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

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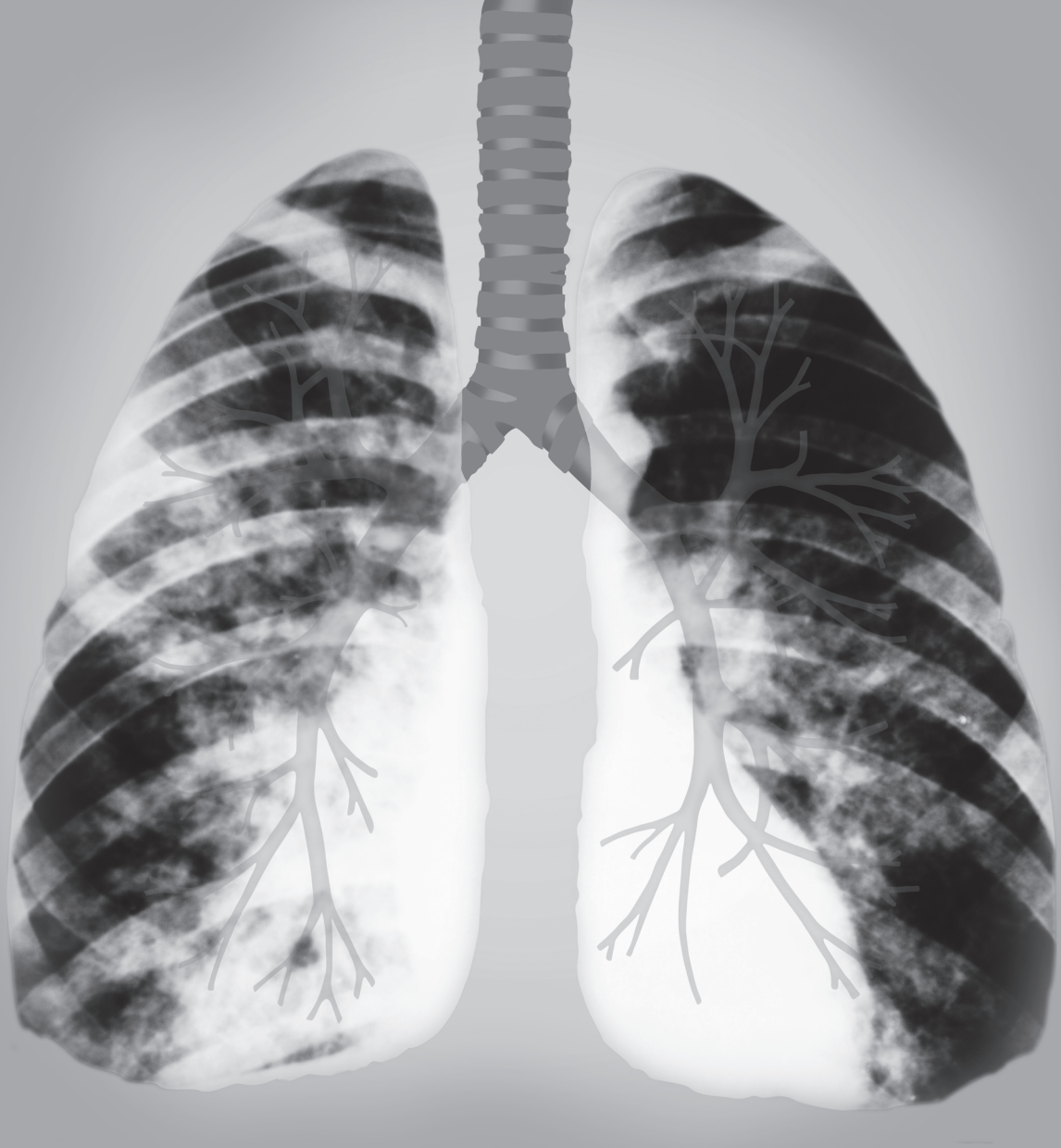

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Summary and general discussion

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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