. Other concerns regarding the Muthuri meta-analysis concerned the potential time-dependent bias (12). In our study, this bias has been reduced by the limited window (48 hours) of adequate treatment and by the time-to-event in the survival analysis of at least three days (12).

Only 176 patients from the complete cohort (n=390) were included in the matched cohort. This is partly due to missing data regarding the CURB-65 score (n=67). This score has not been recorded routinely in the patients´ medical records. Without the availability of this score, patients could not be matched and consequently were not included in the matched cohort. A potential additional weakness is the selection of patients who have been sampled to test for influenza virus infection. In a recent report, test frequency for influenza virus infection is inhomogeneous in various countries. In the Dutch patients in this study, test frequency was, however, high at 72% (33/46) (49). We assume that missing tests were most substantial among the least sick patients (49).

Furthermore, the unmeasured confounders were not considered and we could not rule out the presence of these.

Interestingly, our data show a steady increase in 30-day mortality as the CURB-65 score gets higher. In our study, with 323 laboratory-confirmed hospitalized patients with influenza virus infection for which CURB-65 scores are available, the 30-day mortality rate in the various CURB-65 risk classes corresponds to the risk profile of community-acquired pneumonia (50). In other reports, CURB-65 score predicted 30-day mortality inconsistently (51) or showed higher mortality in each risk class (52, 53).

In conclusion, in our study using propensity score methods, patients with prolonged symptoms, admitted with seasonal influenza virus infection and treated with oseltamivir within 48 hours after hospital admission, had a significantly reduced 30-day mortality and a significantly reduced composite endpoint of 30-day mortality and/or ICU admission >48h after hospital admission. A new cohort of these patients could confirm the benefit of

oseltamivir treatment within 48 hours after hospital admission and could assess the trend in improvement in length of hospital stay and in-hospital mortality.

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SUPPLEMENTARY

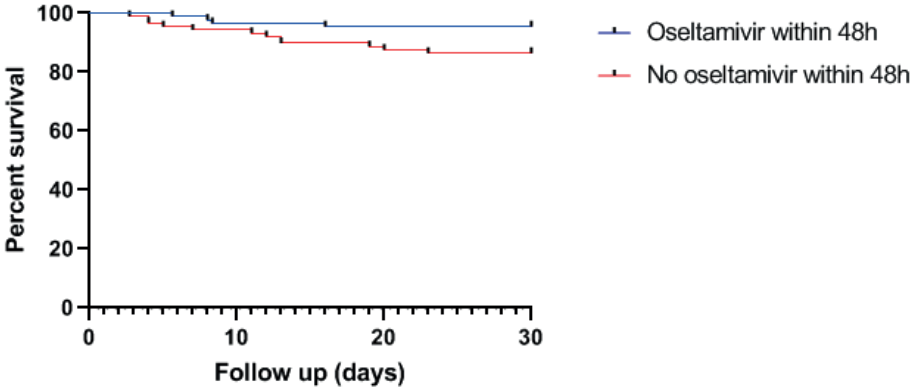


Figure S1. Cumulative 30-day survival in the group of influenza patients treated with oseltamivir within 48 hours after hospital admission versus the group without this treatment

Cumulative 30-day survival in patients treated with oseltamivir within 48 hours after hospital admission (blue) was significantly better than that of patients without oseltamivir treatment within 48 hours after hospital admission (red) (p=0.04).

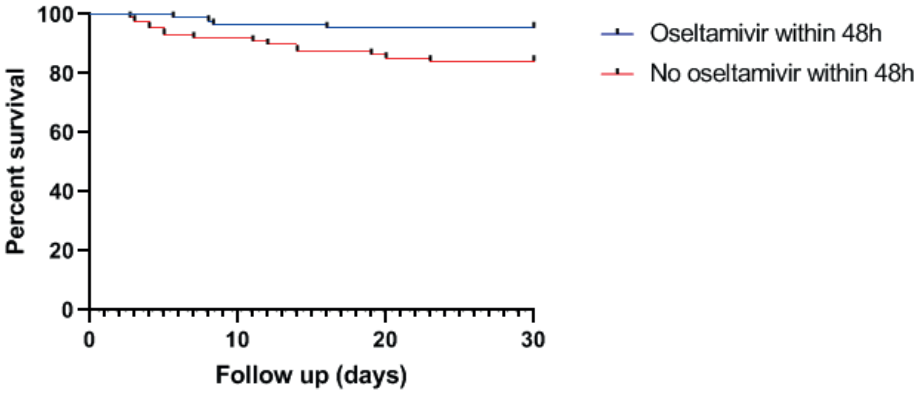
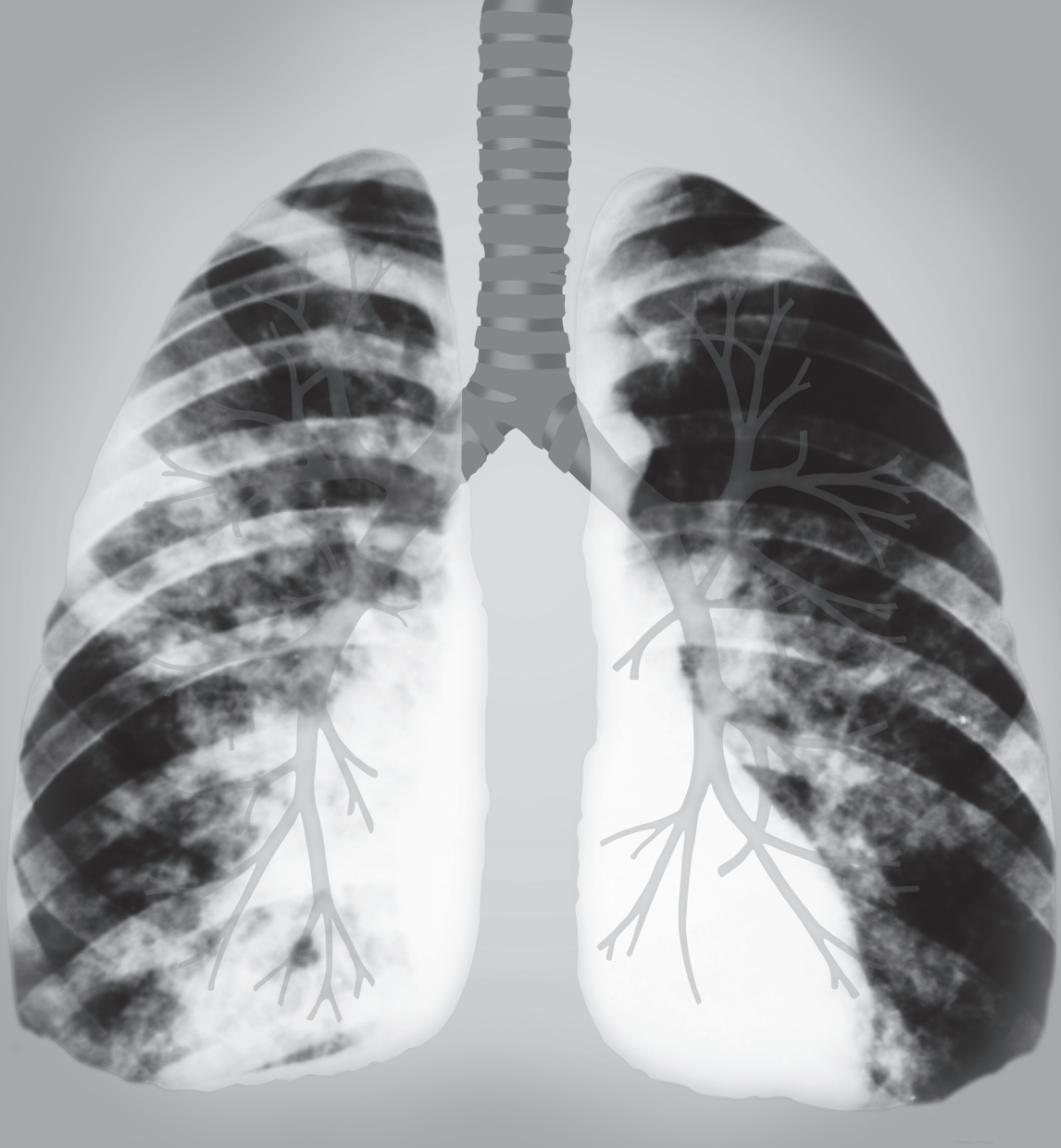


Figure S2. Cumulative 30-day composite endpoint in the group of influenza patients treated with oseltamivir within 48 hours after hospital admission versus the group without this treatment

Cumulative 30-day composite endpoint (ICU admission > 48 hours after hospital admission or 30-day mortality) in patients treated with oseltamivir within 48 hours after hospital admission (blue) was significantly better than that of patients without oseltamivir treatment within 48 hours after hospital admission (red) (p=0.01).



9

Non-lytic antibiotic treatment in community-acquired pneumococcal pneumonia does not attenuate inflammation: the PRISTINE trial.

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ABSTRACT

Background

The inflammatory response in pneumococcal infection is primarily driven by immunoreactive bacterial cell wall components (lipoteichoic acid, LTA). An acute release of these components occurs when pneumococcal infection is treated with β-lactam antibiotics. We hypothesize that non-lytic rifampicin compared to lytic β-lactam antibiotic treatment would attenuate the inflammatory response in patients with pneumococcal pneumonia.

Methods

In the PRISTINE Trial (**P**neumonia treated with **RI**fampicin a**T**tenuates **IN**flammation), a randomized, therapeutic controlled, exploratory study in patients with community-acquired pneumococcal pneumonia, we compare LTA release, inflammatory and clinical response during treatment with both rifampicin and β-lactam compared to treatment with β-lactam antibiotics only (trial number NTR3751).

Results

Forty-one patients with community-acquired pneumonia were included, 17 of them had pneumococcal pneumonia. LTA release, LTA mediated inflammatory response, clinical outcome, inflammatory biomarkers and transcription profiles are not different between treatment groups.

Conclusions

The PRISTINE study demonstrated the feasibility of adding rifampicin to β-lactam antibiotics in the treatment of community-acquired pneumococcal pneumonia but, despite solid *in vitro* and experimental animal research evidence, failed to demonstrate a difference in plasma LTA concentrations, subsequent inflammatory and clinical responses. Most likely, an inhibiting effect of human plasma contributes to the low immune response in these patients. In addition, LTA plasma concentration could be too low to mount a response via TLR2 *in vitro*, but may nonetheless have an effect *in vivo.*

INTRODUCTION

The host inflammatory response in pneumococcal disease contributes significantly to morbidity and mortality.[1] As in other infections with Gram-positive bacteria, the inflammatory response in pneumococcal infection is primarily driven by immunoreactive bacterial cell wall components (lipoteichoic acid) or release of intracellular proteins.[2] Lipoteichoic acid (LTA) is recognized by Toll-like receptor 2 (TLR2), a pattern recognition receptor on macrophages. Binding of LTA to TLR2 induces the release of proinflammatory cytokines (e.g., IL-1, IL-6, TNF) and neutrophil influx.[3,4] Bacterial cell wall components are released when bacteria are killed by autolysis or host immune cells and are important determinants of the severity of inflammation.[5] An acute break down of bacterial cell wall occurs upon exposure to β-lactam antibiotics,[6] the first-line treatment for pneumococcal infections in many guidelines.[7,8]

Reduction of release of bacterial cell wall products may decrease inflammation, reduce tissue damage, and ultimately, reduce morbidity and mortality. Strategies to dampen the host inflammatory response have been studied extensively. Currently, dexamethasone adjunctive treatment in patients with pneumococcal meningitis is used in high-income countries to diminish inflammatory responses and consequently, neurologic sequelae.[9] In community acquired pneumonia, macrolides seem to have an immune modulatory effect by enhancement of the antibacterial effect of neutrophils and by quashing the immune response after bacterial killing.[10] However, in a clinical trial β-lactam monotherapy was non-inferior to macrolide with β-lactam combination therapy.[11]

Another potential approach is to kill the bacteria without immediately lysing them thus preventing release of proinflammatory cell wall products.[12] This would reduce the complete inflammatory trigger by interfering at the beginning of the inflammation cascade.

β-lactam antibiotics disrupt the bacterial cell wall causing lysis of the bacterium and subsequent inflammatory response. A non-lytic antibiotic such as rifampicin causes much less inflammation.[13,14] As an example, *in vitro* studies showed that rifampicin results in less release of LTA and pro-inflammatory compounds from *Streptococcus pneumoniae* than the β-lactam antibiotics ceftriaxone or meropenem, despite similar bacterial killing effects.[14] Furthermore, rifampicin may reduce inflammatory response by downregulating expression of proinflammatory pattern recognition receptors.[15] The killing of *S. pneumoniae* commences instantly after achieving therapeutic drug concentrations. Therefore, rifampicin induced non-lytic killing should start before β-lactam lytic killing.

Although animal models suggest a beneficial effect of rifamycins in the reduction of inflammation during pneumococcal infections,[13] data in humans are not available. Therefore, we hypothesize that non-lytic rifampicin compared to lytic β-lactam antibiotic treatment would attenuate the inflammatory response in patients with pneumococcal pneumonia, shortly after start of treatment.

PATIENTS AND METHODS

The PRISTINE Trial (**P**neumonia treated with **RI**fampicin a**T**tenuates **IN**flammation) is a randomized, therapeutic controlled, exploratory study in patients with community-acquired pneumonia to compare inflammatory responses during treatment with both rifampicin and β-lactam compared to treatment with β-lactam antibiotics only. The study was conducted at the Leiden University Medical Center (LUMC), a tertiary university hospital in the Netherlands. The study was approved by the LUMC Medical Ethical Committee and all patients provided written informed consent. This study was performed in compliance with the Declaration of Helsinki. The trial is registered in the Dutch trial registry, number NTR3751 (EudraCT number 2012-003067-22).

Patients

Patients were recruited at the emergency department. Inclusion criteria were:

- ≥ 18 years of age, and
- hospital admission for community-acquired pneumonia, and
- moderate to severe disease as defined by a CURB-65 score ≥2,[16] or
- one or more of the risk factors for having pneumococcal pneumonia, i.e. pleuritic chest pain, acute onset of symptoms, cardiovascular disease, leukocyte count > 15 x10e9/l, and an alveolar pattern (lobar, segmental or sub-segmental infiltrate) on chest X-ray.[17]

Exclusion criteria were: allergy to rifampicin, rifampicin-induced haemolytic anaemia or thrombopenia in medical history, liver failure, use of voriconazole or protease inhibitors, and pregnancy or breastfeeding.

Treatment

All patients were treated according to the current guidelines in the Netherlands, including at least a β-lactam antibiotic. Since resistance of *S. pneumoniae* to penicillin is extremely rare in the Netherlands,[18] empirical therapy is usually initiated with benzylpenicillin.

Patients were randomized (2:1) between the intervention group and the control group, using a prepared single randomization list. This list is generated and study patients are

assigned by independent persons. Since blinding of rifampicin treatment (with orange secretions) is impossible, this study was open label. The intervention group was treated with rifampicin 600 mg q12h intravenously for 48 hours, in combination with a β-lactam antibiotic. Rifampicin was to be given before the β-lactam antibiotic. β-lactam antibiotic treatment had to be added to the intervention treatment since this is prescribed in current guidelines, and because rifampicin resistant mutants readily appear with rifampicin monotherapy.[19] The control group was treated with a β-lactam antibiotic (without rifampicin).

In severe community-acquired pneumonia (CURB-65 score >2) or in patients with risk factors for Legionella pneumonia, ciprofloxacin is added to the empirical treatment (of patients in either group) to cover *Legionella* infection. This decision and total treatment duration was assigned by the treating physician, according to the Dutch guideline.[20]

Clinical assessment and microbiology

The clinical response was assessed by the research team using the time to clinical stability score and by monitoring the time to defervescence. Thirty and 90 days after start of therapy clinical recovery was assessed by the clinical research team.

Time to clinical stability is defined as the days from admission until: the temperature is ≤ 37.8°C, heart rate is ≤ 100 beats per minute, respiratory rate is ≤ 24 per minute, oxygen saturation ≥ 90%, systolic blood pressure is ≥90 mmHg, mental status is normal, and there is ability for oral intake.[21] If these criteria are not all met on the day of discharge, the day after discharge is defined as the day of clinical stability. Time to defervescence was defined by body temperature < 37.5°C during two consecutive measurements at least eight hours apart. The prescription of antipyretics was not part of the study protocol.

The decision to discharge a patient was left to the attending physician. Criteria to discharge were: recovery of the patient up to the level of being able to take care of themselves, and ability to complete at minimum a five day course of oral antibiotics.

Sputum culture, blood culture, nasopharyngeal swab for viral PCR, BinaxNow pneumococcal urinary antigen test, and a urinary inhibition multiplex immunoassay (IMIA) to detect and serotype pneumococci were performed to identify the causative agents.[22,23] Pneumococcal infection was defined as positive sputum or blood culture with *S. pneumoniae* or a positive BinaxNow or IMIA at inclusion.

At inclusion, at 2, 4, 8, 16, 24, and 48 hours and at 30 days after inclusion a blood sample was taken to determine the TLR2 response and to assay biomarkers. At inclusion, 24

hours, and 30 days after inclusion, blood was collected in PAXgene RNA tubes for multiplex ligation-dependent probe amplification (MLPA) assessment of inflammatory response.[24]

Outcomes

In this exploratory study, primary outcome was the feasibility of adding rifampicin to β-lactam antibiotics in the treatment of community-acquired pneumococcal pneumonia and the difference in LTA release between patients treated in the intervention group versus the ones in the control group. Secondary outcome variables are LTA mediated inflammatory response, clinical response, MLPA results and inflammatory biomarkers. Laboratory procedures to determine LTA response and LTA mediated inflammatory response are described in the supplementary data.

Clinical outcome parameters were: time to clinical stability, time to defervescence, in-hospital mortality and 30-day and 90-day mortality, length of stay in hospital and ICU admission.

Biomarker assessment

The biomarkers C-reactive protein (CRP), procalcitonin (PCT), and midregional pro-adrenomedullin (MR-proADM) were used to define inflammatory responses.[25]

CRP is measured via turbidimetric reaction with antibody-antigen complex (Roche®, Mannheim, Germany, catalogue number 12000951/12000953/04956923190). PCT and MR-proADM were determined with immunofluorescence with Time Resolved Amplified Cryptate Emission (TRACE) technology (Brahms Kryptor®, Hennigsdorf, Germany, catalogue number 82591/82592/825050 for PCT and 82991/82992/829050 for MR-proADM).

In case patients were discharged, blood sampling and biomarker assessment stopped. With clinical recovery we assumed biomarker normalization. To compensate for the missing values, the known half-lives of the biomarkers were applied (with normal value as minimum) to the last measured samples. For CRP, half-life is 19 hours (normal value 1 mg/L), for PCT half-life is 30 hours (normal value 0.15 ng/mL) and for MR-proADM half-life is 4 hours (normal value 0.36 nmol/L).

Difference in biomarkers is defined as a change of value in the first and second 24 hours after the start of treatment.

Multiplex ligation-dependent probe amplification (MLPA)

The dual-color reverse-transcriptase multiplex ligation-dependent probe amplification (dcRT–MLPA) permits accurate RNA expression profiling of 80 selected transcripts to iden-

tify biomarker signatures for host inflammatory responses to infection.[24] A Partial Least-Squares Discriminant Analysis (PLS-DA) was performed to identify components which can discriminate between groups at time point 24 hours. Variable Importance in Projection (VIP) scores is a measure of a variable's importance in the PLS-DA model. The marker with the highest VIP score is the best discriminator.

Statistical analysis

This study was an exploratory study determining the feasibility of adding rifampicin to the standard antibiotic treatment of patients with acute community-acquired pneumonia. As such, the analysis was limited to descriptive statistics and no statistical significance between groups was sought after, and by consequence, no formal power calculation was done.

Continuous variables were summarized as either means with standard deviations or medians with interquartile ranges. T-test or Mann Whitney U test was used as appropriate. Categorical variables were depicted as numbers with percentages, and Chi-squared test or Fisher's exact test was used for hypothesis testing.

To model the effect of LTA release and biomarkers over time in the different treatment groups and to assess their effect, we used a linear mixed model (LMM). We used results from the first 48 hours of sampling since this is the time window of interest.

Following our hypothesis, LTA release and biomarker response after the start of treatment will not have a linear relation. Therefore, we used polynomial splines to model the trend of LTA release and biomarker response. Changes in biomarkers were assessed by comparing change within the first and second 24 hours after treatment with a T-test. Statistical analyses were performed using SPSS (IBM Software) version 23.

RESULTS

Between January 2013 and May 2014, a total of 41 patients with community-acquired pneumonia were included. After the empirical start of antibiotic treatment in all study patients, 17 of them were found to have pneumococcal pneumonia. Of these 17 patients,13 were in the intervention group, while four were in the control group. In these 13 patients, ten completed the 48 hours (four dosages) of rifampicin treatment, two received three dosages and one received two dosages. The median number of infected lobes was one.

The median age of the total cohort was 69 years, 58% was male, and median CURB-65 score was 2 (**Table 1**). Twenty-six patients received ciprofloxacin as empirical treatment on top of a β-lactam antibiotic with or without rifampicin. Since groups are small, some differences exist between the treatment groups. Baseline characteristics are outlined in **Table 1** (and **Table S1**).

Table 1. Baseline characteristics

	Complete cohort n=41	Rifampicin + β-lactam treatment (*S. pneumoniae*) n=13	β-lactam treatment (*S. pneumoniae*) n=4	P value	Rifampicin + β-lactam treatment (all patients) n=28	β-lactam treatment (all patients) n=13	P value
Medical history							
Median age (IQR)	69 (57-75)	69 (58-76)	48 (42-63)	0.03	71 (61-76)	67 (50-71)	0.13
Female gender	17 (42%)	3 (23%)	4 (100%)	0.01	9 (32%)	8 (62%)	0.08
Cardiovascular disease	11 (27%)	4 (31%)	0 (0%)	0.52	8 (29%)	3 (23%)	0.71
Immunocompromised	12 (29%)	3 (23%)	0 (0%)	0.54	8 (29%)	4 (31%)	0.89
Pulmonary comorbidity	18 (44%)	5 (39%)	1 (25%)	0.62	10 (36%)	8 (62%)	0.12
Influenza vaccination	25 (61%)	7 (54%)	1 (25%)	0.31	16 (57%)	9 (69%)	0.46
Objective parameters at presentation							
Median CURB-65 score (IQR)	2 (1-3)	2 (2-3)	2 (1-2)	0.63	2 (2)	2 (3)	0.68
Pneumonia on chest X ray or confirmed by physical examination	39 (95%)	11 (85%)	4 (100%)	1.00	27 (96%)	13 (100%)	1.00
Causative agent*							
S. pneumoniae	17	13	4	-	13	4	0.34
H. influenza	1	0	0	-	0	1	0.32
S. aureus	1	0	0	-	1	0	0.32
Influenza A	3	1	0	1.00	2	1	1.00
RSV	1	0	0	-	0	1	0.32
Metapneumovirus	2	0	1	0.24	1	1	0.54
Human rhinovirus	5	3	0	0.54	4	1	0.55
Human Coronavirus	1	1	0	1.00	1	0	0.32
Parainfluenza virus 1	2	0	1	0.24	0	2	0.10
Parainfluenza virus 2	1	1	0	1.00	1	0	0.32
No pathogen detected	16	0	0	-	12	4	0.46
Bacterial with viral coinfection	6	4	1	0.83	5	1	0.39
Empirical antibiotic treatment							
Benzylpenicillin/cefuroxime	37/4	12/1	2/2	0.12	27/1	10/3	0.16
Ciprofloxacin/no ciprofloxacin	26/15	7/6	3/1	0.45	16/12	10/3	0.46

IQR, Interquartile range. *in some patients more than one causative agent was detected.

The diagnosis of pneumococcal pneumonia in the 17 patients was based on positive blood cultures in five; positive sputum cultures in six; positive BinaxNOW antigen test in nine; and a positive IMIA test in ten patients. Various *S. pneumoniae* serotypes were detected. Interestingly, two patients had an infection with more than one serotype (**Table S2**).

LTA release and LTA mediated inflammatory response

In short, LTA release could not be demonstrated with two commercial ELISA tests. Of two study patients with proven pneumococcal pneumonia with pneumococcal bacteremia, no LTA mediated inflammatory response via TLR2 was detected.

Results of the laboratory work on LTA response and LTA mediated inflammatory response are described in the supplementary data on the laboratory work.

Clinical outcome is not different between treatment groups

Time to clinical stability and time to defervescence in patients with pneumococcal pneumonia did not differ significantly between treatment groups (**Figure 1A** and **1B**). None of the patients with pneumococcal pneumonia died in the hospital or within 30 days, while 90-day overall mortality was 6%. The median length of hospital stay was four days, and there were no significant differences in ICU admissions, adverse events and recovery at 30 and 90 days in the pneumococcal group and the complete cohort. Clinical outcome parameters are described in **Table 2** and **3**.

Table 2. Clinical outcome parameters for patients with microbiologically proven pneumococcal pneumonia

	All patients n=17	Rifampicin + β-lactam treatment n=13	β-lactam treatment n=4	P value
Median length of hospital stay (IQR)	4 (3-9)	5 (4-9)	4 (2-8)	0.36
ICU admission	4	3	1	0.94
Median length of ICU stay (IQR)	4 (2-6)	3 (2-5)	4	0.66
Mechanical ventilation	1	1	0	0.57
Multiple organ failure	5	4	1	0.83
In hospital mortality	0	0	0	-
Day 30 mortality	0	0	0	-
Day 30 recovery				0.28
Complete	4	2	2	
Partial	10	8	2	
No	3	3	0	
Day 90 mortality	1	1	0	0.57
Day 90 complete recovery	11	8	3	0.53

IQR, interquartile range

Table 3. Clinical outcome parameters for all patients

	Complete cohort n=41	Rifampicin + β-lactam treatment n=28	β-lactam treatment n=13	P value
Median length of hospital stay (IQR)	4 (3-8)	4 (3-8)	4 (2-7)	0.46
ICU admission	7	4	3	0.49
Median length of ICU stay (IQR)	3 (2-7)	5 (2-10)	3 (3-4)	0.59
Mechanical ventilation	2	2	0	0.15
Multiple organ failure	6	4	2	0.21
In hospital mortality	1	1	0	0.49
Day 30 mortality	1	1	0	0.49
Day 30 complete recovery	13	10	3	0.47
Day 90 mortality	2	2	0	0.32
Day 90 complete recovery	25	18	7	0.19

IQR, interquartile range

Biomarker and transcription profiles cannot distinguish treatment groups

The biomarkers CRP, PCT, and MR-proADM were measured at various time points before and after the start of treatment (**Figure 2**). Before the start of the treatment, median CRP and MR-proADM were slightly higher in the rifampicin intervention group, whereas median PCT was slightly higher in the group treated without rifampicin. After the start of treatment, biomarker levels were not significantly different between the groups in the linear mixed model (**Figure 2A-2F and Table 4**).

CRP values showed a small increase within the first 24 hours after the start of treatment in both treatment groups (**Figure 2A, 2D**). In patients with pneumococcal pneumonia, all biomarkers show a steady decline between 24 and 48 hours after the start of treatment (**Figure 2A-2C**). The change in the concentrations of the biomarkers were not different between groups in the first and second 24 hours after the start of treatment (**Table 5** and **Table S3**). In four patients, blood samples (n=5) were limited to those taken during hospitalization.

At inclusion, and 24 hours and 30 days after inclusion, RNA expression profiling of 80 transcripts was performed. The MLPA heat map shows colored quantities of the various transcripts in **Figure 3**. Patients with similar transcript profiles are plotted adjacent. Although nine patients with pneumococcal pneumonia with rifampicin clustered together, the gene expression data do not reveal clear patterns associated with treatment or disease status.

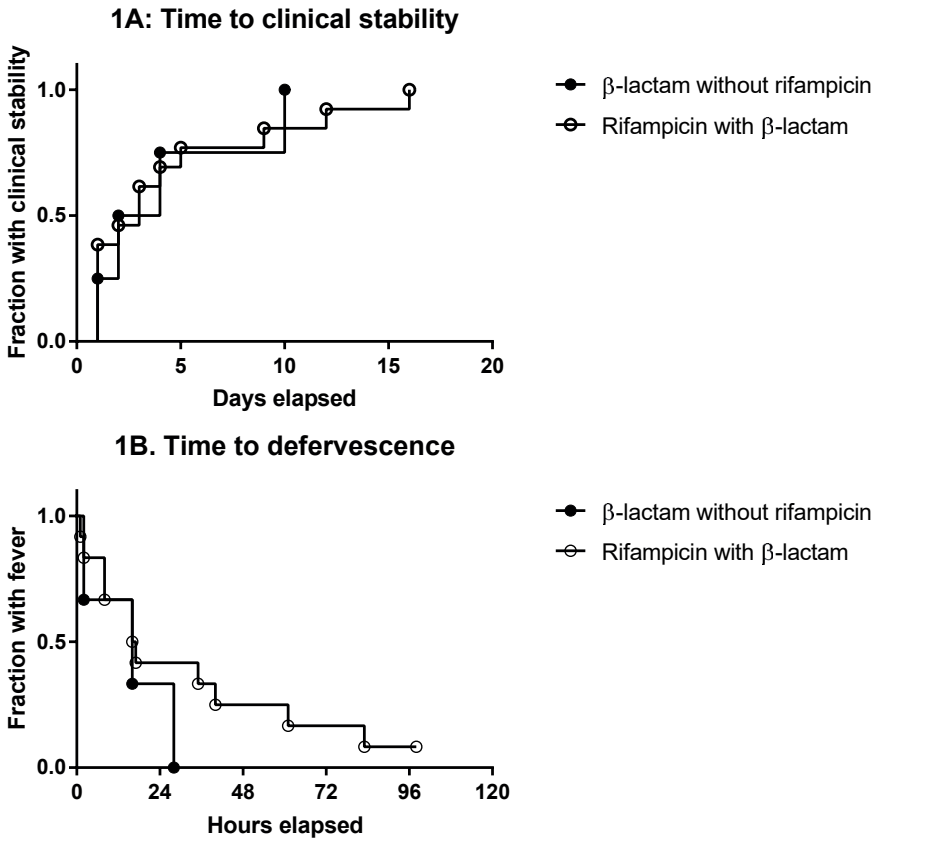

Figure 1. Time to clinical stability and to defervescence in patients with pneumococcal pneumonia.
Kaplan Meier curves for time to clinical stability and time to defervescence in patients with pneumococcal pneumonia treated with rifampicin versus patients treated without rifampicin.

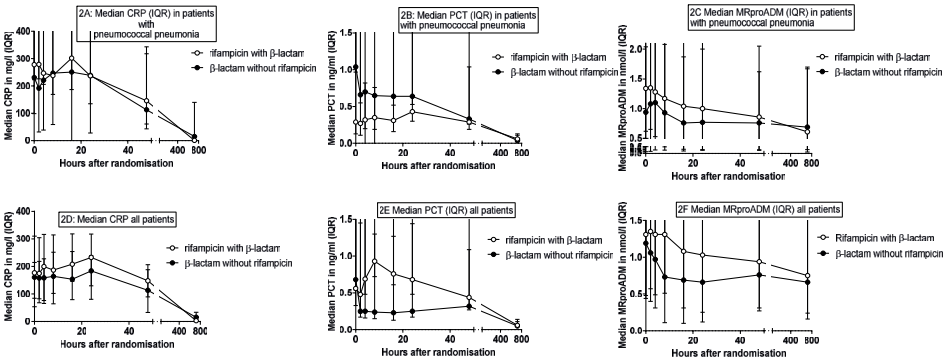

Figure 2. Biomarkers in patients´ plasma before, during and after treatment
The inflammation biomarkers C-reactive protein (CRP), procalcitonin (PCT) and midregional pro-adrenomedullin (MR-proADM) were analysed in plasma. Median biomarker with interquartile range (IQR) over time for patients with pneumococcal pneumonia (2A-2C) and for all patients (2D-2F).

Table 4. Linear mixed model: mean response over time (0-48 hours) in patients with pneumococcal pneumonia treated with rifampicin compared to the control group without rifampicin

Biomarker	Estimate (95% CI)	P value
CRP	37.7 (-32.9 - 108.2)	0.27
PCT	0.00 (-0.07 - 0.07)	0.97
MR-proADM	-0.23 (-0.54 - 0.07)	0.12

CRP, C-reactive protein; PCT, procalcitonin; MR-proADM, midregional pro-adrenomedullin. The group without rifampicin is the baseline comparator.

Table 5. Change in biomarkers over time in patients with pneumococcal pneumonia

	Rifampicin group (n=13)	Group without rifampicin (n=4)	Mean difference (95% CI)	P value*
In the first 24 hours after start of treatment				
ΔCRP	13.7 mg/L	32.8 mg/L	-19.0 (-113.6-75.5)	0.67
ΔPCT	3.70 ng/mL	0.03 ng/mL	3.67 (-12vw.18-19.52)	0.63
ΔMR-proADM	-0.20 nmol/L	-0.21 nmol/L	-0.00 (-0.34-0.35)	0.98
In the second 24 hours after start of treatment				
ΔCRP	-79.3 mg/L	-112.6 mg/L	33.3 (-51.4-117.9)	0.42
ΔPCT	-1.89 ng/mL	-0.29 ng/mL	-1.60 (-6.19-2.99)	0.47
ΔMR-proADM	-0.28 nmol/L	-0.13 nmol/L	-0.15 (-0.69-0.39)	0.57

CRP, C-reactive protein; PCT, procalcitonin; MR-proADM, midregional pro-adrenomedullin. * T-test
Change in concentrations of CRP, PCT and MR-proADM within the intervention (rifampicin) group and within the control group (without rifampicin) in the first 24 hours after start of treatment, i.e. from start of treatment until 24 hours thereafter; and in the second 24 hours after start of treatment, i.e. from 24 to 48 hours after start of treatment. Mean difference between intervention and control group and the P value are shown in separate columns.

To identify transcripts with the highest discriminatory power between pneumococcal versus other infections PLS-DA were run and VIP scores were calculated. Transcripts with the five highest VIP scores are shown in **Figure S1A-E**. Only Chemokine (C-C motif) ligand 5 (*CCL5*) was statistically significant lower at 24 hours after the start of treatment in patients with pneumococcal pneumonia versus patients with non-pneumococcal pneumonia. Treatment with or without rifampicin did not significantly affect the results.

DISCUSSION

The PRISTINE study is the first exploratory clinical trial in humans to determine the feasibility of adding rifampicin to the standard treatment with β-lactams of community-acquired pneumococcal pneumonia. The rifampicin is added to reduce the release of bacterial compounds within the first hours of therapy and thereby attenuate the inflammatory re-

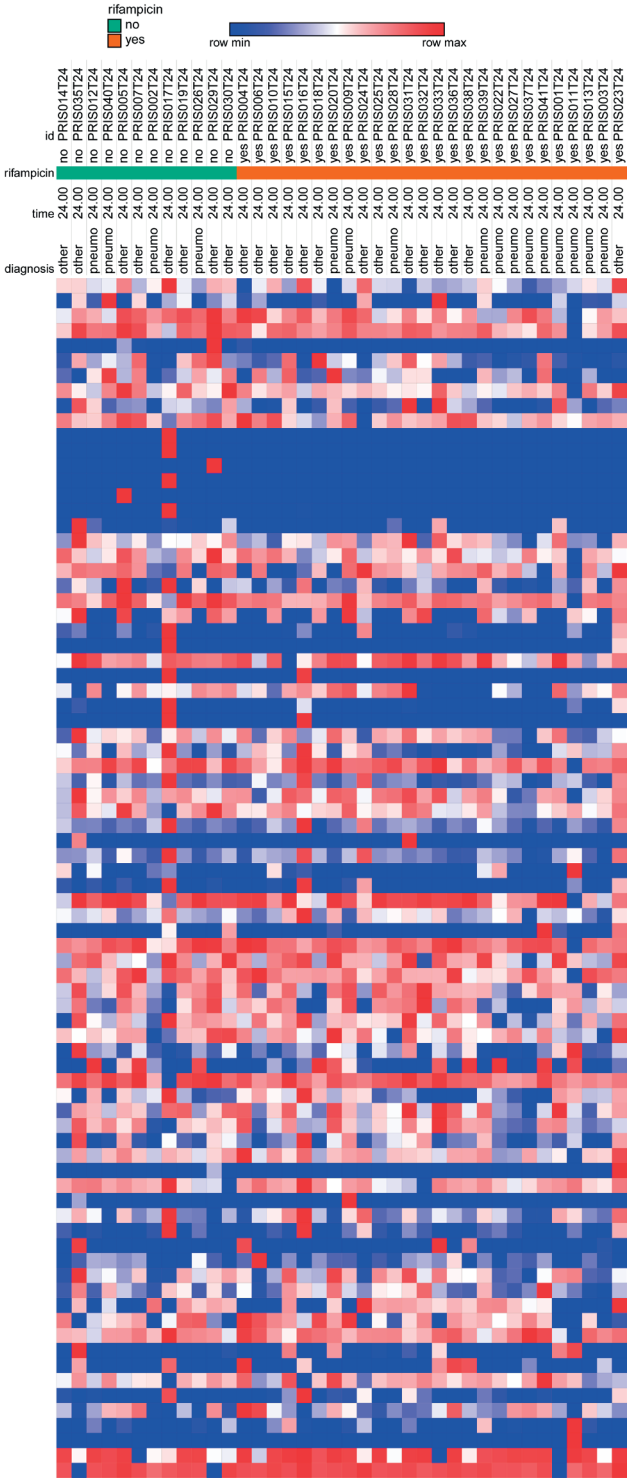

Figure 3. Heatmap of RNA expression results measured by MLPA in all patients

Heatmap at T=24 hours with rifampicin treated patients (brown) versus patients treated without rifampicin (green). Clustering is poor for all genes investigated irrespective of clinical diagnosis.

sponse. In this initial small group, the additional non-lytic rifampicin antibiotic versus lytic β-lactam antibiotic only treatment for pneumococcal pneumonia did not reveal differences in the blood concentrations of various inflammatory biomarkers nor in the clinical response to treatment.

Strengths of our study are the high percentage of pneumococcal infections included, the frequent sequential measurement of a spectrum of biomarkers in the first 48 hours to assess our hypothesis, and the complete biomarker profile to evaluate the specific inflammatory responses. Initially, we included only patients with high severity score (CURB-65≥2) as the percentage of pneumococcal infection is highest in this group, and the high severity would best contrast the possible effects. After inclusion of the eighth study patient, we extended our inclusion criteria to patients having a specific risk factor for pneumococcal pneumonia to speed up inclusions.[17] We applied extensive testing for pneumococcal infection, to ensure the identification of all patients with pneumococcal pneumonia.[23] We were able to confirm a pneumococcal infection in 41% of patients. This percentage is higher than in comparable hospital and intensive care studies with community-acquired pneumonia.[11,26,27]

In vitro studies and animal models demonstrated differences in LTA release and inflammatory response within hours in lytic versus non-lytic antibiotic treatment of *S. pneumoniae*.[12,28,29] Although extensive sampling is a challenge in human trials, it is essential for testing our hypothesis. Therefore, the large number of sequential samples we collected is an important strength of our study. With the extensive sampling, we detected that CCL5 is expressed significantly differently between pneumococcal pneumonia versus non-pneumococcal pneumonia 24 hours after start of treatment. CCL5 is known to be upregulated in pneumococcal infection and to be an essential chemokine in pneumococcal adaptive immunity.[30] Our finding needs to be validated in a larger cohort of pneumonia patients.

A weakness of our pilot trial is the small sample size. This is however in line with the exploratory character of our study. As we anticipated that the LTA and biomarker responses induced by β-lactam treatment would be in a broad range, we included more patients with rifampicin added to β-lactam treatment than β-lactam treatment only, and randomized at a 2:1 ratio. With only four patients with pneumococcal pneumonia treated with β-lactam therapy only, this assumption was imperfect and the small group hindered comparisons. For example, in the analyses of biomarkers for inflammation, at start of treatment, PCT value seems higher in the β-lactam group while CRP and MR-proADM show higher values in the rifampicin group. Since only three samples (one sample was missing) were available in the β-lactam group, the interpretation of these findings is difficult.

We could not detect LTA in plasma nor its direct inflammatory response via TLR2. LTA cell wall components should bind TLR2 and induce the release of a broad range of proinflammatory cytokines leading to neutrophil-mediated lung damage and, with that, morbidity and mortality.[31,32] Most likely, an inhibiting effect of human plasma contributes to the low immune response in these patients. In addition, with a median number of only one infected lung lobe, representing relatively limited pneumococcal load, LTA plasma concentration could be too low to mount a response via TLR2 *in vitro* (see supplementary data), but may nonetheless have an effect *in vivo.*

LTA release may also have been delayed by quinolone treatment.[14,29] Ciprofloxacin was frequently co-administered in our cohort. A delayed LTA release may have decreased the potential difference in inflammatory responses between the two treatment groups.

Finally, another reason for the absence of detectable LTA in our samples could be the serotypes causing pneumococcal pneumonia. Different pneumococcal isolates have different lytic effects.[33] In an experimental meningitis model in rabbits, serotype 23F caused more LTA release and inflammation than pneumococcal serotype 3.[34] In our study, only one patient had a pneumococcal pneumonia with serotype 23F versus four patients with serotype 3.

In contrast to LTA in plasma, LTA can be detected at the site of infection in humans (see supplementary data). For example, in liquor of patients with pneumococcal meningitis, LTA is detectable until 15 days after the start of treatment.[35] Unfortunately, it is not possible to puncture the infected lung lobe for repeated measurements in critically ill human patients. Therefore, human studies to determine the LTA load in the lung during pneumonia have not been done.

Previous *in vitro* and animal studies showed vast differences in LTA release and inflammatory response between lytic versus non-lytic antibiotic treatment. The potential clinical benefit of decreased LTA release and inflammatory response in patients with pneumococcal pneumonia might be substantial. Restrepo *et al.* demonstrated that patients with community-acquired pneumonia who were transferred to the ICU immediately from the emergency department were better off than patients who were initially treated on wards and thereafter transferred to ICU.[36] This secondary deterioration could be caused by inflammation due to LTA release after the start of treatment.

A large randomized trial of patients with Gram-positive *Staphylococcus aureus* bacteraemia showed no adjunctive clinical benefit of rifampicin over standard (most often

flucloxacillin) antibiotic treatment.[37] Long-term endpoints in that trial were used, making a comparison with our short-term outcome measures difficult.

Strategies to dampen inflammatory response in pneumonia have so far primarily focused on corticosteroids. Corticosteroid therapy demonstrated shorter time to clinical stability and limited shortening of hospital stay in patients with non-severe community-acquired pneumonia. Some studies in adults with severe disease, show a reduction in mortality. The quality of these studies is moderate. In all studies, corticosteroid therapy increased the risk of hyperglycemia.[38] Therefore, corticosteroids are not included in current treatment guidelines.[7,8]

Alternative therapeutic options should be explored to attenuate the inflammation.

The effects and benefits of non-lytic antibiotics for the treatment of pneumococcal infections may be easier to detect and prove in pneumococcal meningitis patients. In this group of patients with high morbidity, long-term sequelae, and substantial mortality strategies to improve outcomes are urgently needed.[39] Moreover, the clinical results of our study could have been blurred by the use of antipyretics.

Higher LTA concentration in liquor in human patients with pneumococcal meningitis is associated with worse outcome.[40] In addition, in rabbits with pneumococcal meningitis, rifampicin reduced LTA release and inflammatory response, and improved survival substantially.[13] Therefore, clinical trials with non-lytic antibiotics in pneumococcal meningitis should be developed. Rifampicin would be the antibiotic of choice since it is most effective in killing *S. pneumoniae* while causing the least release of LTA per killed bacterial cell.[41]

Unfortunately, we could not compare monotherapy of a non-lytic (rifampicin) antibiotic versus monotherapy of a lytic, β-lactam, antibiotic. This would be a highly relevant, but different research question. Reasons for this are that the current Dutch guidelines for community-acquired pneumonia recommend β-lactam antibiotic (e.g., benzylpenicillin) treatment and the fact that rifampicin monotherapy may induce resistance during treatment. Therefore, it would have been unethical to withhold this first-line treatment to patients with community acquired pneumonia. A significant difference in LTA release has been demonstrated in a rabbit model of *S. pneumoniae* meningitis, when comparing β-lactam monotherapy with rifampicin followed by β-lactam antibiotic therapy six hours later.[42] In the rifampicin treatment group in our study, rifampicin was frequently (56%) given before β-lactam treatment, but with a median time frame of 5 minutes only (interquartile range –10 minutes to 60 minutes). Therefore, the antimicrobial killing of *S. pneumonia* in both groups might be primarily caused by the β-lactam (lytic) killing effect.

In conclusion, the PRISTINE exploratory study demonstrated the feasibility of adding rifampicin to β-lactam antibiotics in the treatment of community-acquired pneumococcal pneumonia but, despite solid *in vitro* and experimental animal research evidence, failed to demonstrate a difference in LTA and subsequent inflammatory response. Further studies in selected groups of patients, such as those with pneumococcal meningitis, will be necessary to confirm the hypothesis that non-lytic antibiotic treatment attenuates inflammatory response and improves clinical outcome.

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SUPPLEMENTARY DATA

- Part 1: Additional tables and figure (Table S1-S3 and Figure S1)
- Part 2: Laboratory work PRISTINE

Table S1. Additional baseline characteristics

	Complete cohort n=41	Rifampicin + betalactam treatment (pneumococcal pneumonia) n=13	Betalactam treatment (pneumococcal pneumonia) n=4	P value	Rifampicin + betalactam treatment (all patients) n=28	Beta-lactam treatment (all patients) n=13	P value
Medical history							
Hospital admission in the previous year	13 (32%)	4 (31%)	0 (0%)	0.52	10 (36%)	3 (23%)	0.42
Antibiotic use in the previous 3 months	17 (42%)	4 (31%)	3 (75%)	0.12	10 (36%)	7 (54%)	0.27
Help needed with activities of daily living	3 (7%)	1 (7%)	0 (0%)	1.00	2 (7%)	1 (8%)	1.00
Current smoker	11 (27%)	5 (38%)	2 (50%)	0.68	7 (25%)	4 (31%)	0.70
Smoking history	34 (83%)	12 (92%)	3 (75%)	0.43	23 (82%)	11 (84%)	0.85
Median number of pack years (IQR)	20 (6-45)	20 (9-43)	31 (4-50)	1.00	21 (7-49)	20 (4-43)	0.75
Travelled abroad in previous 3 months	12 (29%)	2 (15%)	1 (25%)	1.00	8 (29%)	4 (31%)	0.89
Symptoms at presentation							
Symptoms < 1 week	35 (85%)	11 (85%)	3 (75%)	1.00	24 (86%)	11 (85%)	0.93
Acute onset of symptoms	15 (37%)	6 (46%)	0 (0%)	0.09	11 (39%)	4 (31%)	0.60
Throat pain	12 (29%)	3 (23%)	2 (50%)	0.30	7 (25%)	5 (38%)	0.38
Runny nose	16 (39%)	4 (31%)	3 (75%)	0.12	9 (32%)	7 (54%)	0.19
Cough	35 (85%)	13 (100%)	4 (100%)	-	25 (89%)	10 (77%)	0.30
Sputum production	26 (63%)	10 (77%)	3 (75%)	1.00	17 (61%	9 (69%)	0.60
Dyspnea	33 (80%)	11 (85%)	4 (100%)	1.00	22 (79%)	11 (85%)	0.65
Pleuritic chest pain	16 (39%)	7 (54%)	2 (50%)	0.89	12 (43%)	4 (31%)	0.46
Fever	37 (90%)	12 (92%)	3 (100%)	0.43	26 (93%)	11 (85%)	0.41
Myalgia	12 (29%)	5 (38%)	2 (50%)	0.68	7 (26%)	5 (39%)	0.42
Headache	17 (42%)	8 (62%)	3 (75%)	0.62	13 (46%)	4 (31%)	0.34
Joint pain	10 (24%)	3 (23%)	1 (25%)	0.94	8 (29%)	2 (15%)	0.36
Objective parameters at presentation							
Leukocyte count > 15 x10e9/l	14 (34%)	6 (46%)	2 (50%)	0.89	9 (32%)	5 (39%)	0.69
On chest X-ray: an alveolar pattern (lobar, segmental or sub-segmental infiltrate)	25 (61%)	8 (62%)	4 (100%)	0.14	18 (64%)	7 (54%)	0.52
Median number of lobes infected (IQR)	1 (1-2)	1 (1-2)	1 (1-2.5)	0.47	1 (1-2)	1 (1-1.5)	0.56

Table S2. Pneumococcal serotypes

Pneumococcal serotype	Number of cases
1	2*
3	4*
8	3
11A	1
18C	1*
20	1
23F	1*

* One patient with two serotypes; one patient with three serotypes detected.
These findings are ambiguous and could be caused by infection with multiple pneumococcal serotypes, asymptomatic carriage or previous infection with other serotypes than the one causing the actual infection or false positive test result.

Table S3. Difference in biomarkers in complete cohort (n=41)

Biomarker per time frame	Rifampicin group (n=28)	Group without rifampicin (n=13)	Mean difference (95% CI)	P value*
In the first 24 hours				
CRP	16.8 mg/L	41.8 mg/L	-25.0 (-84.3 - 34.2)	0.40
PCT	2.0 ng/mL	1.4 ng/mL	0.65 (-5.4 - 6.7)	0.83
MR-proADM	-0.08 nmol/L	-0.07 nmol/L	-0.01 (-0.25 - 0.22)	0.90
In the second 24 hours				
CRP	-65.0 mg/L	-50.5 mg/L	-14.5 (-61.0 - 32.0)	0.53
PCT	-1.21 ng/mL	-0.82 ng/mL	-0.40 (-2.44 - 1.65)	0.70
MR-proADM	-0.26 nmol/L	-0.05 nmol/L	-0.21 (-0.50 - 0.07)	0.14

* T-test

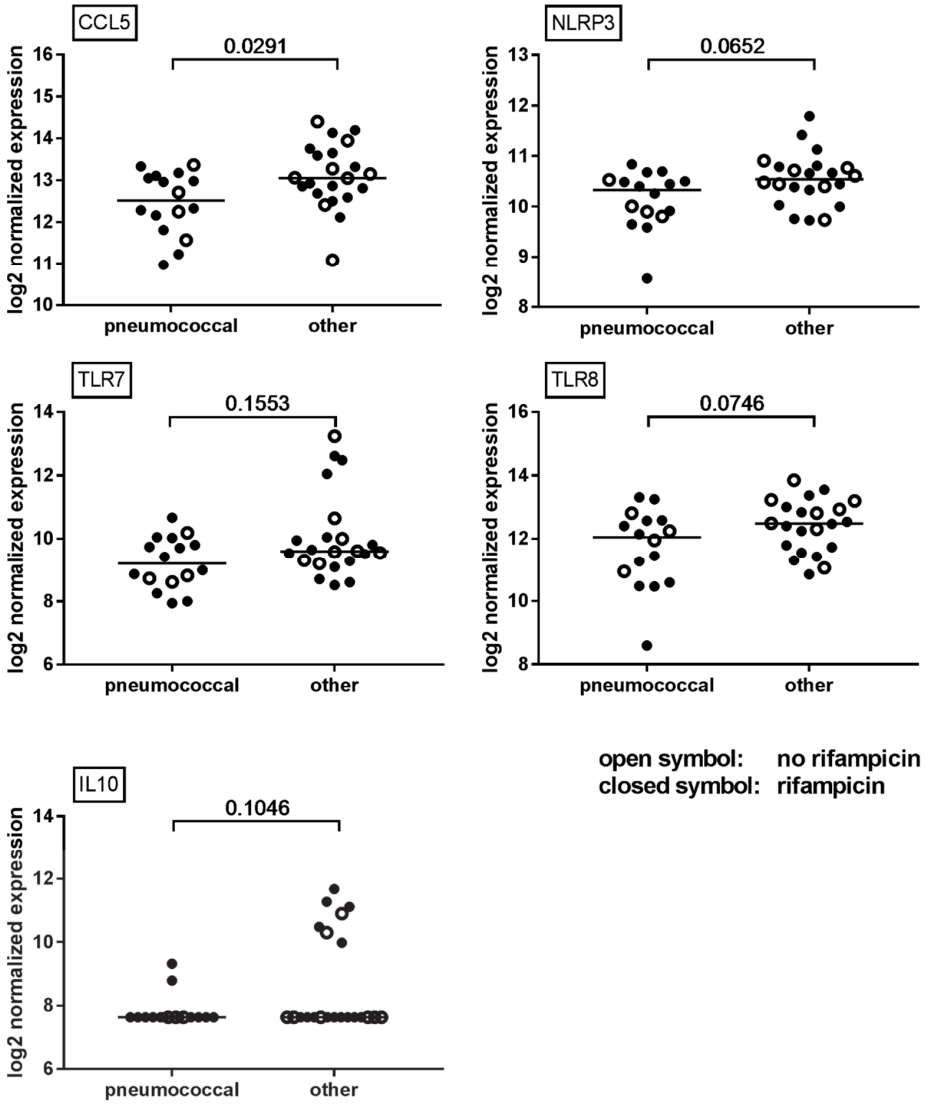

Figure S1. Transcripts with the top 5 VIP scores in the first 24 hours

Transcripts with the five highest VIP scores after 24 hours of treatment to distinguish between pneumococcal pneumonia and non-pneumococcal pneumonia (other). *CCL5* is the only transcript that is significantly different between pneumococcal infection and non-pneumococcal infection 24 hours after the start of treatment.

Laboratory work PRISTINE

During inclusion of study patients in the PRISTINE study, laboratory tests were conducted to detect lipoteichoic acid (LTA) in serum and to test TLR2 transfected Human Embryonic Kidney (HEK) 293 cells (Invivogen®, the Netherlands, catalogue number 293-htlr2cd14) for the ability to produce IL-8 after trigger by pneumococcal cell wall components (i.e. LTA), as measure of inflammatory potential. In humans, lipoteichoic acid (LTA) is recognized by Toll-like receptor 2 (TLR2), the pattern recognition receptor on macrophages. Binding of LTA to TLR2 induces the release of proinflammatory cytokines (e.g. IL-1, IL-6, TNF) and neutrophil influx.[1,2]

To measure LTA we used two commercial LTA ELISA kits (SunRedBio, China, catalogue number 201-12-1911 and EIAab Science Co LTD, China, catalogue number E1405Ge).

Firstly, *Staphylococcus aureus* and *Streptococcus pyogenes* LTA (Sigma-Aldrich, Zwijndrecht, the Netherlands, L2515 and L3140) solution was made with pure water. In various concentrations (0.31-20 ng/mL), the LTA levels remained under the detection limit (0.3 ng/mL) of the ELISA.

Secondly, we cultured *Streptococcus pneumoniae* in vitro. Neither in brain heart infusion growth medium (BHI solution) with *S. pneumoniae*, nor in BHI solution with *S. pneumoniae* and various concentrations of benzylpenicillin nor in BHI with *S. pneumoniae* and various concentrations of rifampicin, significant amounts of LTA were detected with ELISA (Table S4).

In conclusion, both ELISA tests were unable to demonstrate LTA in various concentrations in water and were unable to demonstrate any LTA released from the cell wall in pneumococcal broths with and without two types of antibiotics (lytic and non-lytic).

Thereafter, we determined TLR2 responsiveness with TLR2 transfected Human Embryonic Kidney (HEK) 293 cells (Invivogen®, the Netherlands, 293-htlr2cd14). TLR2 transfected HEK 293 cells were used to measure IL-8 production by ELISA (Invitrogen®, the Netherlands, CHC1303) in response to TLR2 stimulation.[3] Positive control for the HEK293 cells was Pam3Cys-SKKKK (EMC microcollections®, Germany, L2000) and negative control with ultrapure lipopolysaccharide (LPS) (Invivogen®, the Netherlands, tlrl-peklps) was added. IL-8 release was measured quantitatively with ELISA. Higher IL-8 release represents higher TLR2 binding by immunoreactive agents.

In our experiment, *S. aureus* LTA showed higher IL-8 response with stimulation in increasing LTA concentrations (Figure S2).

Table S4. LTA ELISA response in *S. pneumoniae* log culture with and without antibiotics

	Concentration	Time	concentration LTA
	(µg/mL)	(minutes)	(ng/mL)
Without antibiotic		10	0.32
Rifampicin	10	10	0.23
		30	0.32
		60	0.23
		90	0.21
Rifampicin	1	10	0.26
		30	0.35
		60	0.25
		90	0.61
Penicillin	1	10	0.21
		30	0.25
		60	0.22
		90	0.23
Penicillin	0.1	10	nt
		30	0.43
		60	nt
		90	0.32

All *S. pneumoniae* log cultures showed significant and comparable reduction in colony forming units after the start of treatment with penicillin and rifampicin.
nt = not tested
EIAab Science Co LTD, China, catalogue number E1405Ge

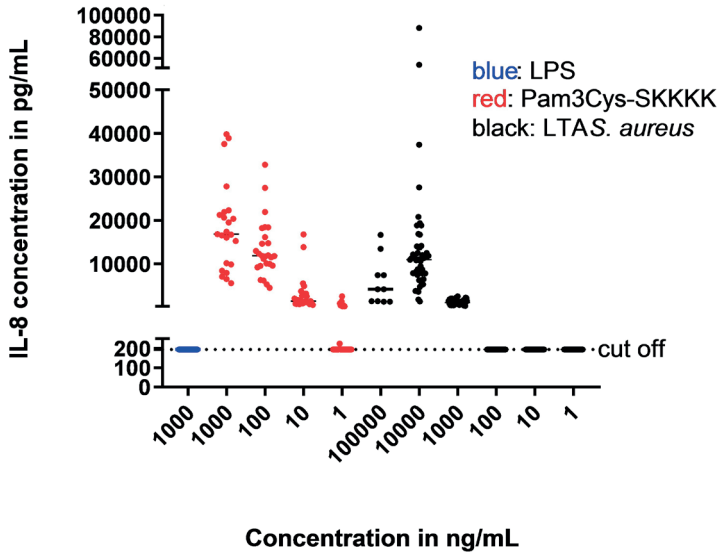

Figure S2. *S. aureus* LTA induced IL-8 response

Upon adding human serum to the *S. aureus* LTA samples, the IL-8 response was reduced. When serum was added to the positive control, Pam3Cys-SKKKK, the IL-8 response was much less reduced. This implied an LTA-inhibiting component in human serum. This inhibiting factor does not affect the functionality of the TLR2 receptor since IL-8 response to a positive control was less reduced (Figure S3).

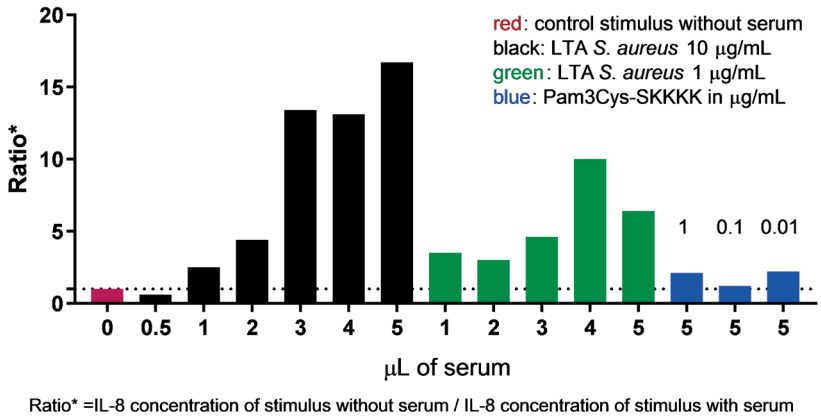

Figure S3. Inhibition of TLR2 response with human serum

The *S. pneumoniae* log culture samples did elicit a TLR2 response. The response in the BHI solution of *S. pneumoniae* with benzylpenicillin was stronger than the response in the solution with rifampicin (Figure S4).

Of two patients from the PRISTINE study with proven pneumococcal pneumonia with pneumococcal bacteremia, plasma was thawed and 5 µL was incubated with 5x10e4 HEK293 cells. Positive control for the HEK293 cells was Pam3Cys-SKKKK, positive control for the LTA was *S. aureus* LTA and negative control with ultrapure LPS was added. IL-8 release was measured quantitatively with ELISA.

No TLR2 response could be detected after adding EDTA plasma to cell cultures. Also after diluting the samples (1:10, 1:25 and 1:50), to remove a potentially inhibiting effect of plasma, no TLR2 response was detected. Similarly, on testing a few urine samples of this subset, in none of them a TLR2 response could be detected.

Of two patients with pneumococcal meningitis and in a third with pneumococcal empyema, a strong TLR2 mediated inflammatory response was measured with cerebrospinal fluid and pleural fluid respectively. These samples were not collected in the PRISTINE study (Figure S5).

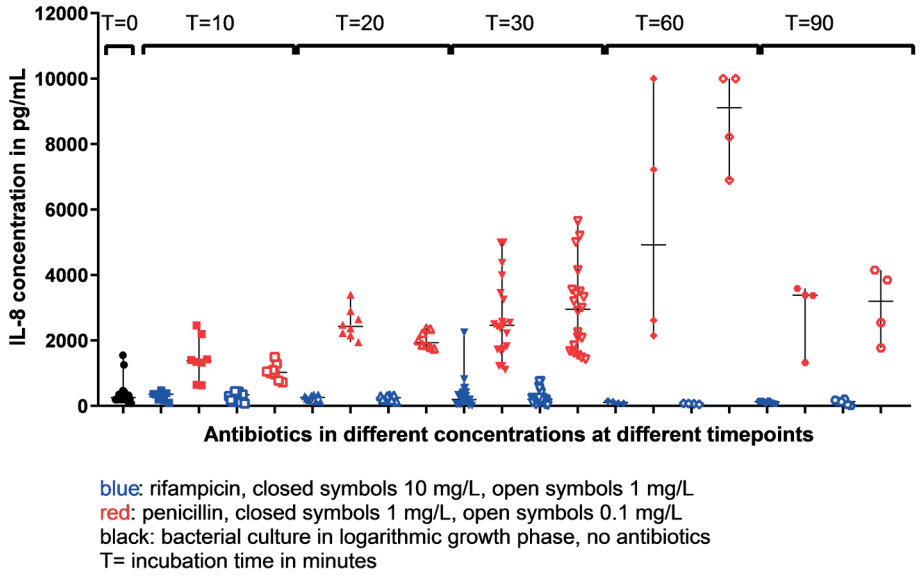

Figure S4. TLR2 response of *S. pneumoniae* with and without antibiotics

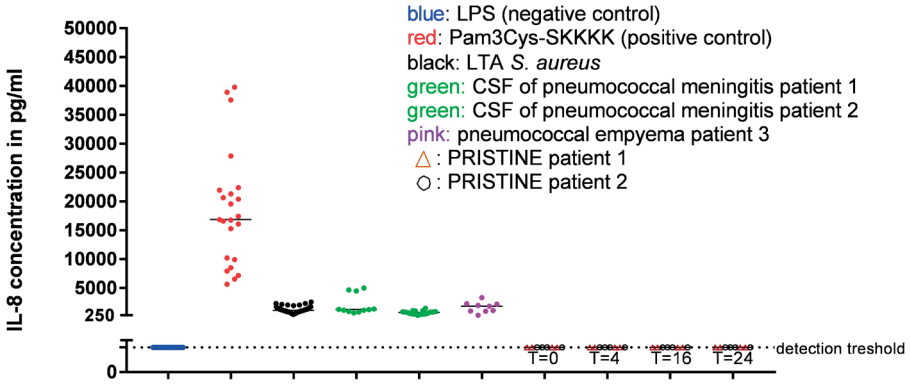

Figure S5. TLR2 mediated inflammatory response in clinical samples
CSF: cerebrospinal fluid

Conclusion

TLR2 transfected HEK 293 cells are able to respond to LTA in *in vitro* samples and in cerebrospinal fluid and pleural fluid, but not in patient plasma samples from the PRISTINE study. Most likely, an inhibiting effect of human serum might contribute to the low immune response in these experiments.

In addition, plasma concentration in patients with pneumococcal pneumonia might be too low to mount an IL-8 response in vitro.

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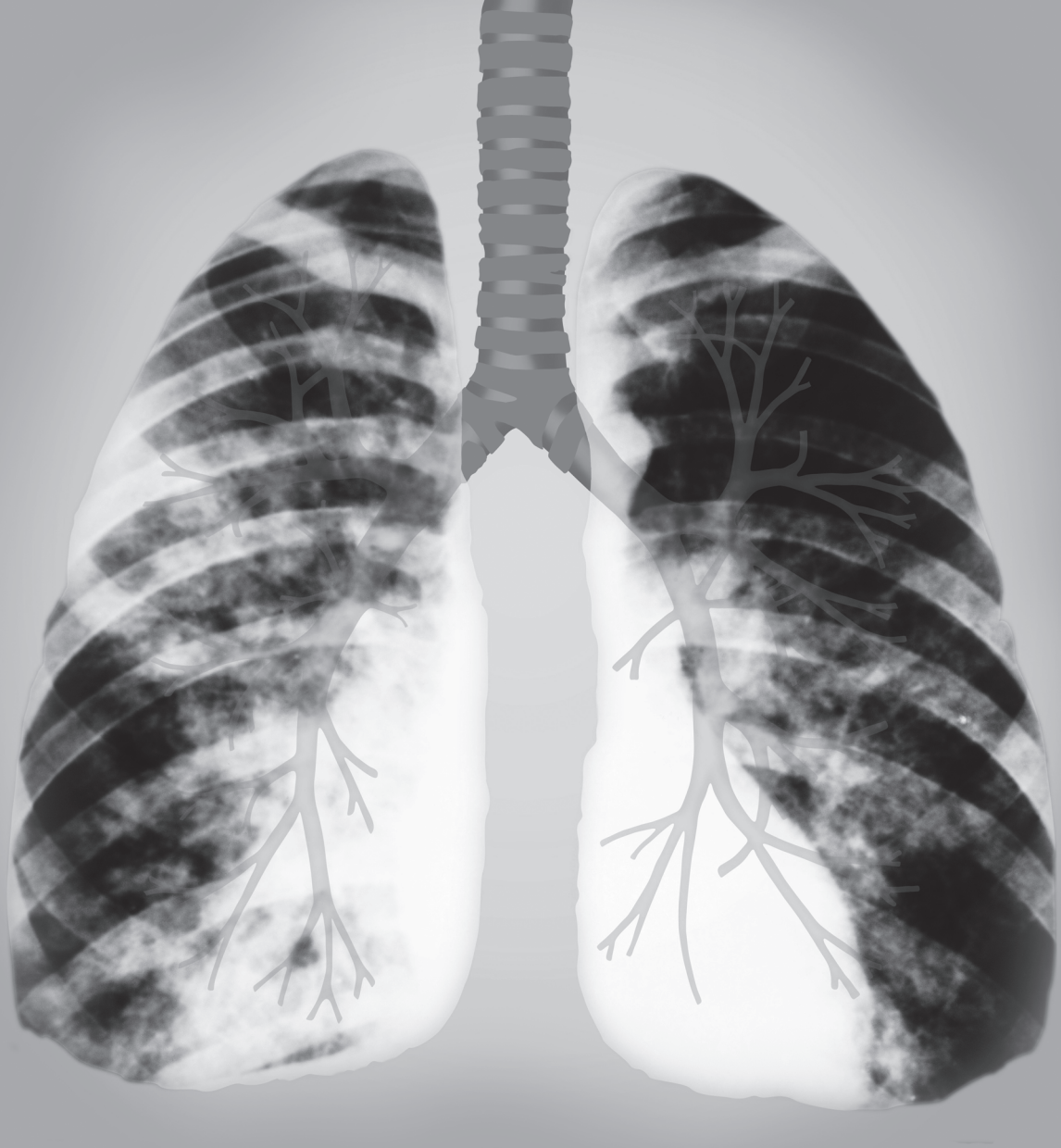

10

Influenza season and ARDS after cardiac surgery.

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INTRODUCTION

A pulmonary inflammatory response after cardiac surgery was described almost 60 years ago as a severe complication of such surgery (1). Still, this Acute Respiratory Distress Syndrome (ARDS) is considered life-threatening with a mortality rate of about 40 % of those affected (2, 3). The pathogenesis of ARDS is complex and not all factors involved are elucidated (4).

One "massive hit" may cause ARDS but it seems more likely that ARDS follows on multiple sequential minor insults (5, 6). Cardiopulmonary bypass (CPB) might be the most important factor causing ARDS postoperatively (7). Transfusion of blood products, complexity of surgery and emergency procedures are additional insults or risk factors (3, 8). Most of these factors are rigid and do not lend to intervention, in an effort to improve outcome after surgery.

Recently, it has become clear that symptomatic viral infections may cause acute lung injury. In particular, severe, symptomatic influenza virus infection can lead to ARDS (9, 10).

Most influenza virus infections in adults, however, are asymptomatic. Thus, in a recent analysis, only 23 percent of influenza virus infections were symptomatic (11).

Moreover, an asymptomatic respiratory virus infection has been demonstrated in a small cohort of patients undergoing elective cardiac surgery (12). Such asymptomatic infections could be a hit contributing to the development of ARDS, e.g. by inducing a low-grade inflammatory response in the lung priming this organ for ARDS in case additional insults would follow.

The aim of the present study was to fill in this deficit and to test in adults, respiratory virus infections as a risk factor for development of ARDS after cardiac surgery.

METHODS

A single-center observational cohort study based on routinely collected clinical data was conducted at the Intensive Care Unit (ICU) of the Leiden University Medical Center (LUMC) between January 2009 and December 2011. This is a tertiary university hospital in the larger metropolitan area of the Netherlands. The ICU is a 25 bed, mixed medical, surgical, neurosurgical and thoracic surgical ICU. All patients ≥ 18 years of age were enrolled at admission on the ICU after cardiac surgery.

The study was approved by the Medical Ethical Committee of the Leiden University Medical Center.

Primary outcome was ARDS within 7 days after cardiac surgery, according to the Berlin definition (13). Secondary outcomes were time on mechanical ventilation, length of stay in ICU and ICU mortality.

Elective cardiac surgery patients were admitted to the hospital one day prior to surgery and were checked to be fit for surgery. In case of fever or clinically apparent respiratory tract infection on the day of surgery, the operation was postponed. A minority of patients underwent surgery in an emergency setting. These patients were not checked for fever or severe respiratory tract infections since their cardiac emergency was life-threatening and therefore warranted immediate surgery.

Cardiac surgery involved coronary artery bypass grafting (CABG), ventricular surgery, valve surgery or aortic surgery.

Induction of anesthesia was done with propofol and remifentanil. Anesthesia was maintained with midazolam or propofol and remifentanil and sufentanil. Tranexamic acid prophylaxis was given to minimize perioperative blood loss, cefazolin was used as antibiotic prophylaxis.

Cardiopulmonary bypass (CPB) with a centrifugal blood pump was initiated. Oxygenation was ensured with a hollow fiber membrane oxygenator, tubing was coated with bio-inert heparin-free polymers. Flow was laminar. Intermittent warm antegrade blood cardioplegia was instituted. During CPB, heparin was used to achieve an activated clotting time > 400s. Patients were ventilated with low pressure and low tidal volume to prevent atelectasis of the lung, except for procedures in which persistent ventilation obstructed surgical procedures. During bypass, core temperature was maintained at 34°C to 36°C. Active cooling was solely used during aortic surgery to prevent brain ischemia. Inotropic and vasoactive agents were administered on indication.

Perioperative care was according to the fast-track protocol (14, 15). During the study period, no changes in protocols of intraoperative mechanical ventilation of cardiac surgery patients have been practiced in our institution. On the ICU, lung protective mechanical ventilation (PEEP 5-8 with small tidal volumes (<6ml/kg) according to the then prevailing mechanical ventilatory standards) has been used.

Demographic data, ASA category, EuroSCORE as preoperative risk assessment (16), APACHE IV score as a marker of severity of disease after surgery (17) were all recorded peri-operatively. Duration of surgery and duration of cardiopulmonary bypass (CPB) were recorded. Administration of blood products was recorded.

Definition of ARDS

Patients were diagnosed with an ARDS if they have met each of the Berlin criteria described below (13):

- Within 1 week of a known clinical insult or new or worsening respiratory symptoms
- Bilateral opacities on chest radiograph or computer tomography scan—not fully explained by effusions, lobar/lung collapse, or nodules
- Respiratory failure not fully explained by cardiac failure or fluid overload
  Need objective assessment (e.g. echocardiography) to exclude hydrostatic edema if no risk factor present
- Oxygenation:
  - Mild: 200 mm Hg < PaO_2:FiO_2 ratio ≤ 300 mmHg with PEEP or CPAP ≥ 5 cm of water
  - Moderate: 100 mm Hg < PaO2:FiO2 ratio ≤ 200 mm Hg with PEEP ≥ 5 cm of water
  - Severe: PaO2:FiO2 ratio ≤ 100 mm Hg with PEEP ≥ 5 cm of water

Abbreviations: CPAP, continuous positive airway pressure; FiO2, fraction of inspired oxygen; PaO2, partial pressure of arterial oxygen; PEEP, positive end-expiratory pressure.

Cardiac surgery was the initial clinical insult. All criteria were measured within the first week after cardiac surgery.

Chest roentgenograms were standardly obtained on admission on the intensive care unit (ICU) and 24 hours after admission to the ICU. If the respiratory condition of patients deteriorated, additional radiographic evaluation, including CT scan, was performed. Presence or absence of bilateral opacities was judged by 3 independent observers on chest radiograph or computer tomography scan. The observers were unaware of the study design. Consensus in interpretation was required. In case of discrepancy in radiograph or scan interpretation, uniformity was achieved by discussion between observers. Prominent hili on both sides were not considered as bilateral opacities. This feature is most likely due to cardiac failure and therefore not a feature of ARDS.

Presence of hypoxia and ventilation prerequisites were monitored continuously throughout ICU stay. In case ARDS had developed, the point of time that the patient first fulfilled the Berlin criteria was recorded.

The worst oxygenation during the first week after cardiac surgery was used to determine severity of ARDS.

Assessment of respiratory virus infection

Preoperative respiratory virus diagnostics is not routinely performed, and thus none of our patients was tested for presence of respiratory viruses. Of note, none of the electively operated patients experienced preoperative respiratory complaints or was febrile on admission which would have justified such diagnostics.

Therefore, we have used influenza season as a proxy for respiratory virus infection.

Surveillance of influenza season is conducted by the Netherlands Institute for Health Services Research (NIVEL) and the WHO European Flu Network. They report the number of patients with influenza-like illness (ILI) in the Netherlands (18, 19). NIVEL primary care database assembles records from sentinel general practices in an information system. Data about ILI come from approximately 120,000 patients recorded in these GP practices and represent a good estimate of the Dutch population.

ILI incidence was calculated per 100,000 persons per week between 2009 and 2011 (20). In temperate climate, infections with most respiratory viruses have a seasonal pattern (21-23) and therefore ILI incidence is fluctuating.

In the Netherlands, influenza epidemic season is defined by > 51 ILI-reports per 100,000 per week for at least two consecutive weeks and by the detection of Influenza virus in respiratory samples (24, 25). An epidemic season ends in the first week ILI-reports fall to ≤ 51 per 100,000 per week.

These patients with Influenza A or B virus infection contribute to a large extent to the seasonal increase in ILI numbers. The percentage of positive Influenza specimens is highest during the ILI epidemic season (26), making this the best indicator for Influenza virus activity (27).

In contrast to the well-defined influenza epidemic season, we have arbitrarily predefined a baseline season as at least two consecutive weeks < 25 ILI-reports per 100,000 per week. This represents a period with low incidence of respiratory virus infections. The baseline periods end in the first week ILI-reports increase to ≥25 per 100,000 per week.

All other weeks were categorized as periseasonal period. These periods represent build up phase to an epidemic season or a period of decreasing ILI activity towards a baseline sea-

son. Comparable baseline and periseasonal periods in surveillance of respiratory viruses have been used previously (28).

Statistical analysis

Continuous variables were summarized as either means with standard deviations or medians with interquartile ranges. Categorical variables were depicted as numbers with percentages or as medians with interquartile ranges. Kruskal-Wallis and Chi-squared tests were performed for comparing baseline data as appropriate. Statistical analysis was performed using SPSS (IBM Software) version 23.

To evaluate whether influenza epidemic season is a risk factor for development of ARDS or ICU mortality, we have used binary logistic regression to calculate odds ratio with 95% confidence intervals.

For continuous secondary outcomes (time on mechanical ventilation and length of stay on ICU), we have used mixed linear modelling and calculated estimates with 95% confidence intervals. All variables that were significant ($p<0.1$) in univariate analysis and variables that were deemed clinically relevant were entered in the multivariable logistic model. Emergency procedure or not was not added to the model since both EuroSCORE and APACHE IV score have this entity within their total score.

EuroSCORE is used as preoperative risk assessment and therefore ASA category is not added to the model.

RESULTS

Between January 2009 and December 2011, in total 2021 patients have undergone cardiac surgery. Of these patients, 8 died during surgery and therefore 2013 were admitted to the ICU postoperatively.

Of these patients, 289 have had surgery during an influenza epidemic season and 740 during a baseline period. The other 984 patients have had surgery during a periseasonal period.

The patient characteristics are shown in Table 1. Patients and research team had no influence on the season of surgery.

No differences were found in demographic and perioperative variables between the different seasons.

The weeks with different influenza-like illness incidence are specified in Figure 1.

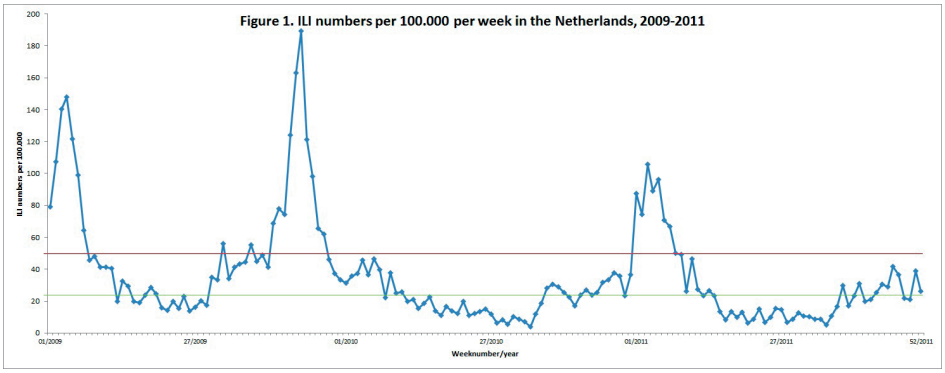

Figure 1. ILI numbers per 100.000 per week in the Netherlands, 2009-2011
The Netherlands Institute for Health Services Research (NIVEL) reports its data on influenza-like illness (ILI) to the WHO European Influenza network. Graphs depict ILI-reports between late 2008 until the end of 2011. The horizontal lines denote baseline (green) and epidemic (red) threshold within the studied period.

Two influenza epidemic seasons were present in 2009 (Fig. 1). The first started in January and a second started in October. The subsequent year 2010 had no influenza epidemic period. In 2011, influenza epidemic season started in January.

Of all patients who had been admitted on ICU after cardiac surgery in baseline weeks, 38 out of 740 (5.1%) developed ARDS. In periseasonal period, 55 out of 984 (5.6%) developed ARDS and in influenza epidemic season 26 out of 289 (9.0%) developed ARDS. All ARDS cases occurred within 26 hours after start of surgery. Of all ARDS patients that underwent surgery during influenza epidemic season, 22 (85%) were admitted within 24 hours before surgery. In the univariate analysis (table 2A and 2B), the odds ratio for ARDS within 7 days after cardiac surgery in influenza epidemic season versus baseline season is 1.83 (95% confidence interval 1.09-3.07).

In the multivariate model (table 3A), odds ratio for ARDS in influenza epidemic season versus baseline season is 1.85 (95% CI 1.06-3.23). Furthermore, duration of mechanical ventilation was significantly increased in the influenza epidemic season compared to the baseline season (Table 3B). Other clinical outcome parameters did not differ significantly between baseline and influenza season (Table 3A and 3B).

Table 1. Demographic data and perioperative details of studied population

Variable*	Baseline season	Periseasonal	Influenza season	P value**
All patients – no.	740	984	289	
Preoperative				
Age in years (IQR)	66 (58-74)	66 (58-75)	66 (58-73)	0.88
Gender - % males	72.2%	67.8%	67.1%	0.10
Body Mass Index - kg/m^2 (IQR)	26 (24-29)	26 (24-29)	26 (24-29)	0.92
ASA category (IQR)	3 (3-3)	3 (3-3)	3 (3-3)	0.68
Emergency procedure (%) EuroSCORE (IQR)	21 (2.8%) 4 (2-9)	27 (2.7%) 4 (2-9)	9 (3.1%) 5 (2-9)	0.95 0.56
During surgery				
Duration of surgery – min (IQR)	360 (300-426)	358 (302-427)	354 (292-428)	0.81
Duration of CPB – min (IQR)	129 (99-184)	131 (94-185)	124 (92-183)	0.79
Units of blood products during procedure (IQR)	0 (0-1)	0 (0-1)	0 (0-1)	0.70
CABG (%)	435 (58.8)	597 (60.7)	167 (57.8)	0.59
Valve surgery (%)	295 (39.9)	403 (41.0)	115 (39.8)	0.88
- 1 valve (%)	195 (26.4)	279 (28.4)	73 (25.3)	
- 2 valves (%)	91 (12.3)	106 (10.8)	34 (11.8)	
- 3-4 valves (%)	9 (1.2)	18 (1.8)	8 (2.8)	
Aortic surgery (%)	80 (10.8)	109 (11.1)	39 (13.5)	0.45
Left ventricular reconstruction (%)	31 (4.2)	36 (3.7)	12 (4.2)	0.84
Postoperative				
Apache IV score (IQR)	48 (37-59)	48 (38-60)	48 (39-61)	0.74
Second surgery required (%)	63 (8.5%)	64 (6.5%)	22 (7.6%)	0.29

IQR = Interquartile range; min = minute; no. = number
*Medians are described for all continuous variables ** Kruskal-Wallis and Chi-squared tests were performed for comparison of baseline data as appropriate.

Post hoc analysis revealed that the number of ARDS cases increases when Influenza A and B circulation in the community per influenza epidemic season increases (Figure 2). This is calculated by multiplying the total number of ILI cases per 100,000 per epidemic season with the percentage of Influenza A and B positive tests.

DISCUSSION

Cardiac surgery during influenza season is an independent risk factor for development of postoperative ARDS compared to surgery during seasons with little respiratory virus transmission.

Table 2A. Univariate analysis of demographic and perioperative parameters for ARDS and mortality

	ARDS within 7 days after surgery			Mortality on ICU		
	OR	95% CI	P	OR	95% CI	P
Age	1.00	0.98-1.01	0.52	1.02	0.99-1.04	0.22
Sexe	1.02	0.68-1.52	0.92	0.95	0.49-1.82	0.87
BMI	1.03	0.99-1.07	0.21	1.00	0.94-1.08	0.91
Apache IV	1.03	1.03-1.04	0.00	1.06	1.05-1.07	0.00
Euroscore	1.04	1.03-1.06	0.00	1.07	1.05-1.09	0.00
Time in surgery (minutes)	1.00	1.00-1.00	0.00	1.01	1.00-1.01	0.00
Blood products during surgery	1.07	1.04-1.10	0.00	1.10	1.06-1.14	0.00
Total time on CPB (minutes)	1.01	1.00-1.01	0.00	1.01	1.01-1.01	0.00
Emergency procedure	3.15	1.51-6.58	0.00	3.62	1.25-10.47	0.02
ASA 3*	4.21	1.32-13.40	0.02	3.39	0.46-25.09	0.23
ASA 4 and 5*	5.88	1.71-20.18	0.01	14.79	1.94-112.59	0.01
Periseasonal**	1.09	0.72-1.67	0.68	1.19	0.60-2.33	0.62
Influenza epidemic season**	1.83	1.09-3.07	0.02	1.48	0.61-3.56	0.39

* ASA 1 and 2 are the reference category.
** Baseline season is the reference category.

Table 2B. Univariate analysis of demographic and perioperative parameters for time on mechanical ventilation and length of stay on ICU

	Time on mechanical ventilation (hours)			Length of stay on ICU (hours)		
	Estimate	95% CI	P	Estimate	95% CI	P
Age	0.39	-0.24-1.02	0.23	0.39	-0.26-1.04	0.24
Sexe	4.09	-12.21-20.38	0.62	4.43	-12.63-21.48	0.61
BMI	0.58	-1.20-2.36	0.53	0.50	-1.40-2.39	0.61
Apache IV	2.17	1.78-2.55	0.00	2.46	2.07-2.86	0.00
Euroscore	3.95	3.13-4.77	0.00	4.64	3.79-5.48	0.00
Time in surgery (minutes)	0.31	0.25-0.38	0.00	0.37	0.30-0.44	0.00
Blood products during surgery	8.66	6.71-10.60	0.00	11.74	9.69-13.80	0.00
Total time on CPB (minutes)	0.40	0.31-0.50	0.00	0.48	0.38-0.57	0.00
Emergency procedure	25.40	-18.85-69.64	0.26	35.13	-12.22-82.48	0.15
ASA 3*	19.32	-6.23-44.87	0.14	26.22	-0.11-52.54	0.05
ASA 4 and 5*	110.23	76.39-144.08	0.00	105.98	72.02-139.95	0.00
Periseasonal**	10.59	-5.73-26.92	0.20	5.83	-11.31-22.97	0.51
Influenza epidemic season**	27.16	3.83-50.50	0.02	25.11	0.59-49.63	0.05

* ASA 1 and 2 are the reference category.
** Baseline season is the reference category.

Table 3A. Multivariate analyses of demographic and perioperative parameters for development of ARDS and mortality on ICU

	ARDS within 7 days after surgery			Mortality on ICU		
	OR	95% CI	P	OR	95% CI	P
Apache IV	1.03	1.02-1.04	0.00	1.05	1.04-1.07	0.00
Euroscore	1.02	1.00-1.04	0.07	1.03	1.01-1.06	0.01
Time in surgery (minutes)	1.00	1.00-1.00	0.61	1.00	1.00-1.01	0.09
Blood products during surgery	1.00	0.95-1.05	0.93	1.01	0.95-1.08	0.76
Total time on CPB (minutes)	1.00	1.00-1.01	0.25	1.00	0.99-1.01	0.83
Periseasonal*	1.09	0.69-1.72	0.71	0.96	0.43-2.11	0.91
Influenza epidemic season*	1.85	1.06-3.23	0.03	1.57	0.58-4.24	0.37

* Baseline season is the reference category

Table 3B. Multivariate analyses of demographic and perioperative parameters for time on mechanical ventilation and length of stay on ICU

	Time on mechanical ventilation (hours)			Length of stay on ICU (hours)		
	Estimate	95% CI	P	Estimate	95% CI	P
Apache IV	1.61	1.20-2.02	0.00	1.79	1.37-2.20	0.00
Euroscore	1.58	0.64-2.51	0.00	1.69	0.77-2.62	0.00
Time in surgery (minutes)	0.19	0.06-0.31	0.00	0.13	0.02-0.24	0.02
Blood products during surgery	2.80	0.45-5.15	0.02	4.68	2.28-7.09	0.00
Total time on CPB (minutes)	0.00	-0.16-0.16	1.00	0.13	-0.02-0.27	0.08
Periseasonal*	10.00	-5.49-25.49	0.21	6.51	-9.20-22.21	0.42
Influenza epidemic season*	22.64	0.47-44.81	0.05	21.08	-1.42-43.58	0.07

* Baseline season is the reference category.

The main finding of the present study is that the risk for the development of ARDS after cardiac surgery is about twice increased during the influenza season as compared to seasons with low burden of respiratory virus infections. Moreover, the influenza season did increase the duration of mechanical ventilation. The influenza season was estimated on the basis of weekly reporting of influenza-like illness within the community by sentinel surveillance at general practitioner offices, confirmed by detecting influenza in nasopharyngeal samples. On multivariate modelling, the influenza season proved to be an independent risk factor for the development of ARDS postoperatively, besides well-known factors like EuroSCORE and total time on CPB.

Our study has several strengths. Firstly, the database is robust and complete. The definitions used to define an influenza epidemic season and ARDS are widely used and accepted

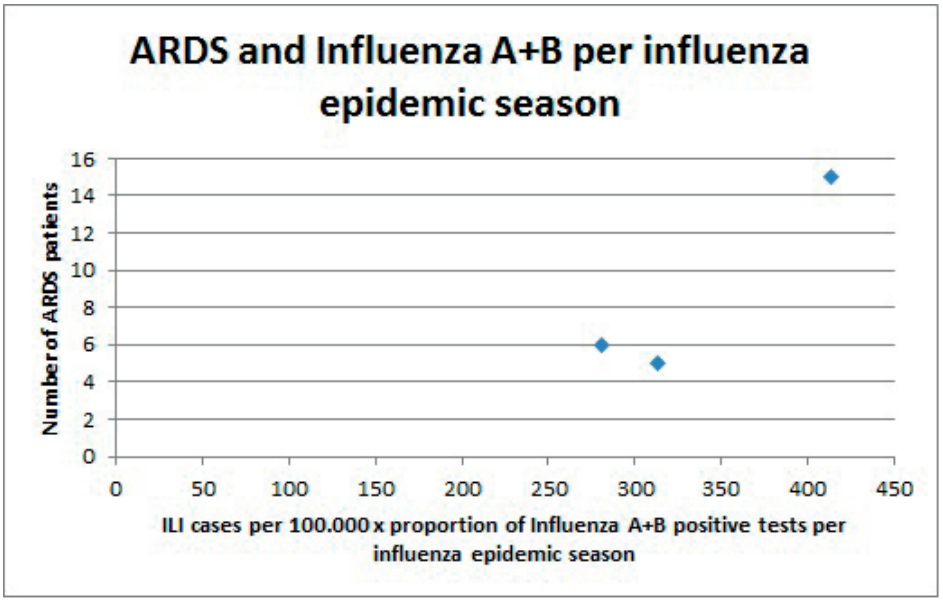

Figure 2. ARDS and Influenza A+B per influenza epidemic season

(13, 18). The ARDS definition by the so-called Berlin criteria makes our results generalizable and readily permissible for comparison with other studies.

Surveillance of influenza-like illness is a good proxy for monitoring burden of influenza virus infection in the community (26, 27). Since our hypothesis is that respiratory virus infection is an extra risk factor for developing ARDS in cardiac surgery patients, our study design is suitable to examine this expectation.

Although previous studies used different definitions of ARDS, the overall percentage of patients developing ARDS in our study (5.9%) resembles that of other studies (3, 8, 29-32).

The in-hospital mortality in patients with ARDS was 17%, which is somewhat less than that reported in other studies (2, 3).

In recent literature, focus on moderate to severe ARDS instead of mild ARDS as a clinically relevant entity has come in use (33). Of note, in our cohort the influenza season was an even stronger risk factor for moderate to severe ARDS than it is for ARDS in general (data not shown).

Our study also has several weaknesses. First and for all, our cohort study shows an association but does not prove a causal relation between viral infection and ARDS in cardiac

surgery patients. There are potential confounders that vary by season, such as vitamin D level or ambient temperature, for which we could not adjust.

The 2009 Influenza A (H1N1) outbreak is remarkable in this aspect. The start of an influenza season in October is uncommon on the northern hemisphere. In this period of the year, temperature was higher than during usual epidemic periods in January to February. In addition, the vitamin D level shortly after summer should have been in the normal range, making these factors as being implicated in ARDS during influenza season less likely.

Still, we cannot exclude that such factors could have confounded our findings (34-36).

Although surveillance of influenza season is robust, the 2009 H1N1 Influenza pandemic might have had an impact on the health seeking behavior of patients. For instance, fear for this new virus infection, might have lowered the threshold for visiting the general practitioner because of respiratory complaints (37, 38) and the definition of influenza epidemic season might have been reached more early. This bias could have underestimated our results.

The number of influenza seasons we studied, three, is too small to draw firm conclusions about secondary outcome variables and the post hoc analysis. Certainly, more influenza seasons with different products of ILI numbers and fraction of Influenza positive tests are required to determine the predictive value for ARDS.

How should we interpret the findings? Studies on the consequences of viral respiratory infection in cardiac surgery patients have mostly been done in the paediatric population. Children with upper respiratory tract infection or with documented rhinovirus infection at the time of cardiac surgery have more postoperative (respiratory) complications (39, 40). On this evidence, it was suggested that an ongoing respiratory virus infection should influence the decision to postpone elective cardiac surgery in children (41). If latent respiratory virus infections are a risk factor for developing postoperative ARDS in adults as well, the chance of developing ARDS can be affected via this risk factor.

Spaeder et al. performed a study in children undergoing cardiac surgery. No differences in postoperative length of stay were detected between ‘viral’ and ‘nonviral’ season. However, in that study the definition of respiratory virus season was much more crude than in our study, their primary end point was different (length of stay) from ours and did not include ARDS, and the sample size was much smaller, making the study underpowered to reveal our association. Furthermore, children with symptomatic viral infections did have an increased risk of morbidity (42). Although viruses in children, their immune response

and seasonality of these viruses are different from that in adults, a comparison of the hypothesis that viral infection is a predictor of worse (respiratory) outcome after surgery is reasonable. To our knowledge, our study is the first to assign respiratory virus season as a risk factor for ARDS in adult patients undergoing cardiac surgery.

H1N1 Influenza virus infections were predominant in the influenza outbreak in October 2009 (23, 43). These infections are therefore presumably the most likely agents contributing to the increase in ARDS.

Our post hoc analysis is in line with this observation. Numbers of ARDS are higher during seasons with relatively more Influenza virus, defined as the ILI numbers multiplied by the proportion of Influenza A and B positive tests per week. This implicates the Influenza virus as the risk factor for ARDS more likely than other factors previously described. Of note, the patients undergoing cardiac surgery were at increased age and therefore less likely to acquire symptomatic H1N1 Influenza virus infection due to cross-reactivity against previously encountered H1N1 Influenza strains (44, 45).

During influenza season, the percentage of positive swabs for other respiratory viruses is relatively high (23, 46). Most likely, other respiratory viruses are equally important risk factors for the increased incidence of ARDS.

Of note, it is well known that symptomatic (H1N1) Influenza virus infection can be a cause of ARDS, with and without prior surgery. However, our study population differs from this group as it does not include patient with manifest respiratory (Influenza or not) virus infection.

Since most patients (97.2%) in our study have had elective surgery, they were checked preoperatively to assess whether they had an (acute) inflammatory disease. Surgery would have been postponed when infection was evident.

Our findings fit with the multiple hit hypothesis of ARDS pathogenesis. ARDS is most likely caused by multiple insults of which cardiac surgery and accompanying cardiopulmonary bypass are the most prominent. This study suggests that asymptomatic respiratory virus infection could prime the lungs for development of ARDS. The finding that ARDS occurs within 26 hours after cardiac surgery reflects our hypothesis that the lungs are primed, perioperatively, by viral infection.

Different studies support this theory.

A controlled randomized study in 1992 revealed that prophylactic antibiotics in patients undergoing aggressive antileukemic chemotherapy, reduced the number of sepsis and ARDS, most likely by removing streptococcal colonization from the upper airways (47). In a rodent model, a low grade immune stimulus in the lungs before pneumonectomy caused aggravated lung injury in the contralateral lung compared to rodents who were not primed with the stimulus (48). The exact mechanism of this lung priming is not elucidated (49).

Previously, we demonstrated in a small cohort that in 18% of elective cardiac surgery patients, respiratory viruses could be detected in mini broncho-alveolar lavage (12). None of these patients had a manifest infection which is in line with a bigger cohort of patients with asymptomatic influenza virus infection (11).

Our population consists of – mostly elderly - cardiac patients, which should have received yearly immunization against influenza viruses. Of all Dutch patients with cardiac illnesses, 77.1 percent was vaccinated against influenza in 2011 (50). Therefore, our study design might be underestimating the association between influenza (season) and ARDS. On the other hand, the influenza vaccine effectiveness is less in older patients and might be insufficient to prevent subclinical influenza replication and infection (about 50% effective in preventing Influenza virus infection in the elderly) and, with that, ARDS (51). Other respiratory viruses with higher incidence during ILI season, for example RS virus, can also be the second hit in causing ARDS.

The majority of patients with ARDS in influenza epidemic season were admitted to the hospital ≤ 1 day before surgery. This essentially excludes nosocomial acquisition of influenza virus in most of the cases.

Future research is needed to test if vaccination of patients could reduce the risk of ARDS post cardiac surgery. Whether vaccination of health care workers could reduce this risk cannot be proven or ruled out by our study. Numbers are too small to draw firm conclusions.

In none of our cardiac surgery patients respiratory virus diagnostics were done before surgery. Remember that none had respiratory symptoms that might have justified such diagnostics.

In the 7 days after cardiac surgery, in only 6 patients respiratory virus diagnostics were done. In none, a respiratory virus was detected and none developed ARDS postoperatively.

Further research will be necessary to reproduce our findings and prospective studies to determine a causal relation is necessary. If confirmed, virus diagnostic testing or vaccination could be useful before high risk cardiac surgery to attenuate the risk of postoperative ARDS.

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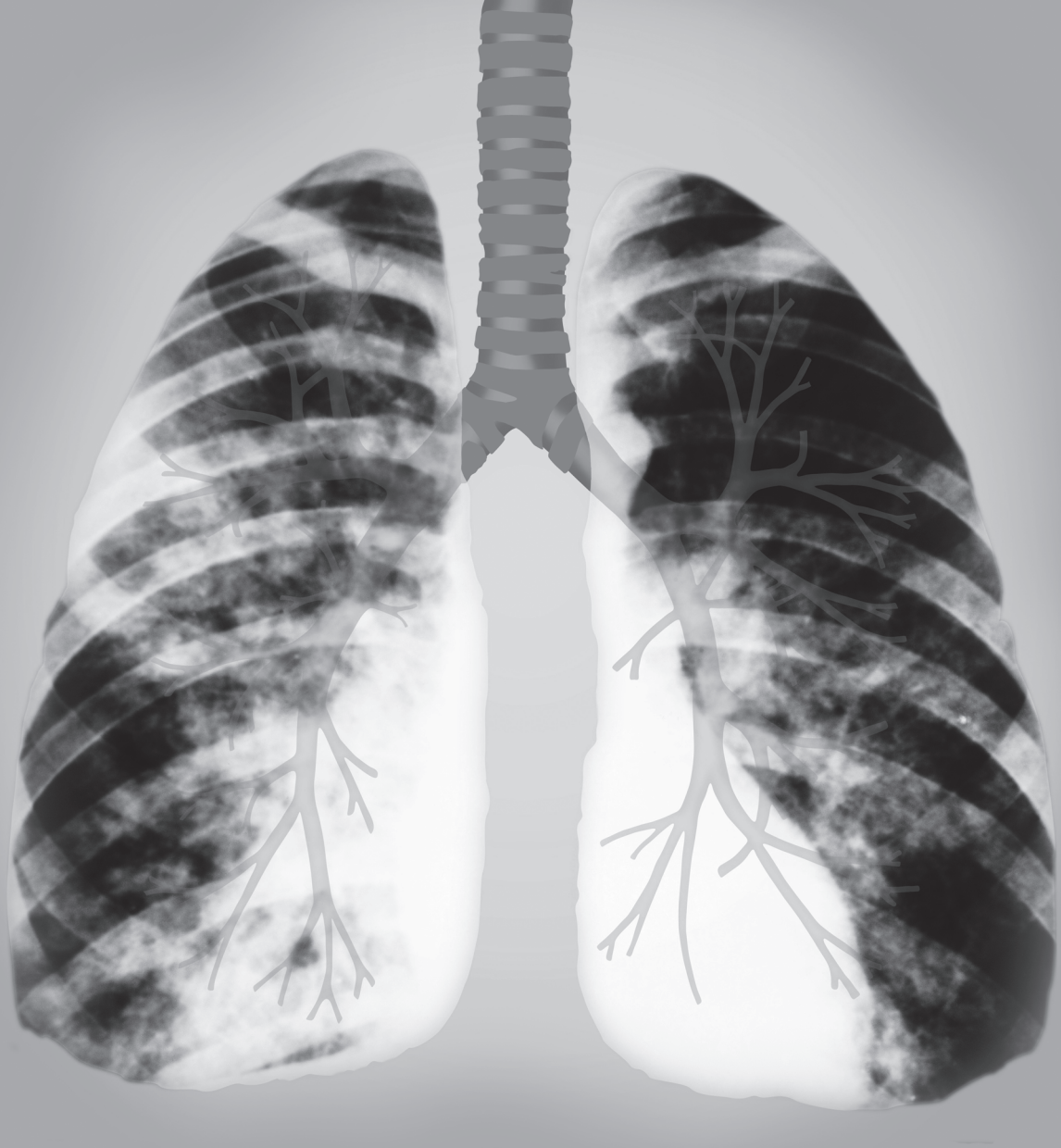

11

Summary and general discussion

SUMMARY AND GENERAL DISCUSSION

Respiratory tract infections are among the most common infections treated by health care practitioners. These infections are characterized by microbes invading the respiratory tract and eliciting an inflammatory response. Lower respiratory tract infections, the infections below the vocal cords, are divided into pneumonia, bronchopneumonia and bronchitis. Acute lower respiratory tract infections are defined by symptoms and signs lasting for less than three weeks. In the Netherlands, the average patient with a community-acquired acute lower respiratory tract infection most likely has either a viral or a bacterial etiology of infection or a combined infection with both a virus and a bacterium playing a role (1-4). Yearly in the Netherlands, such infections account for about 50.000 individuals admitted to hospital (www.zorgatlas.nl). Globally, lower respiratory tract infections are the third cause of death, and responsible for the death of about 3.0 million people worldwide in 2016 (5).

In general, the occurrence and outcome of an infection is determined by the complex interaction of host, microorganism and environment. Also in patients with an acute lower respiratory tract infection the specifics of the host's inflammatory response to the causative microorganism(s) plays a determining role in the course of disease. An uncontrolled or exacerbated inflammatory response may result in 'collateral' damage to the lung tissue, and in severe cases, result in severe acute lung injury (such as Acute Respiratory Distress Syndrome) and consequently, severe morbidity and high mortality (6, 7). Somehow, the host must titrate its inflammatory response in such a way as to ensure a delicate balance between an inflammatory response adequate to eradicate the causative microorganism while precluding inadvertent tissue damage. In other words, the inflammatory response should successfully sterilize the infected part of the respiratory tract without causing 'collateral' damage of lung tissue and lung architecture (7). In some infections, e.g. tuberculosis, the host response does not seek to sterilize the lung tissues but rather mitigate and lock up the microorganisms in delicate granulomas that may remain for life.

Currently, the best way to prevent excessive lung injury is to detect and identify patients with pneumonia or lower respiratory tract infection as early as possible in the course of their disease, in particular those with an increased risk for a complicated course (8). Early and adequate treatment is one of the best predictors of advantageous outcome. In essence, this strategy focuses on the assessment of the intensity of the host inflammatory response as predictor of potential derailing of the immune response. This is done by combining information of the medical status of the host (e.g., any underlying conditions that may deteriorate during the stress of infection or limit his or hers ability to mount an adequate immune response), the current host response (e.g., temperature, shortness of breath, respiratory rate, consciousness) and the assessment of biomarkers reflecting the

host's inflammatory response (e.g., C-reactive protein, procalcitonin and the like). This evaluation is considered in the context of local and current epidemiology of respiratory disease pathogens (e.g., complaints occurring in yearly influenza season, following holiday, etc.) and may result in swift initiation of empiric therapy or a cautious waiting posture and follow-up.

In short, while it may be best to prevent an infection altogether by, for instance, vaccination, clinician's efforts should be directed at the early detection of potentially severe respiratory tract infections, and their ability to distinguish these from harmless ones. Finally, in case of a severe infection, treatment strategies should be directed at attenuation of an overwhelming host immune response that causes excessive tissue damage. Most of these aspects are covered in this thesis, some more loosely and some in detail, and are summarized in the following paragraphs.

PREVENTION BY VACCINATION

To prevent lower respiratory tract infections, in many cases, it is possible to vaccinate against the pathogens causing these infections like *Streptococcus pneumoniae* and the influenza virus. Against other pathogens like RSV, vaccines are being developed. For respiratory pathogens against which a vaccine is available, immunization is a cost-effective way to prevent respiratory tract infection. Among others, influenza vaccination is recommended in the Netherlands for subjects with an increased risk for influenza complications, for example elderly, immunocompromised, and patients with comorbidities (9). The influenza virus causes yearly epidemics, which on average last for eight to twelve weeks. The clinical spectrum varies from asymptomatic infection, mild to moderate illness, to severe acute respiratory tract infection (SARI) which requires hospitalization and can even result in death in a small minority of patients (10).

Influenza vaccination provides the best protection in individuals who mount the most robust immune response, namely the young and immunocompetent subjects. The ability of the influenza vaccine to induce protection is reduced in the elderly, frail, and immunocompromised patients (11-13). These vulnerable patients would benefit most from an adequate protection by vaccination as these are the patients with an increased risk of a complicated course of the disease. Primarily in this group of patients, prevention would lead to a reduction in the societal burden of disease and mortality due to the influenza virus. If a weak or even absent immune response to vaccination in these vulnerable patients cannot offer protection, vaccination of close contacts may prevent the introduction

of a pathogen in the proximity and thereby preclude exposure and infection in the most vulnerable (i.e., through 'herd immunity').

Hospitalized patients or patients in long term care facilities are most often older, frail, or immunocompromised. Among others, these include individuals in need for care after surgery, after an acute cardiovascular event, receiving cancer treatment, and geriatric patients with cognitive impairment. Studies have shown that these individuals have a relatively weak immune response to the influenza vaccine and are therefore only marginally protected against influenza infection after vaccination. On the other hand, health care professionals, who are at increased risk of acquiring influenza from their close contact with symptomatic and asymptomatic influenza patients (14), generally will mount an adequate, protective immune response after vaccination. Thus, immunizing health care professionals against influenza virus may help to protect vulnerable patients by minimizing, or at least reducing, exposure to this pathogen.

Unfortunately, influenza vaccination coverage has been low among health care professionals, both physicians and nurses alike. In Europe, this coverage was below 30%, and in Dutch hospitals in 2012 median vaccination coverage amounted to 13% (15, 16). Still, the low acceptance of vaccination such as the one against influenza, goes against the principles of 'first do no harm', i.e., delivering safe care, and the low vaccination rate among care providers puts vulnerable patients at risk for acquiring influenza infection during hospitalization (17, 18).

In **Chapter 7**, we discuss the severe influenza season 2017/2018 and note the low percentage of health care professionals who had received the influenza vaccination in the months prior to the season. Hospitals struggled to meet the demand for care, with high numbers of patients with influenza and its complications visiting the hospitals, and at the same time decreased hospital capacity due to flu-related sick leave of hospital staff. A call was made for 100% influenza vaccine coverage among health care professionals. High coverage will prove beneficial to the employer and employees since non-attendance among employees will be reduced during peak demand and thus ensure continuity of care capacity. It will also have a positive impact in terms of patient safety and will boosts professionalism, through improved protection of vulnerable patients against nosocomial influenza infection.

Many strategies have been implemented to improve vaccination coverage among health care workers, some with more success than others. In recent years a few best practice hospitals increased their coverage to 50%, by using both education and easy-access vaccination, information via various media, stimulating internal competition and a public

debate about the importance of safe care for susceptible patients. The chapter contributes to this debate by reviewing evidence regarding different strategies and prioritized vaccination of health care professionals in all domains of health care institutions. In October 2018 the Netherlands Federation of University Medical Centres (Nederlandse Federatie van Universitair Medische Centra, NFU), the Dutch Hospital Association (Nederlandse Vereniging van Ziekenhuizen, NVZ), the Dutch Association of Medical Specialists (Federatie Medisch Specialisten, FMS) and the Dutch Association of Nurses and Nursing Assistants (Verpleegkundigen & Verzorgenden Nederland, V&VN) initiated a campaign to improve influenza vaccine coverage among health care professionals. So far, education and campaign materials have been made available for all health care institutions.

These improvements and initiatives come along with a public discussion about vaccine policy in general. For example, the measles outbreak in Europe has led to public discussion about mandatory vaccination of children attending daycare (19). As unvaccinated children pose a risk of introducing measles into the daycare center, parents and policymakers discuss the obligation of protecting an individual child and its playmates that are attending the same daycare center, or the parents' right to choose what they think is best for their kids. In the daycare settings, how should we weigh a parents' right not to vaccinate their child against the rights of parents to a safe environment for their young children until vaccination can effective protect their child? Similarly, in healthcare, the discussion regarding influenza vaccination for health care workers and the patients they care for is in the same spectrum. So why do healthcare professionals refrain from yearly influenza vaccination?

Impediments for healthcare workers are, among others, their own good health status, the fact they do not perceive symptomatic influenza virus infection as a problem themselves, accessibility, or time constraints. A mandatory influenza vaccine for health care workers could overcome the majority of these issues but does not seem feasible in the Netherlands with respect to employee autonomy. In the United States, temporary mandatory influenza vaccination has resulted in a sustained high influenza vaccine coverage among health care workers (20), even after stopping the mandatory nature of the vaccination.

Another explanation may be that it is not vaccination in general that is the issue that causes hesitancy, but rather the influenza vaccine for which vaccination needs to be repeated every year. The lack of sustained protection and the lack of assurance about a protective effect may cause restraint among health care workers. However, currently the vaccination is the best we have.

These factors could be equally important since hepatitis B vaccination has never led to much controversy in the Netherlands, and coverage is between 85 and 93% among

European countries (21). Introduction of the quadrivalent influenza vaccine during the 2019 influenza vaccination campaign may improve both protection against the circulating influenza strains in the forthcoming season and the confidence of healthcare workers in this vaccine. A major improvement in protection is expected from universal influenza vaccines; these are however still in the early stages of development (22, 23).

Another target group in which improvement in vaccination coverage is needed is the increasing number of patients treated with immune checkpoint inhibitors (immunotherapy). In recent years, immunotherapy has become standard treatment for several malignancies across all tumor stages, for example, against lung cancer, melanoma, and head and neck cancer. The immune system plays a critical role in fighting off cancer by detecting and controlling the proliferation of malignant cells (24, 25). T-cells are key players in the anti-tumor immune response, and these cells have, therefore, been an important target for immunotherapeutic interventions. Tumor cells interfere with immune checkpoints on activated T cells to trigger inhibitory pathways that downregulate the intensity and the extent of the immune response, thus giving tumor cells a chance to proliferate. The anti-tumor response of the immune system can be enhanced by blocking these checkpoints with specific inhibitors. These immune checkpoint inhibitors have side effects reflecting their pharmacodynamic properties as they may lead to immune (activation)-related adverse events (e.g., encephalitis, colitis, pneumonitis) by 'overstimulation' of the immune system (26). Consequently, 17-54% of patients on various immune checkpoint inhibitors will have an (auto)immune-related adverse event (27).

Patients with cancer are often older and have (pulmonary) comorbidity and would, therefore, benefit from influenza vaccination. In addition, the influenza-like illness may lead to temporary interruptions of cancer treatment. Therefore, cancer patients, in particular the ones receiving chemotherapy, should be given yearly influenza vaccinations (28). This would apply for cancer patients treated with immune checkpoint inhibitors as well. However, in 2018, a Swiss research group observed an increased incidence of immune-related adverse events after influenza vaccination in a small cohort of patients treated with immune checkpoint inhibitors (n=23) (29). This observation has withheld physicians from advising influenza vaccination for their patients receiving immunotherapy.

In **Chapter 6,** we describe that seasonal influenza vaccination is safe in patients who are treated with immune checkpoint inhibitors. In our retrospective cohort of lung cancer patients treated with immunotherapy, there was no difference in immune-related adverse events between patients who received the influenza vaccination and the ones that did not. Moreover, our results were recently confirmed in a cohort of 370 patients receiving immune checkpoint inhibitors in New York. The incidence of immune-related adverse

events among influenza vaccine recipient was not higher than the incidence reported in previous reports (30). Although there are some methodological concerns about that study (31), both that and our study demonstrate that influenza vaccination can safely be administered to patients who are treated with immune checkpoint inhibitors. Along with the biological implausibility of increased immune-related adverse events triggered by influenza vaccination, these studies strongly advocate influenza vaccination for cancer patients receiving immunotherapy. Still, an Italian group is planning to conduct a prospective study to confirm our findings (32).

Interestingly, the study in New York also demonstrated a very low incidence of influenza virus infection in patients treated with immune checkpoint inhibitors who received influenza vaccination (30), lower than the incidence in the rest of the institution. This observation may be explained by the fact that these agents enhance vaccine-induced protection. In accordance, another study demonstrated a significantly higher seroconversion rate in patients with immune checkpoint blockade, indicating a more potent immune stimulation (29). Similarly, in a rhesus macaque model, an immune checkpoint inhibitor (PD-1 blockade) caused an increased T cell response after vaccination with adenovirus vectors encoding SIVgag (33). This enhanced T cell response could improve vaccine response and effectiveness. The exact mode of action (e.g., enhanced humoral or cellular responses?) still needs to be determined, and strategies to use this mechanism for vaccine improvement need to be evaluated in clinical trials.

In the group of patients on immunotherapy, the balance between too much inflammation leading to side effects, enough inflammation leading to a robust immune response against cancer and vaccine antigens, and too little immune response leading to tumor cell proliferation, is delicate. In any case, seasonal influenza vaccination can safely be advocated in cancer patients receiving immune checkpoint inhibitors.

Regarding influenza vaccination in health care workers, future studies should determine which (combination of) strategies are best to improve vaccination coverage, ideally up to 100%. For the patients on immune checkpoint inhibitors, assessment of the enhanced immune response against vaccination and its mode of action, will provide a basis to design (adjuvants for) an improved influenza vaccine.

EARLY DETECTION OF LOWER RESPIRATORY TRACT INFECTIONS: THE COMMUNITY PERSPECTIVE

Early detection of an infectious disease in an individual patient is essential to be able to initiate treatment as early as possible, to – as demonstrated in several studies – improve outcome. Early detection of an infection is, however, not only essential for the individual concerned but can also be beneficial for the community around this patient, by limiting ongoing exposure and spread of disease.

Dutch public health care authorities detect and monitor potential outbreaks of infectious diseases. In three diverse ways, curative care partners notify public health care authorities. First, microbiological laboratories and doctors are obliged to report infections from the list of notifiable diseases. Since a microbiological diagnosis is usually required, there is a time lag in this way of notifying diseases. Secondly, clusters of disease, for example diarrhea in institutions such as nursing homes, are reported to local public health care authorities. These outbreaks are most often local, affecting a single institution. Thirdly, any other unusual number of patients with a syndrome (a specific set of signs and symptoms) of likely infectious etiology that could potentially threaten public health, should be reported within 24 hours. In current practice, the third type of outbreaks is hardly ever reported.

In order to help preclude major regional outbreaks like the Legionella cluster in 1999 and the Q fever outbreak in the first decade of the twentieth century, and to automate the third pillar of the notification system, we developed and tested an automated, real-time cluster detection tool for infectious diseases. This Integrated Crisis Alert and REsponse System (ICARES) covers all regional health care facilities where patients would present with a new infectious disease (general practices and hospitals, 24/7 coverage). In **Chapter 2**, we describe that ICARES was able to detect and monitor local outbreaks of infectious diseases in real-time. We used the current coding systems in primary care (ICPC) and hospital setting (DBC/DOT coding for reimbursement from insurance companies). The codes in these systems represent syndromes. In addition to respiratory tract infections, i.e., a prevalent syndrome presenting to both GP and hospital with a seasonal pattern, we evaluated hepatitis and meningoencephalitis. These are less frequent infectious diseases without a clear seasonal pattern. Meningoencephalitis is a severe disease that is most likely diagnosed in hospitals, whereas a potential outbreak of hepatitis could be diagnosed at primary care or in hospital, depending on the type of outbreak.

ICARES demonstrated that it is possible to monitor and follow the numbers of patients with the three syndromes in real-time. During the study, ICARES detected a local outbreak of meningoencephalitis. Later, this small outbreak turned out to be part of a national

increase in the incidence of patients with enterovirus meningoencephalitis. Although the effort needed from general practitioners and hospitals to make the system work was limited, and the daily effort to check the ICARES dashboard by public health care authorities seemed well-arranged and limited, implementation of the ICARES tool proved difficult.

After completion of the ICARES study, we hypothesized that insufficient involvement of knowledge users during protocol development and execution of the study could have affected final implementation. Public health interventions are often complicated because the breadth of the public health base is vast, encompassing not only medical, but also social, political, economic, and cultural factors (34). The absence of a robust, automated, real-time cluster syndromic surveillance system in public health seems a critical omission. However, we may have assessed the public health need for such a swift and almost instantaneous notification system insufficiently. For instance, the system may put too much emphasis on the delay between syndromic surveillance and microbiological diagnostics, and uncertainties what to do in the interval. This may have contributed to insufficient implementation of ICARES in the daily practice of local health care authorities in the Leiden-The Hague area and may have hampered further efficiency study of ICARES in the Netherlands. In addition, the ICARES study team could have put more emphasis on effectiveness and implementation of ICARES by using accessible and preferred formats for public health workers (35).

The syndromic surveillance approach is nevertheless promising. Syndromic surveillance systems rely on automated data collection and analysis from various healthcare sources, for example, hospitals and general practitioners, on a near real-time basis. Most often, existing data are used. These systems monitor the spread and impact, or absence of impact, of known or as yet unknown events, often an infectious disease, in the population based on the presentation of signs and symptoms (36). As microbiological diagnostics take time and because for new, emerging infections diagnostic tests are not readily available, signs and symptoms are the first expressions of disease. In case this involves multiple patients, these could be the first signs of a potential outbreak. Syndromic surveillance appears to be a useful tool for public health preparedness in multiple settings. Larger outbreaks, such as influenza, are consistently detected in a timely manner. However, the data source determines what kind of outbreak can be detected, and the performance of the syndromic surveillance may vary geographically and seasonally (37). Syndromic surveillance is also useful to provide real-time data about the burden of disease, in particular to reassure policymakers and the public during an outbreak with only a marginal burden of disease (38). Besides surveillance properties, public health requires credible and rapidly available information to allow informed decisions on response and control of emerging (infectious) threats (39).

During the ICARES project, another use for our syndromic surveillance tool became evident. In 2015, the National Institute for Public Health and the Environment in the Netherlands (RIVM) started the project 'Severe acute respiratory infections, the missing link in the surveillance pyramid'. In line with recommendations of the World Health Organization (WHO) and the European Centre for Disease Prevention and Control (ECDC), this project developed and implemented sustainable surveillance of severe acute respiratory infections (SARI) in the Netherlands (40). As most preventive strategies are aimed at reducing the burden of disease in the most severely ill patients, SARI surveillance is required to monitor this. From that perspective, SARI surveillance has added value to surveillance of influenza-like illness (ILI) in general practice. Because syndromic surveillance is useful to detect and monitor respiratory infections, we adapted the ICARES tool to provide syndromic data from two regional hospitals to this SARI surveillance (37).

In **Chapter 3**, we describe the differences in incidence in ILI in general practice and SARI in hospitals. Interestingly, in the majority of respiratory infection seasons, the peak in incidence in severe acute respiratory infections (SARI) in hospitals precedes the peak in primary care (ILI). Reasons for this are unclear from our study, and several hypotheses should be evaluated. For instance, we hypothesize that other viruses than influenza could contribute to the early peak in SARI patients. New data confirming the specific viral cause are needed to determine whether this hypothesis is correct. As the source of our data were DBC codes, SARI surveillance does not provide information about the causative agent. In new outbreaks, early disease confirmation is paramount to initiate an adequate response. The various causative agents may have different sources and thereby require different control measures. An association between the peak in SARI incidence and microbiological surveillance systems, such as the national virologic surveillance (41) could be evaluated retrospectively. This does, however, not provide causal relation between the cases from the SARI surveillance and the virologic data since a patient identifier is unavailable in the latter system. Also, historical data do not allow a response to an outbreak or epidemic, and annual peaks may not be caused by the same pathogen every year. A new prospective design would be more practical, with syndromic surveillance for early detection and monitoring of the burden of disease combined with microbiological results of the individuals with the syndrome to allow appropriate measures for source detection and response. However, to build an automated link between the individual patient who is part of a cluster of cases, and their microbiological test results, is controversial. Within the hospital data in ICARES, an encrypted patient identification number is enclosed. Encryption ensures that the data do not contain identifiable patient information. Only the principal investigator at the hospital is able to decrypt these codes. Privacy concerns could become an obstacle for this linkage (42).

In addition, the demand for hospital admission is high in the frailest, high-risk part of the population who are infected with a respiratory virus. Yearly influenza epidemic coincides with an increase in mortality in the elderly (>65 years of age). Influenza is very likely an important contributor to the observed excess mortality among the elderly (43). SARI surveillance incorporating demographic data of individual patients may contribute to the understanding of the presumed causal relation between respiratory virus infection, i.e., influenza, and the excess in mortality during flu season.

Moreover, influenza dynamics may vary between different age groups (44). Transmission dynamics in the elderly are different from that in other age groups. Elderly in a long-term care facility may transfer respiratory virus infections readily to their roommates, thus facilitating a small and more severe peak in influenza incidence. The frail elderly subgroup is the group that is likely to visit a hospital. The frailest are likely the first to present with disease, and this may be (part of) the explanation why the hospital peak in SARI cases precedes the peak in influenza-like illness cases in the community.

Prospective studies on dynamics of ILI and SARI incidence should validate our finding. In addition, understanding of the differences are important to target preventive strategies in the future. Outbreak detection and follow up with syndromic surveillance could be improved when microbiological results are added. Research using these two data sources is necessary, for example in regions with emerging infectious diseases.

EARLY DETECTION OF LOWER RESPIRATORY TRACT INFECTIONS: THE PRIMARY CARE PERSPECTIVE

The individual patient with an acute lower respiratory tract infection in primary care is only mildly to moderately ill and can usually be treated and managed by the GP, at home. Respiratory tract infections in this setting are most often viral and are self-limiting. Viral respiratory tract infection often presents as upper respiratory tract infection or as bronchitis, a manifestation of lower respiratory tract infection. These infections have an excellent prognosis, and a wait-and-see strategy is generally appropriate (45-47). Another form of lower respiratory tract infection is pneumonia. This type of disease is often caused by bacteria and therefore, pneumonia frequently requires antibiotic treatment. However, in primary care patients with an acute lower respiratory tract infection, the differentiation between the ones who benefit from antibiotic treatment, i.e., having pneumonia, and the ones that do not, i.e., having bronchitis, is difficult. Clues to determine the diagnosis are needed. Unfortunately, history and physical examination lack sensitivity and specificity to diagnose pneumonia (48). Recently, several studies evaluated the use of biomarkers

to determine their added value in combination with clinical characteristics to positively diagnose or rule out pneumonia (49). Compared to procalcitonin (PCT) and midregional proadrenomedullin (MR-proADM), C-reactive protein (CRP) proved to be the only useful predictor for the presence of pneumonia on a chest X-ray (**Chapter 5**). However, none of the various prediction rules for pneumonia have sufficient sensitivity and specificity to predict pneumonia (50, 51).

In the Dutch guideline 'Acute Cough', a diagnostic algorithm is defined to help the GP to identify the patients with an acute respiratory tract infection who would benefit from antibiotic treatment and the ones who would not. The most severely ill patients with abundant inflammation most likely have bacterial pneumonia and should, therefore, be treated with antibiotics. Mildly ill patients most likely have viral disease and a wait-and-see strategy without antibiotics is recommended. The moderately ill group is the most challenging group to select appropriate treatment for. In these, a low CRP can exclude pneumonia with reasonable certainty, irrespective of history, comorbidity, and physical examination, while an elevated CRP greatly increases the chance of pneumonia (51, 52). A recent meta-analysis ascertained that even when clinical variables are taken into account, the CRP test can help to confirm or exclude pneumonia (49). For these reasons, a CRP test is indicated in the Dutch guideline in moderately ill patients. A low CRP value (< 20 mg/l) rules out pneumonia; these patients should not be treated with antibiotics. On the other hand, a high CRP value (> 100 mg/l) makes pneumonia more likely and these patients should be treated with antibiotics. With intermediate results (CRP values between 20 and 100 mg/l), the decision whether or not to start antibiotics is left to the clinician evaluating the clinical presentation and risk factors for a worse outcome (8). Studies that evaluate whether the CRP point-of-care test reduces the number of antibiotic prescriptions show variable results (53, 54).

The 'gold standard' for establishing pneumonia is the chest X-ray. A chest X-ray in outpatients, however, does not improve outcome (55, 56). Moreover, a chest X-ray is not readily available in primary care; patients must be referred to a hospital. For these reasons, a chest X-ray is not routinely recommended in patients attending their general practitioner (GP) with suspicion of community-acquired pneumonia. General practice guidelines do not provide clear guidance when to order a chest X-ray in specific patients with acute respiratory infections (8, 57). Despite that, in 22% of patients with a suspected lower respiratory tract infection, a chest X-ray is requested (58).

In **Chapter 4** we describe the use of the above additional diagnostic tests among GPs in the Netherlands. GPs who have the CRP test at their disposal (54% of the GPs in our study) tend to request fewer chest X-rays. This is in line with a previous Scandinavian study (59).

The most important reason to request a chest X-ray is to confirm or rule out other abnormalities than pneumonia. Lung cancer was the most frequently reported condition GPs wanted to exclude. Uncertainty about the presence or absence of pneumonia is not the most frequently used reason. GPs feel quite confident about their diagnosis of the respiratory tract infection, based on clinical signs and symptoms, with or without CRP test. Still, GPs overestimate the pre-test chance that a consolidation will be present in the patients that they refer for chest X-ray. The overestimation in this subgroup of patients is however not reflected in the overall antibiotic prescribing behavior of Dutch GPs. Antibiotics are used more restrictively by GPs in the Netherlands than by many of their colleagues in other European countries (60).

It would be of interest to determine the added value of a biomarker in the patients with an acute respiratory tract infection who are referred for other reasons than to confirm or rule out pneumonia. A consolidation on the chest X-ray in these patients would compromise the detection of other pathologies, such as a lung tumour. If clinical signs and symptoms combined with a biomarker, result in a high pre-test chance of the presence of pneumonia, it would be feasible to initiate antibiotic treatment and postpone the chest X-ray a few weeks until the suspected pneumonia has resolved and a potential malignancy can be ruled out or confirmed more confidently.

In the study described in **Chapter 5**, we evaluate a cohort of patients with an acute respiratory tract infection who had been referred by their GP for a chest X-ray, so that we could identify predictive factors for the presence of pneumonia. The findings of this study might have been complicated by the inhomogeneous patient population at the radiology department if a considerable proportion was not referred to confirm or rule out of pneumonia. However, this study only included patients for whom the GP asked to determine the presence or absence of pneumonia. If the chest X-ray has been requested to exclude other pathology, the GP will, in 90% of the cases, state this on the X-ray application form.

We demonstrated that CRP measurement, in addition to clinical signs and symptoms, did not improve prediction of pneumonia in patients who were subsequently referred for chest X-ray. However, CRP measurement did help to guide antibiotic treatment; from the group with a moderate chance (2.5-20%) of having pneumonia, 23 out of 146 (16%) were reclassified in the high risk (>20%) group warranting antibiotic treatment.

Based on the Dutch guideline ‘Acute Cough’ and the results of these two studies, the guidelines for additional diagnostic testing in primary care to confirm or rule out pneumonia need further improvement. First, moderately ill patients with an acute respiratory tract infection with an intermediate CRP level (20-100 mg/l) may benefit a from chest X-ray. As

the decision to start antibiotic treatment was left to the physician and comorbidity should guide the start or withholding of antibiotic treatment, this subgroup of patient needs more robust guideline. Insight in current antibiotic usage for these patients would elucidate the potential for treatment improvement and good antibiotic stewardship.

Secondly, in patients with an acute respiratory tract infection for whom the GP would currently request a chest X-ray, low-risk patients (based on signs and symptoms only) actually do not have pneumonia. In patients with intermediate risk, the CRP test can improve the decision whether or not to prescribe antibiotics since a substantial proportion (16%) of this subgroup is reclassified as belonging in the high-risk group.

Finally, the intermediate groups are the most difficult to diagnose pneumonia in and to decide for whether to prescribe antibiotic treatment. The informed decision to initiate antibiotic treatment is equally important to the informed decision to withhold antibiotic treatment. Antibiotics have side effects, and stewardship is the most important strategy to keep infections treatable in the (near) future. Antibiotics are used more restrictively by Dutch GPs than by their European colleagues (60). These differences are an expression of the complexity of the consideration of whether or not to prescribe an antibiotic but also an expression of cultural differences. As an example, a Swiss group presented the results of an intervention trial to demonstrate that their biomarker-based therapeutic strategy compared to standard care could reduce antibiotic use in patients with a lower respiratory tract infection. They reported significantly reduced mean duration of antibiotic treatment from 13 to 11 days (61). In the Netherlands, however, standard treatment duration of community-acquired pneumonia is only five days. Therefore, results of their and our findings are difficult to extrapolate to other settings but GP's in the Netherlands, who use antibiotics prudently, should aim to improve local policy further to improve care for our own patients and to serve as a best practice example for other communities.

Future studies, targeting patients at the general practice, should identify the patients who benefit from chest X-ray. In our questionnaire GPs reported to use CRP test for other indications than an acute respiratory tract infection. Apparently, there is clinical need for a biomarker to support the decision making in this patient category and future studies are needed to determine sensitivity and specificity for the diagnosis, the indication for antibiotic use and prognosis.

STRATEGIES TO ATTENUATE THE IMMUNE RESPONSE

Although the immune response against a microbe is an essential component of the host response to help overcome an infection, an uncontrolled or overwhelming inflammatory response may be associated with serious acute lung injury and consequently, severe morbidity and mortality (6, 7). Strategies to attenuate this immune response without interfering with the antimicrobial effect, focus on early initiation of treatment and concomitant anti-inflammatory interventions.

In patients with an influenza virus infection that are severely ill and need hospital admission, i.e., patients with severe acute respiratory tract infection (SARI) caused by influenza, morbidity and mortality are significant. In a cohort of 390 patients admitted with influenza virus infection, described in **Chapter 8**, median length of hospital stay was 5.0 days, 70 patients (18%) needed to be admitted to the ICU, and 30-day mortality was 30 out of 390 (7.7%). In a recent report from Spain, mortality was 12% in patients hospitalized with influenza virus infection (62).

The time window for the treatment of influenza-infected patients has been regarded as very small since treatment of otherwise healthy volunteers ≥48 hours after first symptoms has no added benefit compared to no treatment (63, 64). In these patients with relatively limited inflammation, delayed initiation of treatment that stops viral replication would not significantly attenuate inflammation and thereby time to clinical resolution. However, patients hospitalized with influenza virus infection may represent a distinct group with prolonged viral replication and a more pronounced inflammatory response. In these patients, the therapeutic time window may be larger, and inhibition of ongoing viral spread in the (lower) respiratory tract by neuraminidase inhibitors could perhaps lead to attenuation of inflammatory response and more rapid recovery. For instance, younger patients that were admitted with H1N1pdm09 influenza virus infection had reduced mortality when neuraminidase inhibitor treatment was initiated within 48 hours after the start of symptoms, but this effect remained, although less pronounced, until treatment initiation within five days after symptom onset (65).

The effectiveness of delayed initiation of neuraminidase inhibitor treatment in patients with seasonal influenza who are elderly, frail, or immunocompromised and at high risk for developing complications, is unknown. During the influenza season, this remains a daily challenge since the majority of these patients present to a hospital with symptoms that have been present for more than 48 hours (65, 66).

In **Chapter 8**, we describe the benefit of starting oseltamivir treatment within 48 hours after hospital admission rather than after start of first symptoms. Patients with seasonal influenza virus infection who need hospital admission are either severely ill or vulnerable due to comorbidity. With a propensity score model, we found that oseltamivir treatment significantly reduced 30-day mortality, as well as the composite endpoint of ICU admission >48 hours after hospitalization or 30-day mortality. There was also a trend in reduced length of stay. An importantly distinct improvement in the patients treated with oseltamivir was present in the subgroup with pronounced ongoing viral replication and inflammation, represented by the presence of pneumonia on chest X-ray.

Our study is the first study examining the benefit of oseltamivir treatment in the Dutch healthcare system. In the Netherlands, general practitioners are important gatekeepers for hospital care. This is one of the reasons why hospitalized patients represent only the tip of the iceberg of all seasonal influenza cases (67). In the Netherlands, patients are not admitted unless they have severe influenza disease, exacerbation of comorbid illness, or when they are vulnerable, e.g., due to comorbidity.

In this study, with three large hospitals and over three influenza seasons, we included elderly patients with comorbidity and severe disease (16% was admitted to the ICU within 48 hours after hospital admission, 48% had a CURB-65 score ≥2). The median time of hospital admission after symptom onset was 4.0 days. Our cohort appears to be an excellent representation of the total burden of hospitalized influenza patients in the Netherlands. The findings of a reduction of 9% in 30-day mortality, 11% in the combined endpoint 30-day mortality or ICU admission > 48 hours after hospital admission, and the trend in reduced length of hospital stay (2 days) are in line with the findings in a large meta-analysis in 2014 (65). The magnitude of the effect, the consistency and precision of the results, and robustness of the evidence (68) contribute importantly to the quality of evidence for the benefit of oseltamivir treatment in hospitalized patients with influenza.

In only 35% of patients in our cohort, oseltamivir was initiated within 48 hours after hospital admission. This low percentage reflects the current lack of confidence that many Dutch clinicians have on the level of evidence supporting treatment in these patients who present >48 hours after symptom onset. Despite the biological plausibility of the benefit of oseltamivir treatment in severely ill patients, the lack of randomized controlled trials has been an important reason for the ongoing debate about the presence or absence of clinical benefit of oseltamivir in hospitalized patients. With these new findings, however, we should work on the implementation of oseltamivir treatment in patients admitted with influenza virus infection. In the near future, awaiting better treatment options, all patients admitted with influenza virus infection should be treated with oseltamivir.

A severe complication of influenza virus infection is the development of acute respiratory distress syndrome (ARDS). ARDS is an inflammatory response with epithelial and alveolar cell damage leading to bilateral opacities on chest X-ray with marked hypoxia occurring within seven days after a clinical insult (69, 70). Since the 2009 H1N1 influenza outbreak, numerous reports appeared indicating that influenza virus infection may in rare cases cause ARDS (71, 72). Of note, ARDS can occur after many other unrelated triggers as well, for example, sepsis, trauma, inhalation of exogenous toxins, or major surgery (73). ARDS is likely caused by the occurrence of several, sequential hits to the lung (74, 75). We hypothesized that a combination of these triggers, including a subclinical influenza infection, would increase the risk of ARDS.

In a cohort of 2013 patients undergoing cardiac surgery, described in **Chapter 10**, 6% developed postoperative ARDS. We wanted to determine whether a concomitant influenza virus infection was an additional risk factor for ARDS. Unfortunately, in none of the patients in this cohort respiratory virus diagnostics had been done preoperatively, as none had preoperative symptoms that justified such diagnostics. However, the majority of influenza virus infections be it symptomatic or not, occur during the yearly influenza season. Therefore, we used the influenza season as a proxy for influenza virus infection. The influenza season is defined by high numbers of patients (>51/100,000) with influenza-like illness visiting their general practitioner and influenza virus detected in respiratory samples (76, 77).

In **Chapter 10**, we describe the observation that cardiac surgery during influenza season is indeed a risk factor for postoperative ARDS. In a retrospective database, we compared cardiac surgery during various seasons and adjusted for potential confounders. The odds ratio for ARDS in the influenza season compared to baseline season (with few cases of influenza-like illness) was 1.85 (95% confidence interval 1.06-3.23). There was a trend in the increase in absolute length of ICU stay (21 hour increase, p=0.07), and time on mechanical ventilation (23 hour increase, p=0.05). Furthermore, the number of ARDS cases increased in severe influenza seasons when Influenza A and B virus circulation in the community was increased. With these data, we show that influenza virus infection (or, less likely, other respiratory virus infections) could be a risk factor for ARDS after cardiac surgery.

During the influenza season, the majority of infected persons remains asymptomatic (78). Therefore, asymptomatic elective cardiac surgery patients can also be infected with influenza. In our hypothesis, asymptomatic viral infection may prime the lungs, leading to increased incidence of ARDS postoperatively. Several studies demonstrate the importance of priming of lung endothelium leading to endothelial activation and vascular leak after a second hit. For example, evidence from another setting showed that influenza virus

infection predisposes to ARDS upon exposure to *S. aureus* (79). In a rat model, an intratracheal LPS immune stimulus before pneumonectomy caused aggravated lung injury in the contralateral lung compared to rats who were not primed with LPS (80). After proof of the causal relation between (asymptomatic) influenza virus infection and ARDS after cardiac surgery, studies to unravel the pathophysiological mechanisms, and studies to assess preventive measures such as adequate vaccination uptake or viral diagnostics at the day of surgery are needed.

Pathophysiological similarities may exist between CMV (and other Herpesviridae) reactivation during critical illness and ARDS after cardiac surgery in influenza virus-infected patients. Critically ill patients suffer from a certain degree of immune paralysis, an immunodeficient status characterized by T cell immunosuppression and alteration of NK cell function (81, 82). This immune suppression may facilitate CMV reactivation but could also enable (influenza) virus replication, thus causing asymptomatic (influenza) virus infection to become symptomatic, tissue destructive, and thereby the second hit for ARDS development.

Similarly, asymptomatic rhinovirus infection is detected in 14-50% of children (83). Children with upper respiratory tract infection, with documented rhinovirus infection at the time of cardiac surgery, were found to have more postoperative (respiratory) complications (84, 85). In these cases, surgery seems more of a second hit, after rhinovirus infection.

If the causal relationship between asymptomatic viral infection and ARDS after cardiac surgery is confirmed, strategies to help prevent at least part of the ARDS cases and improve outcome are needed. This should be the focus of future studies.

In this scenario, improved influenza vaccine effectiveness and improved influenza vaccination coverage among patients who are scheduled for cardiac surgery might prevent ARDS after cardiac surgery. Among patients with cardiovascular diseases, i.e., the ones that could undergo cardiac surgery, vaccination coverage is declining and was only 61% in 2017 (86). Viral diagnostics (and, if positive, postponement of surgery) on the day of cardiac surgery seems logistically challenging. In a small cohort of 69 asymptomatic children, preoperative screening for respiratory viruses was not an effective strategy to predict infants at risk of complications after cardiac surgery (87).

Infection is defined as a noticeable immunological reaction, be it the formation of antibodies, the demonstration of an elicited cellular response, and commonly in the acute phase, a local inflammatory response. In some infections, this inflammatory response appears abundant and ill-directed, and strategies to attenuate this response might

improve outcome. For instance, in pneumococcal meningitis, dampening of the local inflammatory response at the level of the meninges and brain improves outcome of the infection. In other settings, these effects are less clear. For instance, in cells from patients with community-acquired pneumonia, macrolides have a positive immune modulatory effect by enhancement of the antibacterial effect of neutrophils and by "quashing the immune response after bacterial killing" (88, 89). However, this effect noted in vitro was not observed in a clinical trial in which β-lactam monotherapy was non-inferior to macrolide with β-lactam combination therapy (3). Concomitant corticosteroid immune suppression during antimicrobial therapy of community-acquired pneumonia would improve short-term but not long-term outcome measures but at the cost of a large number of side effects. It is not recommended in treatment guidelines (90, 91).

Similar to the small margin between appropriate inflammation that led to killing the microorganism, and an overwhelming response causing severe collateral damage, the margin of adjunctive immunosuppressive therapy during infection seems limited. To improve outcome in infectious diseases, we should target our therapy not only at killing microbes but also at attenuation of the immune response, without losing its antimicrobial properties, to reduce collateral damage, i.e., morbidity, long term sequelae, and mortality. Thus, we need to gain insight into the meaning of immune-reactive biomass (i.e., the load of immune-reactive components released of viruses or bacteria) as opposed to the arrhythmic of infection by enumeration of bacterial or viral numbers, to better understand what exactly trigger a specific degree of inflammation. This degree of inflammation is usually assessed using a clinical scoring system, with or without addition of biomarkers (**Chapter 5**). Clearly, the actual immune-reactive biomass is much more difficult to define and grasp than determining the mere presence of living or death bacteria during treatment, in localised infections such as pneumonia.

Taking the serum lipoteichoic acid concentration as measure to assess immune-reactive biomass in patients with pneumococcal pneumonia, we used Toll-like receptor 2 (TLR2)-transfected Human Embryonic Kidney (HEK) 293 cells. These cells respond in vitro by IL-8 release after binding of pneumococcal cell wall components to the TLR2. IL-8 release after exposure of the cells to plasma samples from patients can be measured quantitatively, thereby determining pneumococcal cell wall load, i.e., the immune-reactive biomass. Studies using TLR2-transfected HEK293 cells have focused on signalling, for example in *Burkholderia* infections, but have not assessed these cells as sensors for immune-reactive biomass (92, 93).

In **Chapter 9**, we describe the PRISTINE (Pneumonia treated with RIfampicin aTtenuates Inflammation) study in which we have tried to determine the pneumococcal immune-

reactive biomass in patients with pneumococcal pneumonia and targeted this immune-reactive biomass with an antimicrobial immune modulator rifampicin. Proinflammatory bacterial cell wall components are released when bacteria are killed by autolysis or host immune cells and are important determinants of the severity of inflammation (94). An acute break down of bacterial cell wall occurs upon exposure to β-lactam antibiotics, lysing the bacteria (95). β-lactam antibiotics are the first-line treatment for pneumococcal infections in many guidelines (96). A method to potentially attenuate the immune response is to kill the bacteria without immediately lysing them, thus preventing the release of proinflammatory cell wall products (97). This approach would reduce the complete inflammatory trigger by interfering at the beginning of the inflammation cascade. In vitro studies showed that non-lytic rifampicin antibiotic treatment results in less release of LTA and other pro-inflammatory compounds from *Streptococcus pneumoniae* than the β-lactam antibiotics ceftriaxone or meropenem, despite similar bacterial killing effects (98). In animal models, rifampicin was beneficial as it reduced both the release of bacterial cell wall components and animal mortality (99). Non-lytic killing could be an immune-reactive biomass-targeted treatment to attenuate inflammation in pneumococcal infections.

In the appendix of **Chapter 9**, results of IL-8 release from TLR2-transfected HEK293 cells as sensor of immune-reactive biomass are described. In vitro, purified LTA could be determined quantitatively, but LTA/pneumococcal cell wall components could not be detected in plasma. This can be explained by the lack of measurable plasma concentrations of LTA both before and shortly after the start of treatment. In addition, an inhibiting effect of human plasma may contribute to the low immune response. We were able to detect IL-8 release from TLR2-transfected HEK293 cells in a pneumococcal empyema sample and in two meningitis (CSF) samples, in which bacterial load is obviously higher.

The PRISTINE trial, described In **Chapter 9**, assessed whether treatment with non-lytic rifampicin in addition to β-lactam for pneumococcal pneumonia could attenuate the inflammatory trigger, i.e., lipoteichoic acid (LTA) release from the bacterial cell wall. Despite solid in vitro and experimental animal research evidence, we failed to demonstrate differences in plasma LTA concentrations, subsequent inflammatory responses, and clinical responses in this pilot study. Apparently, the model we chose was not sensitive enough to reveal such differences, or alternatively, the hypothesis is simply not correct in humans. Besides the reasons above why we could not determine TLR2 response with patient plasma samples, this could be explained by the observation that the β-lactam treatment was given shortly after (or even before) rifampicin treatment. As we could not use rifampicin monotherapy, this may have hindered proper comparison between lytic and non-lytic therapy. Consequently, the killing of the streptococci may well have been

induced by the lytic β-lactam antibiotic. And this may have obscured the detection of a potential difference.

Based on our results, we hypothesize that LTA concentration is high at the site of infection but low in plasma. Therefore, the non-lytic killing of gram-positive microorganisms might still be beneficial in infections with abundant local inflammation and subsequent local tissue damage. This would be the case in pneumococcal meningitis. For this infection, attenuation of the inflammatory response by reducing the trigger, i.e., cell wall components, before and more importantly after the start of treatment, could decrease morbidity, long term sequelae, and mortality. In future studies with clinical endpoints, the inflammatory response should be followed up at the site of infection, i.e., in liquor samples instead of plasma samples.

Nevertheless, in severe pneumococcal pneumonia or other gram-positive infections, non-lytic antibiotic treatment can still be a strategy to decrease inflammation and to improve outcome. However, rifampicin has a considerable number of potential side effects, has interaction with multiple other drugs after several days of treatment, and monotherapy could lead to resistance during treatment. These important drawbacks may hinder rifampicin from being the most attractive non-lytic antibiotic drug. New drugs in development should lack these disadvantages and would then be more suitable candidates for killing gram-positive microorganisms without causing an overwhelming immune response. In general, this should apply for all antimicrobials in development. New drugs should not only kill (resistant) microorganisms, but their mechanism of action should also reduce the inflammatory response. If we combine antimicrobial and anti-inflammatory properties in one drug, immune-reactive biomass-targeted therapy would less likely lead to side effects or unintentional immune suppression.

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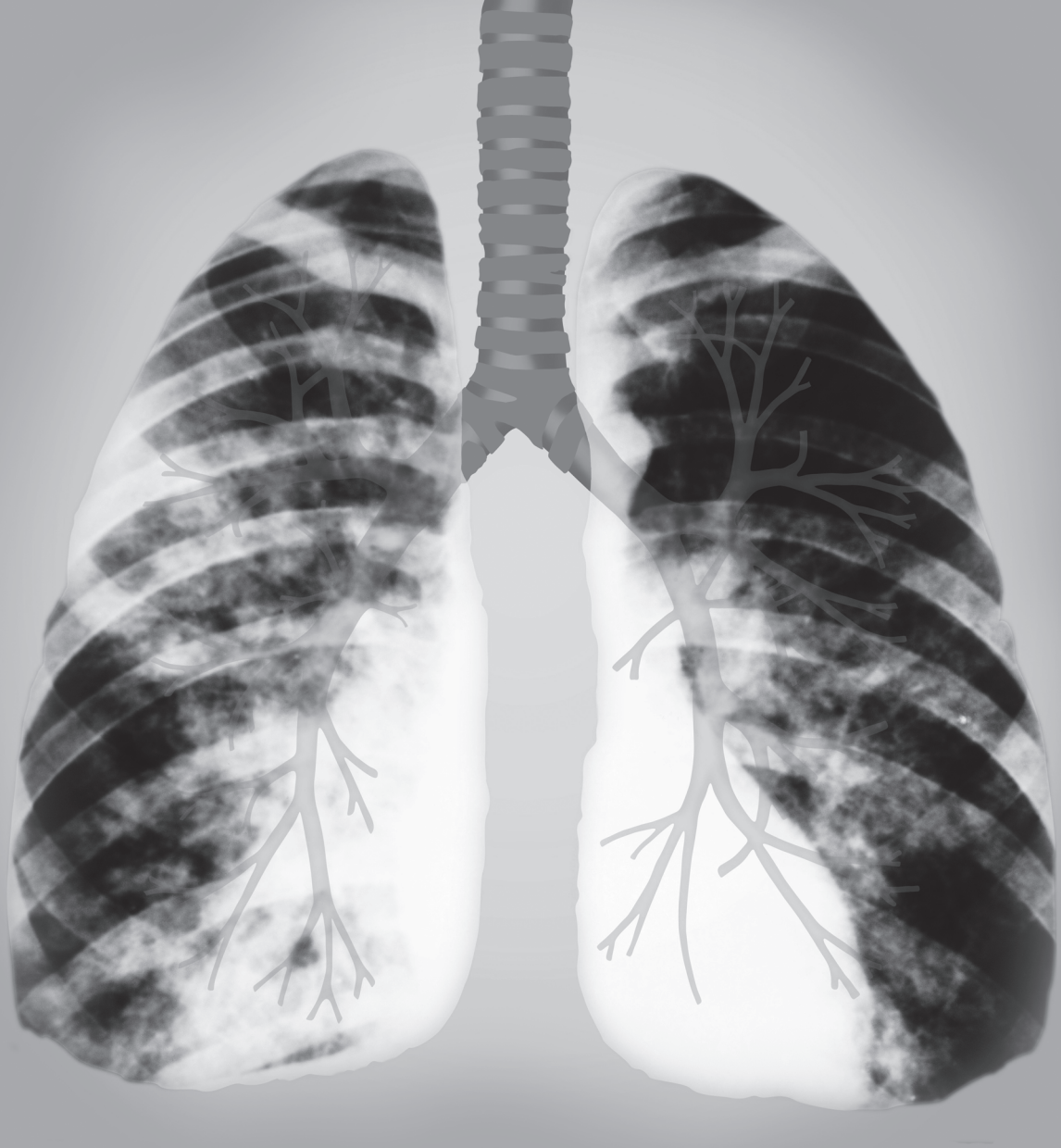

Nederlandse samenvatting

SAMENVATTING

Luchtweginfecties zijn een van de meest voorkomende infecties waarvoor mensen een dokter raadplegen. Deze infecties worden gekarakteriseerd door micro-organismen die de luchtwegen binnendringen en daar een ontstekingsreactie veroorzaken. Lage luchtweginfecties, de infecties onder het niveau van de stembanden, worden onderverdeeld in longontsteking en bronchitis. In Nederland worden luchtweginfecties meestal veroorzaakt door een virus of een bacterie. In sommige gevallen is er een gecombineerde infectie, met zowel een virus als een bacterie. Jaarlijks worden in Nederland ongeveer 50.000 mensen opgenomen met een acute lage luchtweginfectie (www.zorgatlas.nl). Wereldwijd zijn lage luchtweginfecties de derde doodsoorzaak; in 2016 stierven er circa 3,0 miljoen mensen aan de gevolgen van een luchtweginfectie.

Het optreden, beloop en uitkomst van een infectie wordt bepaald door de complexe interactie van gastheer, micro-organisme en omgeving. Ook bij patiënten met een acute luchtweginfectie spelen karakteristieken van de ontstekingsreactie van de gastheer op de specifieke microbiële verwekker een bepalende rol in het verloop van de ziekte. Een ongecontroleerde ontstekingsreactie kan resulteren in 'collateral damage' aan het longweefsel en in ernstige gevallen overgaan in acuut long letsel (zoals 'shocklong' ofwel ARDS – acute respiratory distress syndroom). ARDS leidt tot ernstige morbiditeit en kent een hoge sterfte. De gastheer moet de ontstekingsreactie op zodanige wijze titreren dat een delicaat evenwicht wordt gevonden tussen een ontstekingsreactie die afdoende is om de verwekker te elimineren en een zo beperkt mogelijke weefselbeschadiging. Met andere woorden, het geïnfecteerde deel van de luchtwegen moet met succes gesteriliseerd worden zonder blijvende restschade van longweefsel.

Tijdige en adequate behandeling is een van de beste voorspellers van de uitkomst van longinfecties. In essentie richt deze strategie zich op de beoordeling van de intensiteit van de ontstekingsreactie van de gastheer als voorspeller van beloop en mogelijke ontsporing van de infectie. Hiertoe combineert de arts medische informatie over de onderliggende status van de gastheer (bijvoorbeeld zijn/haar afweer en co-morbiditeit), de huidige status van de gastheer (de mate van ziek zijn) en bepaling van biomarkers als kwantitatieve afspiegeling van de ontstekingsreactie (bijvoorbeeld C-reactief proteïne of procalcitonine). De arts beoordeelt deze medische informatie samen met epidemiologische gegevens over het voorkomen van ziekteverwekkers (bijvoorbeeld klachten die zich voordoen in het jaarlijkse influenza seizoen, of in aansluiting op een vakantie). Op grond hiervan neemt de arts een besluit tot een prompte empirische therapie of slechts een afwachtende houding en opvolging, thuis of in het ziekenhuis.

Kortom, hoewel het misschien het beste is om een infectie volledig te voorkomen door bijvoorbeeld vaccinatie, zullen de inspanningen van artsen gericht zijn op het tijdig opsporen van potentieel ernstige luchtweginfecties. De arts moet dergelijke infecties onderscheiden van onschuldige infecties, en ten slotte, bij ernstige infecties adequate behandeling starten zonder overmatige weefsel- en restschade op te laten treden. De meeste van deze aspecten worden behandeld in dit proefschrift, waarvan sommige in detail, en worden in de volgende paragrafen samengevat.

PREVENTIE DOOR VACCINATIE

In **hoofdstuk 7** bespreken we het intensieve griepseizoen 2017/2018 en de gevolgen van de lage vaccinatiegraad onder zorgmedewerkers. De vaccinatiegraad tegen griep is laag onder zorgmedewerkers. Dit geldt voor zowel artsen als verpleegkundigen. In Europa was deze vaccinatiegraad onder de 30% en in Nederlandse ziekenhuizen was de vaccinatiegraad slechts 13% (bepaald in 2012). Door een toestroom van patiënten met griep en de complicaties daarvan en de afgenomen opnamecapaciteit van ziekenhuizen door absentie van zorgpersoneel door griep, waren veel ziekenhuizen in het seizoen 2017/2018 overbelast. In dit hoofdstuk wordt een pleidooi gehouden voor 100% griepvaccinatie onder zorgmedewerkers om ziekteverzuim te voorkomen op momenten dat de vraag naar zorg in het griepseizoen, groot is. Niet alleen persoonlijke bescherming en collegialiteit spelen een rol: de griepprik voor zorgmedewerkers heeft ook een positief effect op de patiëntveiligheid. De kans dat een kwetsbare patiënt griep krijgt van een niet-gevaccineerde zorgmedewerker wordt immers geminimaliseerd.

Zorgprofessionals moeten samen met ziekenhuis- en instellingsbestuurders én bedrijfsartsen het gesprek aangaan met twijfelende collegae die als zorgmedewerker contact met patiënten hebben en hen met feiten en juiste argumenten overtuigen. Deze discussie is nog onvoldoende breed en indringend gevoerd waardoor een vaccinatieplicht – zoals wel toegepast in de Verenigde Staten – op dit moment in Nederland een te ingrijpend middel is.

Ook bij patiënten die met zogenaamde checkpoint remmers (een vorm van immuuntherapie) worden behandeld, moet de griepvaccinatiegraad verbeterd worden. De afgelopen jaren is immuuntherapie onderdeel van de standaard behandeling geworden voor diverse tumoren in verschillende stadia, bijvoorbeeld voor longkanker, melanoom en hoofd- en halstumoren. Het immuunsysteem speelt een belangrijke rol bij de natuurlijke afweer tegen kanker, en bovengenoemde behandelingen zijn erop gericht de afweerreactie tegen de tumorcellen te versterken. Een bekende bijwerking van deze immuuntherapie betreft

de ‘overstimulatie’ van de afweerreactie die hierbij kan optreden, met als gevolg een auto immuun ontsteking van bijvoorbeeld long, dikke darm of schildklier. Van alle patiënten die met checkpoint remmers wordt behandeld, krijgt 17 tot 54% een dergelijke bijwerking.

Patiënten met kanker komen vaak in aanmerking voor de jaarlijkse griepvaccinatie, al was het maar om onderbreking van de (vaak chemotherapeutische) behandeling door griep te voorkomen. Echter, er is aarzeling onder behandelaren om hun patiënten griepvaccinatie te adviseren. Een belangrijke reden hiervoor is dat in een klein cohortonderzoek het optreden van ‘overstimulatie’ verhoogd was bij patiënten die een checkpoint remmer gebruikten en de griepprik kregen.

In **Hoofdstuk 6** weerleggen we de bevindingen uit dit cohortonderzoek en beschrijven we dat griepvaccinatie veilig gegeven kan worden aan patiënten die worden behandeld met checkpoint remmers. Uit retrospectief onderzoek bleek dat het optreden van bijwerkingen en ‘overstimulatie’ van het afweersysteem niet verschilde tussen patiënten die wel en patiënten die geen griepvaccinatie hadden gekregen. Ook een onderzoek uitgevoerd in New York, bevestigde deze bevinding.

Omdat checkpoint remmers de afweerreactie stimuleren, is het niet uitgesloten dat bescherming door vaccinatie beter is in de groep patiënten die deze immuuntherapie gebruikt in vergelijking met patiënten die geen immuuntherapie krijgen. Of dit inderdaad zo is, en of we met een dergelijk werkingsmechanisme de vaccineffectiviteit kunnen verbeteren, moet verder worden onderzocht.

VROEGE HERKENNING VAN LAGE LUCHTWEGINFECTIES: HET BELANG VOOR DE VOLKSGEZONDHEID

In Nederland vindt surveillance plaats naar het voorkomen van infectieziekten, waaronder luchtweginfecties. Medewerkers in de zorg informeren daartoe de Gemeentelijke Gezondheidsdiensten (GGD’s) over het voorkomen van ziektegevallen met een infectieziekte. Dit gebeurt op de volgende manier. Ten eerste, dokters en microbiologische laboratoria zijn verplicht infectieziekten te melden die op de lijst met meldingsplichtige ziekten staan. Ten tweede, clusters van ziekten, zowel onder de bevolking als in een instelling, bijv. meerdere gevallen van diarree in een verpleeghuis, worden gemeld aan de GGD; dit kan ook door een bestuurder van een instelling of schoolonderwijzer gedaan worden. Ten derde, elk ongebruikelijk aantal patiënten met één bepaald syndroom (zoals diarree, huidinfecties, geelzucht, en dergelijke) dat potentieel een gevaar voor de volksgezondheid zou kunnen

betekenen, moet worden gemeld. In de praktijk wordt er nauwelijks gebruikt gemaakt van deze derde mogelijkheid.

In **Hoofdstuk 2**, beschrijven we ICARES (Integrated Crisis Alert and REsponse System), een geautomatiseerd, real-time systeem opgezet om clusters van infectieziekten tijdig op te sporen. Het systeem blijkt in staat om uitbraken van enkele specifieke ziektebeelden real-time te detecteren en te vervolgen. We maken daarbij gebruik van bestaande coderingssystemen uit de eerstelijn (ICPC) en vanuit het ziekenhuis (DBC/DOT codes, bedoeld om financiële vergoeding te krijgen van verzekeringsmaatschappijen). Deze codes representeren een syndroom. Naast luchtweginfectie als een veel voorkomend syndroom in de eerste en in de tweede (en de derde) lijn, onderzoeken we ook geelzucht en meningoencefalitis. Deze laatste twee syndromen komen minder vaak voor en minder in een seizoensgebonden patroon. Meningoencefalitis (hersen(vlies)ontsteking) is een ernstig ziektebeeld dat meestal in het ziekenhuis wordt gediagnostiseerd.

ICARES toont aan dat clusters van patiënten met elk van deze drie syndromen real-time kunnen worden gemonitord. Tijdens het project detecteerde ICARES een lokale uitbraak van meningoencefalitis. Analyse leerde dat dit cluster onderdeel was van een landelijke stijging in het aantal gevallen met een enterovirus meningoencefalitis. Het is een volledig geautomatiseerd systeem waardoor de gevraagde inspanning van huisartsen en ziekenhuismedewerkers zeer beperkt is. Toch bleek de implementatie van ICARES lastig. Onvoldoende betrokkenheid van de lokale GGD bij de opzet en uitvoer van de studie kan hebben bijgedragen aan de tekortkoming. In de pilotfase konden we de behoefte aan een dergelijk meldingssysteem onvoldoende beoordelen. Ook benadrukt het systeem de vertraging tussen syndroom surveillance en microbiologische diagnostiek en leidt tot onzekerheid wat te doen in het interval. Interventies in de publieke gezondheidszorg zijn complex door samenkomen van medische aspecten met sociale, politieke, economische en culturele factoren.

ICARES is ook ingezet voor de surveillance van ernstige luchtweginfecties. In 2015 is het RIVM het project ‘Ernstige acute luchtweginfecties, de missende schakel in de surveillance pyramide’ begonnen. Met dit project moet de surveillance van ernstige luchtweginfecties waarvoor ziekenhuisopname noodzakelijk is (SARI), ontwikkeld en geïmplementeerd worden in Nederland. Omdat morbiditeit, mortaliteit en kosten het meest uitgesproken zijn in de meest zieke patiënten, heeft SARI surveillance meerwaarde bovenop de al bestaande surveillance van griepachtige ziektebeelden in de eerstelijn. We hebben het ICARES systeem aangepast om het aantal patiënten opgenomen met een acute luchtweginfectie te kunnen monitoren als SARI surveillance.

In **Hoofdstuk 3** beschrijven we de verschillen in voorkomen van griepachtige ziektebeelden in de huisartsenpraktijk en de incidentie in SARI, de tegenhanger van het griepachtige ziektebeeld in de tweedelijn. In de meerderheid van de seizoenen met luchtweginfecties viel de piekincidentie van SARI vóór de piek in griepachtige ziektebeelden bij de huisarts. Op dit moment is niet bekend wat hiervoor de verklaring is. Het zou kunnen dat andere virussen dan het griepvirus bijdragen aan de vroege stijging in incidentie van SARI. Omdat de bron van de SARI cases de DBC/DOT code is, geeft onze huidige SARI surveillance geen inzicht in de verwekker van het ziektebeeld. Mogelijk wordt de vroegere piek in het ziekenhuis mede verklaard door een andere virusinfectie, bijvoorbeeld RSV. Een andere verklaring kan gezocht worden in de kwetsbaarheid van de patiëntenpopulatie die opgenomen wordt in het ziekenhuis. Mogelijk presenteren kwetsbare ouderen zich relatief vroeg in het ziekenhuis en verklaart dat de vroege piekincidentie van SARI patiënten.

VROEGE HERKENNING VAN LAGE LUCHTWEGINFECTIES: HET PERSPECTIEF VAN DE HUISARTS

De patiënten met een acute luchtweginfectie die zich in de huisartsenpraktijk presenteren zijn meestal mild tot matig ziek en kunnen door de huisarts thuis worden behandeld. Meestal betreft het immers een virale infectie die zich presenteert als bovenste luchtweginfectie of als bronchitis. Een andere vorm van een lage luchtweginfectie, is een longontsteking. Deze aandoening wordt vaker veroorzaakt door bacteriën en een behandeling met een antibioticum is dan vaak geïndiceerd. In de praktijk is het onderscheid tussen een bacteriële en een virale luchtweginfectie moeilijk te maken, en daarmee ook wie wel en wie geen antibiotische behandeling nodig heeft. Anamnese en lichamelijk onderzoek zijn onvoldoende sensitief en specifiek voor het diagnosticeren van een longontsteking. Hulpmiddelen zijn nodig. De afgelopen jaren is de toegevoegde waarde van diverse biomarkers onderzocht. In vergelijking met enkele andere biomarkers zoals procalcitonine (PCT), bleek C-reactive proteine (CRP) een goede voorspeller van longontsteking (zoals vastgesteld door middel van een thoraxfoto) (**Hoofdstuk 5**). In de Nederlandse richtlijn ‘Acuut hoesten’ worden de diagnostiek, voorlichting en behandeling van infectieuze oorzaken van de klacht acuut hoesten besproken. In deze richtlijn is een diagnostisch algoritme opgenomen dat gebruikt wordt om patiënten met longontsteking die baat hebben bij behandeling met een antibioticum, te identificeren. De ziekste patiënten hebben waarschijnlijk een bacteriële longontsteking en moeten daarom behandeld worden met een antibioticum. Mild zieke patiënten hebben waarschijnlijk een virale aandoening. Een afwachtende strategie zonder het voorschrijven van een antibioticum, is bij deze groep aangewezen. De beslissing om te behandelen met antibiotica is met name moeilijk te nemen bij matig zieke patiënten. Een met vingerprik vastgestelde lage CRP waarde in het

bloed kan in deze groep een longontsteking gevoeglijk uitsluiten, terwijl een hoge CRP waarde de kans op aanwezigheid van een longontsteking vergroot. Deze meerwaarde geldt ook als rekening gehouden wordt met de klinische kenmerken van de patiënt. Daarom is de CRP test opgenomen in de Nederlandse richtlijn als discriminerend diagnosticum bij matig zieke patiënten met een acute luchtweginfectie. Een lage CRP waarde (<20 mg/l) sluit een longontsteking uit en een antibioticum is dan niet nodig. Een hoge CRP waarde (>100 mg/l) maakt een longontsteking meer waarschijnlijk en een antibioticum is daarom geïndiceerd. Bij een matig verhoogde CRP waarde (tussen 20 en 100 mg/l), wordt de beslissing om wel of geen antibioticum te starten weer overgelaten aan de klinische inschatting van de huisarts. Onderzoeken die nagingen of de CRP test het antibioticum voorschrijfgedrag van artsen kon verminderen, toonden overigens wisselend succes.

De gouden standaard voor de bevestiging van de diagnose longontsteking is de

röntgenopname van de long. Een longfoto wordt echter niet routinematig geadviseerd, mede omdat een longfoto niet op de huisartsenpraktijk beschikbaar is en patiënten dus naar het ziekenhuis zouden moeten komen. Onbekend is in welke omstandigheden een longfoto meerwaarde kan hebben.

In **Hoofdstuk 4** beschrijven we het gebruik van aanvullende diagnostiek onder Nederlandse huisartsen. Huisartsen die de CRP test in hun praktijk beschikbaar hebben (54% van het totaal), geven aan minder vaak longfoto's aan te vragen dan hun collega's die niet beschikken over een CRP test. Overigens, ruim 60% van de huisartsen gebruikt de CRP test ook voor andere infecties dan luchtweginfecties ofschoon bewijs voor meerwaarde hier ontbreekt.

De belangrijkste reden om een longfoto aan te vragen bij patiënten met een acute luchtweginfectie is om andere aandoeningen aan te tonen of uit te sluiten. Longkanker is een aandoening die huisartsen vaak willen uitsluiten. Onzekerheid over de aan- of afwezigheid van een longontsteking is minder vaak de reden om een longfoto aan te vragen. Opvallend vaak overschatten de huisartsen de voorafkans op longontsteking.

Om de groep patiënten die voor een acute luchtweginfectie door de huisarts is verwezen voor een longfoto beter in kaart te brengen, onderzochten we een cohort patiënten op verschillende radiologie afdelingen in de regio Leiden-Den Haag. In **Hoofdstuk 5** beschrijven we de uitkomsten. Of de patiënt zich ziek voelt en de afwezigheid van een loopneus zijn de belangrijkste klinische voorspellers voor longontsteking. Het toevoegen van de CRP test aan het predictie model verbeterde de voorspelling van longontsteking niet. Wel her-classificeerde 23/146 (16%) van de patiënten die initieel in de groep zaten met een

redelijke kans (2,5-20%) op een longontsteking naar de groep met een hoge kans op longontsteking (>20%). Dit kan de huisarts helpen in de beslissing om een antibioticum voor te schrijven omdat voor de groep met een hoge kans op een longontsteking een antibioticum is aangewezen. De interpretatie van deze resultaten wordt natuurlijk beïnvloed door de bevindingen in **Hoofdstuk 4**. Hoewel we patiënten includeerden waarbij de huisarts vroeg om de aan- of afwezigheid van een longontsteking te bevestigen, kan het zo zijn dat een deel toch om een andere reden is verwezen, bijvoorbeeld het uitsluiten van een andere aandoening.

De huidige Nederlandse richtlijn 'Acuut hoesten' en de resultaten van deze twee studies, tonen aan dat het diagnostisch proces in de eerstelijn beter kan. Ten eerste, matig zieke patiënten met een acute luchtweginfectie en een CRP waarde tussen de 20 en 100 mg/l, kunnen baat hebben bij het maken van een thoraxfoto, omdat hiermee duidelijk wordt of ze antibiotische therapie moeten krijgen. Immers, de beslissing om een antibioticum voor te schrijven wordt in deze moeilijke 'middengroep' overgelaten aan de inschatting van ziekte ernst en de beoordeling van co-morbiditeit. Ten tweede, patiënten met een acute luchtweginfectie met een laag risico op longontsteking (gebaseerd op symptomen, zonder medeweging van de CRP waarde) waarbij de huisarts een longfoto zou aanvragen, hadden in ons cohort geen longontsteking. Bij deze patiënten die matig ziek waren, kan de CRP test helpen om wel of geen antibioticum voor te schrijven.

STRATEGIEËN OM DE ONTSTEKINGSREACTIE TE DEMPEN

Hoewel een adequate ontstekingsreactie essentieel is om de infectie te genezen, kan een ongecontroleerde of overmatige ontstekingsreactie leiden tot ernstige longschade en, dientengevolge, morbiditeit en mortaliteit.

In een onderzoekcohort van SARI patiënten met influenza, beschreven in **Hoofdstuk 8**, was de mediane opnameduur 5 dagen, en moesten 70 patiënten (18%) op de IC worden opgenomen en bedroeg de 30 dagen mortaliteit 30/390 (7,7%).

De tijd tussen aanvang van griepverschijnselen en het moment waarop behandeling met oseltamivir, een griepvirusremmer, nog zinvol is, is altijd als beperkt beschouwd. Bij gezonde volwassenen heeft het starten van behandeling als de klachten al ≥48 uur bestaan, geen meerwaarde. Echter, patiënten die opgenomen worden met griep representeren een geheel andere categorie waarbij soms sprake is van aanhoudende virale replicatie. De effectiviteit van een late start van oseltamivir bij patiënten die opgenomen worden met

influenza, is niet bekend. Dit zijn veelal oudere, kwetsbare patiënten, vaak met verminderde afweer.

In **Hoofdstuk 8**, beschrijven we de resultaten van een retrospectieve analyse naar de effectiviteit van oseltamivir bij behandeling van opgenomen patiënten met influenza in drie Nederlandse ziekenhuizen. Met een propensity score model hebben we geprobeerd de groepen (wel oseltamivir binnen 48 uur na ziekenhuis opname versus geen oseltamivir in die periode) zo goed mogelijk te kunnen vergelijken ('pseudo randomisatie'). Een behandeling met oseltamivir reduceert de 30 dagen mortaliteit en de samengestelde uitkomstmaat IC opname > 48 uur na opname of 30 dagen mortaliteit. Er was bovendien een trend naar kortere ziekenhuisopname. In de subgroep patiënten met een infiltraat op de longfoto als uiting van voortgaande virusreplicatie en ontsteking, voorkwam oseltamivir behandeling zowel 30 dagen mortaliteit als de samengestelde uitkomstmaat.

Onze studie is de eerste in Nederlandse setting. De Nederlandse gezondheidszorg wordt gekenmerkt door een uitgebreid huisartsennetwerk. De huisarts fungeert als poortwachter voor het ziekenhuis. Dit is een van de redenen dat opgenomen patiënten slechts het topje van de ijsberg vormen van alle patiënten met griep in het griepseizoen. In Nederland worden patiënten alleen opgenomen als ze ernstig ziek zijn, een ontregeling van een onderliggende aandoening hebben door de griep, of wanneer ze kwetsbaar zijn, bijvoorbeeld door comorbiditeit.

In onze studie, waaraan het Jeroen Bosch ziekenhuis, het UMCU en het LUMC meededen, hebben we oudere patiënten met comorbiditeit en/of ernstige ziekte geïncludeerd. De mediane tijd tussen start van klachten en ziekenhuisopname was 4,0 dagen. Dit onderzoekscohort is een uitstekende afspiegeling van de patiëntengroep die jaarlijks in verband met griep moet worden opgenomen. De afname in 30 dagen sterfte sluit aan bij de uitkomst van een meta-analyse waar de effectiviteit van de behandeling met oseltamivir tijdens de H1N1pdm09 influenza werd onderzocht.

Het bewijs voor de effectiviteit van oseltamivir bij opgenomen patiënten met griep zou moeten volgen uit een gerandomiseerd onderzoek. Dit onderzoek ontbreekt echter en dat draagt bij aan het wisselend gebruik van oseltamivir bij deze patiënten. In ons cohort had slecht 35% van de patiënten oseltamivir gekregen binnen 48 uur na ziekenhuisopname.

Een zeldzame, ernstige complicatie van influenza infectie is de ontwikkeling van een Acute Respiratory Distress Syndrome (ARDS, ook wel aangeduid met 'shocklong'). ARDS is een ernstige ontstekingsreactie in de long. Naast influenza, kan een ARDS veroorzaakt worden door andere triggers zoals sepsis, trauma of grote chirurgie. Meer waarschijnlijk wordt

ARDS veroorzaakt door meerdere achtereenvolgende triggers die schade veroorzaken aan de longen. In een cohort van 2013 patiënten die tussen 2009 en 2011 hartchirurgie hadden ondergaan, ontwikkelde 6% postoperatief een ARDS. Zou een bijkomende influenza virus infectie een extra risicofactor kunnen zijn?

In **Hoofdstuk 10**, beschrijven we onze bevinding dat hartchirurgie tijdens het griepseizoen een risicofactor is voor het ontstaan van ARDS na de operatie. In de retrospectieve database vergeleken we complicaties na hartchirurgie tijdens verschillende seizoenen en corrigeerden voor mogelijk storende factoren. De kans op ('odds ratio voor') ARDS in het influenza seizoen in vergelijking met het laagseizoen was 1,85. Er was een trend in afname van ICU ligduur op de intensive care en de tijd aan de beademing. Als de influenza A en B virus circulatie in het griepseizoen stijgt, dan neemt ook het aantal gevallen met ARDS toe. Het lijkt er dus op dat influenza een risicofactor is voor het ontstaan van postoperatief ARDS. Het onderzoek liet niet toe de oorzaak van dit verband vast te stellen. Als een oorzakelijk verband tussen asymptomatische (influenza) virus infectie en ARDS na hartchirurgie kan worden bevestigd, dan biedt dit mogelijkheden om het risico op ARDS te verminderen, bijvoorbeeld door verhogen van de griepvaccinatiegraad of het verbeteren van het griepvaccin.

Infectie is gekenmerkt door een ontstekingsreactie. Soms schiet deze ontstekingsreactie zijn doel voorbij en beschadigt meer dan het goed doet.

In **Hoofdstuk 9**, beschrijven we de PRISTINE studie (Pneumonia treated with RIfampicin aTtenuates Inflammation) waarin we nagaan of gebruik van een ander antibioticum dat minder immuunreactieve bestanddelen doet vrijkomen uit pneumokokkenbacteriën, het herstel bespoedigt en minder ontstekingsreactie veroorzaakt dan de gebruikelijke behandeling met benzylpenicilline. Een longontsteking wordt vaak veroorzaakt door een pneumokok. Immuunreactieve, ontstekingsbevorderende bestanddelen komen vrij uit de bacteriële celwand als pneumokokkenbacteriën worden gedood door antibiotica. Deze bestanddelen, bijvoorbeeld lipoteichoine zuur, zijn belangrijke triggers van de ontstekingsreactie van de gastheer.

In de gerandomiseerde, gecontroleerde PRISTINE studie onderzochten we of rifampicine, een antibioticum dat voorkomt dat immuunreactieve bestanddelen vrijkomen, samen met de standaard benzylpenicilline behandeling bij patiënten met een longontsteking, de ontstekingsreactie in het lichaam beperkt. Ondanks in vitro en dierexperimenteel onderzoek dat in deze richting wees, konden we geen verschil in ontstekingsreactie in bloedcellen aantonen en evenmin verschil in het herstel van patiënten die wel of niet rifampicine kregen naast de penicilline. De reden hiervoor is niet duidelijk geworden, maar mogelijk

was de door ons gekozen infectie, longontsteking door pneumokokken, een te weinig sensitief model om dergelijke verschillen duidelijk te maken.

De uitkomsten van het onderzoek beschreven in dit proefschrift leiden tot de volgende vervolgvragen:

- Op welke manier krijgen we meer gezondheidsmedewerkers gevaccineerd en leidt een hogere vaccinatiegraad tot betere continuïteit van zorg?
- Kan de (griep) vaccineffectiviteit verbeterd worden met gebruik van checkpoint remmers?
- Welke groep patiënten met een acute luchtweginfectie zou de huisarts moeten verwijzen voor een thoraxfoto opdat de klinische uitkomst in deze groep verbetert?
- Als een asymptomatische griepvirusinfectie bij patiënten die aan hun hart worden geopereerd, tot ARDS kan leiden, kunnen we de prognose in deze groep dan verbeteren door het verhogen van de griepvaccinatiegraad of het verbeteren van het griepvaccin?
- Kunnen we nieuwe antibiotica dusdanig laten werken, dat de bacteriën gedood worden zonder dat er te veel immuunreactieve bestanddelen vrijkomen?

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CURRICULUM VITAE

Geert Groeneveld werd geboren op 6 oktober 1978 als oudste zoon van Ymte en Henriëtte Groeneveld. Samen met zijn zus Iris en broer Matthijs groeide hij op in Leiden. Na het behalen van zijn Gymnasium diploma in 1997 aan het Bonaventura college in Leiden, begon hij de studie geneeskunde aan de Rijksuniversiteit Leiden. Tijdens zijn studie was hij onder andere werkzaam als onderzoeksassistent bij de afdeling longziekten van het LUMC, onder leiding van prof. dr. P.J. Sterk, waar hij werkte aan onderzoek naar COPD.

Na een wetenschapsstage in Gambia naar de relatie tussen malaria en pre-eclampsie, behaalde hij in 2002 zijn doctoraal Geneeskunde. Aansluitend begon hij zijn coschappen in het Bronovo ziekenhuis in Den Haag en eindigde deze met een keuzestage tropische infectieziekten in het Havenziekenhuis in Rotterdam.

In 2004 behaalde hij zijn artsdiploma en ging hij aan het werk als ANIOS interne geneeskunde in het voormalig Medisch Centrum Haaglanden (MCH). Ook zat hij in het bestuur van korfbalvereniging Pernix. De opleiding tot internist begon in 2006 in het MCH (opleider dr. P.H.L.M. Geelhoed-Duijvestijn) en vanaf 2010 in het LUMC (opleiders prof. dr. J.A. Romijn/ prof. dr. J.W.A. Smit/prof. dr. J.T. van Dissel/prof. dr. J.W. de Fijter). In mei 2010 startte hij met het aandachtsgebied infectieziekten (opleiders prof. dr. J.T. van Dissel/ prof. dr. L.G. Visser). Vanaf 2012 combineerde hij het onderzoek zoals beschreven in dit proefschrift met de opleiding tot internist-infectioloog.

In januari 2014 registreerde hij zich als internist en begon als staflid bij de acute interne geneeskunde. In september 2014 volgde ook de registratie als infectioloog.

Hij is in juni 2011 getrouwd met Sanne Pera. Samen kregen zij drie kinderen (Imke 2013, Job 2015 en Lise 2017).