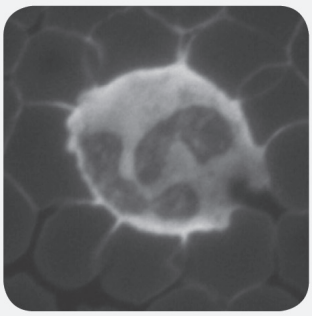
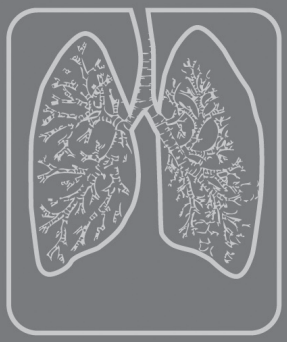
MARKERS FOR DISEASE SEVERITY



Pharmacoepidemiological studies on

obstructive lung disease



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Cover design and
lay-out inside work: *Francis te Nijenhuis, zonnezijn creaties, 's-Hertogenbosch*
Printed by: *Optima Grafische Communicatie, Rotterdam*

The work presented in this thesis was conducted at the Division of Pharmacoepidemiology and Pharmacotherapy of the Utrecht Institute for Pharmaceutical Sciences, Faculty of Science of Utrecht University in affiliation with the Department of Clinical Chemistry and Haematology and the Department of Respiratory Medicine, Division of Heart and Lungs, University Medical Centre Utrecht, The Netherlands.

Parts of this work were conducted in collaboration with:

- PHARMO Institute for Drug Outcome Research, Utrecht, The Netherlands
- Directorate of Information Services and Finance, University Medical Centre Utrecht, The Netherlands
- Abbott Diagnostics, Hoofddorp, The Netherlands
- Abbott Diagnostics, St. Clara, California, United States of America

CIP-gegevens Koninklijke Bibliotheek, Den Haag

Velthove, K.J.
Markers for disease severity – pharmacoepidemiological studies on obstructive lung disease
Thesis Utrecht University – with ref. – with summary in Dutch
ISBN/EAN: 978-90-393-5263-2

MARKERS FOR DISEASE SEVERITY: PHARMACOEPIDEMIOLOGICAL STUDIES ON OBSTRUCTIVE LUNG DISEASE

MATEN VOOR ERNST VAN ZIEKTE:
FARMACO-EPIDEMIOLOGISCHE STUDIES
NAAR OBSTRUCTIEVE LONGZIEKTEN
(met een samenvatting in het Nederlands)

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. J.C. Stoof, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op vrijdag 19 februari 2010 des middags te 2.30 uur

door

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geboren op 26 september 1981 te Rotterdam

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The research described in this thesis has been funded by an unrestricted grant from GlaxoSmithKline.

Publication of this thesis was financially supported by:
- ComputerBasics, schoolvoorbeeld voor ICT-oplossingen,
- E.C. Noyons Stichting ter Bevordering van de Klinische Chemie in Nederland,
- het Nederlands Astma Fonds, en
- Stichting KNMP fondsen.

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chapter 1

General introduction, aims and outline of the thesis

DISEASE SEVERITY IN PHARMACOEPIDEMIOLOGICAL STUDIES

Physicians evaluate diagnosis and disease severity during clinical visits according to patient characteristics, symptoms, physical examination, imaging techniques or laboratory testing. Disease severity is an important parameter in order to obtain a personalised treatment. Disease severity can increase progressively, like Chronic Obstructive Pulmonary Disease (COPD) with GOLD stages I to IV,[1] or fluctuate in time as in inflammatory bowel diseases.[2] Diseases like asthma manifest in several disease phenotypes with different severity.[3,4] In pharmacoepidemiological studies using health care databases usually not all clinical information is available and is mostly limited to information regarding hospitalisation, diagnosis, drug prescriptions, and laboratory test results. Characterisation of disease severity plays an important role in pharmacoepidemiological studies, as illustrated by the following example.

In the 1990s, there was discussion about the association between β_2-agonists and the risk of asthma death. Several studies found that use of β_2-agonists was associated with an increased risk of mortality among asthma patients, but issues regarding confounding by disease severity were raised.[5-8] Much has been said about the β_2-agonist controversy,[9] and eventually confounding by disease severity was indicated as the most probable cause of the association between use of β_2-agonists and asthma death.[5-11] More severely ill patients are likely to use more medication and are at increased risk of having disease exacerbations because of disease severity. Therefore, effects of disease severity and drug exposure are mixed-up, leading to spurious associations. It is essential to minimize this type of confounding in order to get a true risk estimate in epidemiological studies. Therefore, disease severity should be measured to be able to adjust for this factor.

Disease severity can be measured in several ways, among others by symptoms and questionnaires. Examples are different degrees of burn injury,[12] the type 2 diabetes symptom checklist,[13] the Glasgow and Full Outline of Unresponsiveness (FOUR) coma scales,[14] and the specific Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain questionnaire, a validated instrument to assess knee pain in osteoarthritis.[15] Disease severity measured by symptoms or questionnaires could be subjective, however.[16] Therefore, more objective markers are needed to assess disease severity. In this thesis, we will focus on medication use and biomarkers as marker for disease severity.

MEDICATION USE AS MARKER FOR DISEASE SEVERITY

In pharmacoepidemiological studies, drug prescriptions play an essential role. Besides evaluating use of prescription in terms of exposure of interest or outcome, prescriptions can be used as a marker for disease severity in order to select only severe patients or to adjust for disease severity.[17-19] Evaluation of drug prescriptions is a well-accepted proxy parameter for the presence of disease. Medication use could be evaluated by means of all medication of a patient, or by means of specific medications.

The Chronic Disease Score is a validated instrument developed by Von Korff to establish the extent of comorbidity based on pharmacy records and has been used in numerous pharmacoepidemiological studies.[20] Another example is the Rheumatoid Arthritis medical Records-Based Index of Severity (RARBIS) using indicators of disease severity divided into five categories, one of them being medication use.[21,22]

Furthermore, specific medications are often used as a proxy for disease severity. TNF-α antagonists are often used as marker for disease severity among rheumatoid arthritis patients [19] as these drugs are prescribed to severe patients only. Insulin can be used as a proxy for more severe diabetes mellitus type 2.[23] Glucocorticoids are central in the treatment of many inflammatory diseases like rheumatoid arthritis, inflammatory bowel diseases, and obstructive lung disease.[24] Short courses of systemic glucocorticoids only or in combination with antibiotics are often used as marker for exacerbation in obstructive lung disease.[18,25] Among asthma patients, one possible marker for disease severity is montelukast use as in most European countries montelukast is prescribed for asthma patients not controllable with regular asthma medication.[26]

BIOMARKERS FOR DISEASE SEVERITY

In addition to medication use, biomarkers could be used as a measure of disease severity.[27] The Biomarkers Definitions Working Group has defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention".[28] There are several advantages of biomarkers over hard clinical endpoints. Biomarkers are often cheaper, easier, more quickly and earlier to measure than clinical endpoints. Also, measuring biomarkers is more ethical when the biomarker is measurable before tissue damage occurs.[29]

Biomarkers already provided medical innovations and are used in clinical practice. Examples are blood glucose concentration and HbA1c in diabetes mellitus,[29,30]

biomarkers to predict response to therapy as HER-2 in breast cancer,[31] prostate specific antigen (PSA) as a diagnostic marker in prostate cancer,[32] and creatine kinase levels after coronary stenting as biomarker for survival.[33] Other biomarkers are used in research settings like markers in Alzheimer disease,[34] infection and sepsis,[35] heart failure,[36] and complication during warfarin treatment.[37]

Biomarkers can be used to monitor disease progression, but also allow for earlier identification of disease deterioration. These results might be interesting from a clinical point of view. Moreover, early detection and identification of exacerbations is warranted in epidemiological studies for statistical power purposes, to avoid misclassification, but also in order to study severity pathways in patients with occurrence of an exacerbation but with no or little clinical symptoms.

Most biomarkers were discovered only after extensive gathering of data for a specific research question, but in recent years, large health care databases containing routinely collected medical data are increasingly used for research. Information from these databases can provide important tools for identification and development of new biomarkers for disease severity in epidemiological studies [38,39] in collaboration with laboratories and clinical practice.

LINKING CLINICAL, LABORATORY, AND EPIDEMIOLOGICAL TECHNIQUES

Pharmacoepidemiology and clinical chemistry and haematology laboratories are increasingly focused on the identification and development of new markers for disease, that can be used in clinical practice.[28,30,40] Therefore, these three disciplines need to cooperate to evaluate new biomarkers that are associated with disease severity to create objective markers, improve molecular characterisation of phenotypes, and create possibilities to adjust for confounding by disease severity in epidemiological studies. Moreover, availability of these markers allows conducing smaller, more focused clinical trials and better matching treatment to the molecular phenotype profile.[4,41,42]

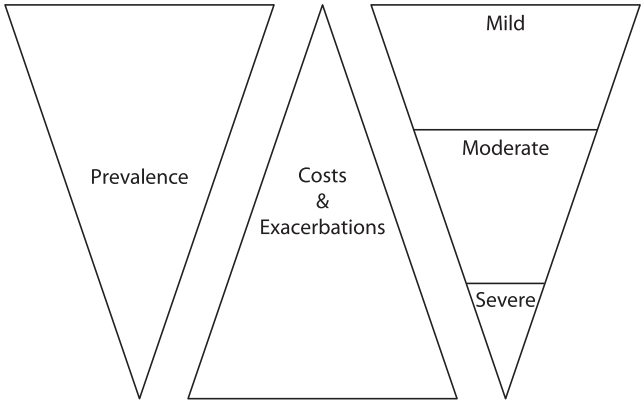
However, using biomarkers in a research setting alone will be of limited value. Often, biological specimens are available with no or limited linkage to clinical data.[43] Linking laboratory parameters and clinical data can provide many new opportunities to search for biomarkers in close conjunction to clinical practice. The Utrecht Patient Oriented Database (UPOD) is an infrastructure of relational databases comprising administrative data on patient characteristics, laboratory test results, medication orders, discharge diagnoses and medical procedures for

all patients treated at the University Medical Centre Utrecht, a 1042 bed tertiary teaching hospital in the centre of the Netherlands.[44] This database was established in January 2004 and is especially suitable to study potential biomarkers in clinical research questions by linking laboratory parameters with clinical data.

DISEASE SEVERITY IN OBSTRUCTIVE LUNG DISEASE

In this thesis, obstructive lung disease will be used as a tool to study markers for disease severity in epidemiological studies. Obstructive lung disease includes two complex diseases with multiple phenotypes, asthma and COPD, both leading causes of morbidity and mortality.[1,45] The global prevalence for asthma ranges from 1–18% in different countries and fluctuates over time.[45,46] Asthma prevalence has increased over recent decades, but tends to stabilize recently.[47,48] Causes of this increase and stabilization are mainly unknown. Although there are arguments in favour of the hygiene hypothesis,[49-52] it is more likely that better diagnosis might play a major part in explaining the increasing prevalence of asthma.[48] The prevalence of COPD is 9–10% of adults aged ≥ 40 years old, and it is estimated that COPD is number three in rank causes of death in 2030.[53,54] Patients with mild disease will occur most

Figure 1 Association between disease severity and prevalence, costs, and exacerbations



Proceeding from mild to moderate to severe obstructive lung disease, prevalence is decreasing while the costs and exacerbation rate increase.

frequently, while patients with severe disease are less prevalent. Moreover, disease severity is associated with costs and exacerbation rates, as illustrated in Figure 1.
Although asthma and COPD are distinct disease entities, issues of disease severity in obstructive lung disease apply to both conditions. When studying medication use and hospitalisation as markers for disease severity, asthma and COPD are clustered to obstructive lung disease in this thesis. Studying neutrophil morphology, a well-defined study population was used by including asthma patients only.
Classically, asthma is described as an eosinophilic inflammatory disease, whereas COPD is associated with neutrophilic inflammation,[55-57] but it has been recognised that several phenotypes of asthma and COPD exist.[3,4,54,58-60] A phenotype is defined as a subtype of disease, based functionally or pathologically by a molecular mechanism or by treatment response.[58,61,62] Examples for asthma phenotypes are intrinsic asthma versus extrinsic asthma, adult versus childhood asthma, brittle versus stabile airflow limitation, aspirin-sensitive asthma, and nocturnal asthma.[63,64] In COPD there are also different phenotypes with the classic phenotypes of 'pink puffers' and 'blue bloaters' as an example.[60]
However, the existing way of phenotyping leads to multiple (sub)phenotypes that have considerable overlap[4,61,62] and we approach the boundaries of current phenotyping. Therefore, the classification of the heterogeneous diseases asthma and COPD needs to be re-evaluated. Stricter phenotyping is needed for understanding the molecular mechanism of disease, and therefore better prediction of outcomes in patients with these phenotypes, new therapeutic innovations and effective phenotype-based treatment.[43]
Difficult-to-treat asthma (DTA) is a heterogeneous phenotype with characteristics of both asthma and COPD and is known under various terms (Table 1). It is

Table 1 Examples of terminology of difficult-to-treat asthma[64-67]

(Near) fatal asthma	Severe asthma	Poorly controlled asthma
Difficult-to-treat asthma	Severe persistent asthma	Brittle asthma
Difficult asthma	Difficult-to-control asthma	Symptomatic asthma
Difficult acute asthma	Steroid-dependent asthma	Life-threatening asthma
Difficult chronic asthma	Corticosteroid-resistant asthma	Irreversible asthma
Acute severe asthma	Steroid-insensitive asthma	Difficult/therapy-resistant asthma
Chronic severe asthma	Corticosteroid-dependent asthma	
Refractory asthma	Therapy-resistant asthma	

estimated that DTA occurs in five to ten percent of asthma patients.[55,65,68] Patients with DTA have a lower quality of life and account for about fifty percent of the total health care costs for asthma.[47,54,55,65,68-70] There is increasing evidence that some difficult-to-treat asthma patients are non-responsive to glucocorticoids and have high neutrophil counts.[4,55,64-66,71-73] These patients keep having symptoms and exacerbations in spite of treatment according to guidelines. Therefore, the diagnosis DTA and exacerbations in obstructive lung disease could be used as a measure for disease severity in obstructive lung disease.

Identification and development of biomarkers for disease severity in obstructive lung disease is needed for both clinical and epidemiological purposes. Examples of biomarkers in sputum or exhalation are sputum eosinophil counts,[74,75] exhaled NO (FeNO),[76-78] or biomarkers detected by the 'electronic nose', which is able to detect volatile organic compounds in exhaled breath.[79] Use of exhaled biomarkers in clinical practice for asthma management is controversial as there is discussion about the added value above measuring clinical symptoms and lung function.[54,80-82] Some studies investigated biomarkers for disease severity in asthma and COPD that were accessible through peripheral blood, like CRP, TNF-α, and copeptin,[83-87] but none were currently found to be implemented in clinical practice. As asthma and COPD are heterogeneous inflammatory diseases, there is a need for biomarkers to measure disease severity and to discriminate between phenotypes, in which the type of underlying inflammation is the distinctive parameter.[3,4,27,45,65]

OBJECTIVES AND OUTLINE OF THIS THESIS

The overall aim of the studies presented in this thesis is to identify and evaluate (bio)markers that are associated with disease severity. We conducted studies in the framework of markers for disease severity in obstructive lung disease in routine clinical practice, combining (molecular) clinical, laboratory medicine and pharmacoepidemiological techniques using the UPOD database. To study medication use in the pathways to hospitalisation, the PHARMO Record Linkage System was used, including hospitalisations and complete medication histories of more than two million community-dwelling residents in the Netherlands from 1985 onwards.[88]

The specific aims of this thesis are:

- To evaluate pathways to hospitalisation and medication use as marker for disease severity in obstructive lung disease.

- To evaluate the absolute neutrophil count, neutrophil morphology, and montelukast use as potential biomarkers for disease severity in obstructive lung disease.
- To evaluate methodological aspects in biomarker studies with regard to disease severity in clinical practice, confounding by glucocorticoid use when using the absolute neutrophil count as a biomarker and testing bias in requests for neutrophil counts in clinical practice.

Chapter 2 deals with medication use and hospitalisation as classical markers for disease severity. In *Chapter 2.1* different exacerbation markers in obstructive lung disease are evaluated where exacerbations with and without hospitalisation are contrasted. *Chapter 2.2* focuses on readmission for obstructive lung disease. In *Chapter 2.3*, medication changes prior to hospitalisation for obstructive lung disease are evaluated. **Chapter 3** concentrates on three biomarker studies evaluating the use of the absolute neutrophil count, neutrophil morphology, and montelukast use as biomarker for disease severity in obstructive lung disease. These three studies underline the translational character of this thesis by using different outcome parameters. In *Chapter 3.1*, hospitalisation is used as clinical outcome parameter for disease severity, in *Chapter 3.2* clinical laboratory parameters are used with focus on neutrophil morphology and *Chapter 3.3* focuses on pharmacoepidemiology by using montelukast use as an easy to measure marker for disease severity among asthma patients. **Chapter 4** addresses methodological aspects in biomarker studies. *Chapter 4.1* describes disease severity in daily clinical practice. In *Chapter 4.2*, it is evaluated whether the absolute neutrophil count can be used as a biomarker for disease severity by studying the effects of systemic glucocorticoids on the absolute neutrophil count. In *Chapter 4.3*, testing bias for requested neutrophil counts is evaluated. Finally, in **Chapter 5** the main findings are discussed and put in general perspective of disease severity in pharmacoepidemiological studies.

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chapter 2

Pathways to hospitalisation for obstructive lung disease

2.1

Measuring exacerbations in obstructive lung disease

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Accepted in
Pharmacoepidemiol Drug Saf

ABSTRACT

Background

Using hospitalisation always has been seen as a solid measurement for exacerbation in pharmacoepidemiology, but might lead to an underestimation of disease exacerbation because of a trend towards outpatient care. The aim of this study was to quantify the incidence of different exacerbation markers in obstructive lung disease and to identify predictors for these exacerbation markers.

Methods

We conducted a cohort study using the Pharmaco-Morbidity (PHARMO) record linkage system, including demographic details and complete medication histories of more than two million community-dwelling residents in the Netherlands from 1985 onwards. Eligible patients were adult users of inhaled corticosteroids (ICS). Outcome parameters were hospitalisation and short courses of systemic corticosteroids. Patients were allowed to have multiple exacerbations during follow-up.

Results

We identified 5327 patients. During follow-up, 8635 exacerbations occurred in 2332 patients with a trend in time towards treating exacerbations out of the hospital (p-value 0.003). Of all patients with exacerbations, 73% was not hospitalised during follow-up. Exacerbations were associated with high-dose ICS use (adjusted relative risk [RR] 1.4; 95% confidence interval [95%CI] 1.2–1.7) and chronic systemic corticosteroid use (adjusted RR 1.9; 95%CI 1.6–2.2).

Conclusions

Using hospitalisation only as exacerbation marker leads to underestimating the exacerbation rate, because of exacerbation treatment out of the hospital. Patients with obstructive lung disease using chronic systemic corticosteroids or high-dose ICS use are more prone to exacerbations. This implies that these patients should be monitored carefully to prevent recurrent exacerbations which are detrimental for their prognosis and quality of life.

BACKGROUND

Obstructive lung disease is a leading cause of morbidity and mortality worldwide.[1,2] The prevalence of Chronic Obstructive Pulmonary disease (COPD) is 9–10% of adults aged 40 years and older, and it is estimated that COPD will be the number three in rank causes of death in 2030.[3,4] Approximately 5% of adults suffer from asthma,[5] although prevalence estimates vary worldwide from 1–18%.[2]

The main goal of treatment as mentioned in all guidelines is to minimize exacerbations.[1,2,6] Large observational studies in Europe and the United States have shown that 30–80% of patients have uncontrolled disease. One of the consequences of uncontrolled obstructive lung disease is the occurrence of disease exacerbations. Such exacerbations are important from a clinical, social, and economic perspective. Exacerbations put severe constraints on patients' daily life,[2,7] and have substantial implications with respect to health care utilization and economic costs.[7-9] Hospitalisations in particular contribute a disproportionate amount to the cost of management of obstructive lung disease.[10]

Epidemiological studies among patients with obstructive lung disease have focussed on hospitalisations as a marker for exacerbation.[11-13] However, using hospitalisation as such might lead to an underestimation of disease exacerbations because of a trend towards outpatient care.[14-17] Therefore, other exacerbation markers need to be taken into account. The objective of this study was to quantify the incidence of different exacerbation markers in obstructive lung disease and to identify predictors for these exacerbations.

METHODS

Setting

The setting of the study was the Dutch Pharmaco-Morbidity Record Linkage System (PHARMO RLS) (www.pharmo.nl). At present, the PHARMO RLS includes the demographic details and complete medication history of more than two million community-dwelling residents of more than twenty-five population-defined areas in the Netherlands from 1985 onwards.[18] This database is also linked to hospital admission records and several other health registries, including clinical laboratory and pathology findings and general practitioner data. Since virtually all patients in the Netherlands are registered with a single community pharmacy, independent of prescriber, pharmacy records are virtually complete with regard to prescription drugs. For this study drug dispensing data and hospitalisation data were used. The computerized drug dispensing histories contain information

concerning the dispensed drug, dispensing date, the prescriber, amount dispensed, prescribed dosage regimen, and the estimated duration of use. Drugs are coded according to the Anatomical Therapeutic Chemical (ATC) classification. The hospitalisation register comprises all hospitalisations in the Netherlands, including detailed information concerning the primary and secondary discharge diagnoses, diagnostic, surgical, and treatment procedures, type and frequency of consultations with medical specialists and dates of hospitalisation and discharge. All diagnoses are coded according to the International Classification of Diseases, 9th edition (ICD-9-CM).[18] All PHARMO linked research is in accordance with Dutch privacy and ethical regulation.

Study cohort

We conducted a retrospective cohort study among patients using inhaled corticosteroids (ICS) in 1993. The date of the first ICS prescription in 1993 marked the start of follow-up. Patients were eligible for inclusion in the cohort when they were 18 years or older at cohort entry and had at least two ICS prescriptions during follow-up. Moreover, each patient required at least one year history prior to cohort-entry and at least one year of follow-up after cohort-entry. Follow-up ended at the end of data collection (December 2002), censoring of the patient in the PHARMO RLS or when more than one year elapsed between subsequent ICS prescriptions, whichever came first. Patients were considered to be at risk for having an exacerbation for 365 days after the last ICS prescription. Asthma patients who were hospitalised for more than 60 days or patients who were hospitalised for asthma and COPD were excluded from the cohort because of uncertainty of correct diagnosis.

Outcome definition

We defined two markers for disease exacerbations: I) hospitalisation for obstructive lung disease, defined as a primary diagnose with ICD-9-CM code 491, 492, 493, or 496; and II) short courses of systemic corticosteroids with a duration of less than 30 days.[15,16,19,20] Antibiotics only were not considered as marker for disease exacerbation because of the uncertainty of their indication of use,[14] but could occur in association with one of the other two marker for disease exacerbation (hospitalisation or short courses of systemic corticosteroids). Because exacerbations can occur in clusters, exacerbation markers were grouped together as a single exacerbation when the interval between individual exacerbation markers was 21 days or less.[14] These exacerbation cluster combinations were categorized as exacerbations with

and without hospitalisation. Patients were allowed to have multiple exacerbations during follow-up.

Exposure and covariate assessment

Drug use was assessed in a six-month period prior to cohort entry. Prevalent ICS users were defined as patients filling at least one ICS prescription in the year before cohort entry. When no ICS prescription has been filled in 1991-1992, a cohort member was considered as an incident ICS user. ICS prescriptions were counted for each patient and divided by follow-up time, as a measure for disease severity. The time at risk for each patient was calculated as the time from cohort-entry to censoring or the end of the follow-up period minus the cumulative duration of exacerbations. As ICS prescriptions usually cover a period of three months in the Netherlands, regular use implicates a dispensing rate of at least one prescription every three months.[21,22] The expected ICS prescription count was calculated as follow-up time divided by 90 days and ratios of observed/expected ICS prescription count were calculated. Taking 80% patient compliance into account,[23,24] regular ICS use was defined as ratios between 0.80 and 2.0, whereas irregular ICS use was defined as ratios < 0.80 and high-dose ICS use as ratios ≥ 2.0. For systemic corticosteroids defined daily dose (DDD) equivalents per day were calculated by dividing the DDDs of all prescriptions by follow-up time. We defined 0.5 DDD/day (equal to 5 mg of prednisone per day) as chronic systemic corticosteroid use.[25]

The number of different respiratory drugs used (ATC-code R03) in the six-month period prior to cohort entry was measured as a proxy for disease severity in accordance to other studies.[16,20,21,26] Considered comorbidities were rhinitis (defined as use of antihistamines and/or nasal preparations), diabetes mellitus (use of antidiabetics), cardiovascular disease (use of β-blockers, calcium antagonists, ACE inhibitors, diuretics and/or statins), dyspepsia (use of H2-receptor antagonists or proton pump inhibitors), pain or inflammation (use of NSAIDs), depression (use of antidepressants), and anxiety disorders (use of benzodiazepines), as these comorbidities frequently occur in obstructive lung disease.[14,27] Furthermore, we calculated the Chronic Disease Score in a one year period prior to cohort entry, measuring chronic disease during a one year period where the score increases with the number of different chronic diseases.[28] Age was categorized into three categories, as a proxy for asthma and COPD, where the 18–44 year group contains mainly asthma patients, the group of 65 years or older contains mainly COPD patients, and the group of 45–64 year olds serves as reference category, in accordance to other studies.[20]

Table 1 Baseline characteristics of the study population (n=5327)

Characteristics	n=5327 (100%)
Age; mean years (standard deviation)	55.9 (17.8)
18–44	1501 (28.2%)
45–64	1748 (32.8%)
≥ 65	2078 (39.0%)
Gender	
male	2580 (48.4%)
female	2747 (51.6%)
Follow-up time; median years (interquartile range)	4.4 (2.0-9.4)
Death during follow-up	944 (17.7%)
Incident inhaled corticosteroid (ICS) user	1468 (27.6%)
Prevalent ICS user	3859 (72.4%)
ICS use	
regular use	2622 (49.2%)
irregular use	2475 (46.5%)
high-dose use	230 (4.3%)
Chronic systemic corticosteroid use [a]	222 (4.2%)
Other respiratory drugs than ICS	
short-acting β_2-agonists	2834 (53.2%)
long-acting β_2-agonists	247 (4.6%)
short-acting muscarinic antagonists	946 (17.8%)
cromones	256 (4.8%)
systemic adrenergics	251 (4.7%)
xanthine derivatives	779 (14.6%)
Number of respiratory drugs [b]	
1–2	3992 (74.9%)
3	904 (17.0%)
> 3	431 (8.1%)
Drugs for comorbidities	
rhinitis	1394 (26.2%)
diabetes	239 (4.5%)
cardiovascular disease	1428 (26.8%)
dyspepsia	536 (10.1%)
pain or inflammation	1077 (20.2%)
depression	164 (3.1%)
anxiety disorder	1255 (23.6%)
Chronic Disease Score [c]	
≤ 3	2649 (49.7%)
> 3–6	1603 (30.1%)
> 6	1075 (20.2%)

Legend Table 1

a) Cut-off value of chronic systemic corticosteroid use is 0.50 defined daily dose/day = 5 mg/day.
b) Respiratory drugs included are: ICS, β_2-agonists, muscarinic antagonists, cromones, systemic adrenergics, and xanthine derivatives.
c) According to Von Korff.[28]

Data analysis

The time at risk was used to calculate incidence density rates to adjust for varying duration of follow-up. The percentage exacerbations with and without hospitalisation was determined by calculating the percentage at each exacerbation date from the cumulative number of exacerbations at each date. Kaplan-Meier survival curves and multivariate Cox proportional hazards regression for recurrent events analysis (Anderson-Gill model) was used to estimate the strength of the association between parameters and exacerbation markers, expressed as relative risks (RR) with 95% confidence intervals (95%CI). All exacerbations from each patient were used and time between the exacerbations was calculated. Hence, each exacerbation was treated as a separate record and corrections for recurrent exacerbations from the same patient were incorporated in the model, according to other studies.[14] Parameters were included in the regression model if they were independently associated with the outcome (at a p-value< 0.2 level). Variables least associated with the outcome in the multivariate model were excluded in order to achieve the most simple model with sufficient predictive value (at a p-value < 0.05 level). All analyses were conducted using SPSS 14.0, and STATA 10.0.

RESULTS

Patient characteristics

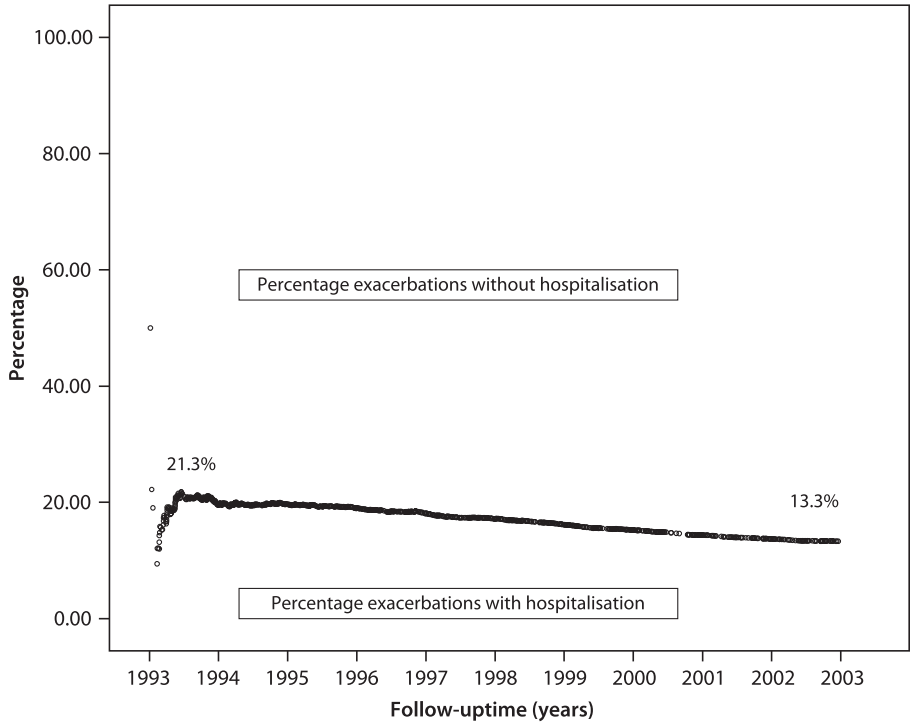
We identified 5327 ICS users who were followed for a median period of 4.4 years (interquartile range 2.0–9.4 years). The characteristics of the study population are described in Table 1. The mean age was 56 years (standard deviation 18 years) and 51.6% were women. Almost three quarters of patients (n=3859; 72.4%) were prevalent ICS users. With regard to ICS use, 2622 patients (49.2%) used ICS regularly, whereas 2475 (46.5%) patients used ICS irregularly, and 230 (4.3%) were high-dose ICS users. Of all patients, 222 (4.2%) used chronic systemic corticosteroids. Regarding use of other respiratory drugs, short-acting β_2-agonists were prescribed most frequently (53.2%), followed by short-acting muscarinic antagonists (17.8%) and xanthine derivates (14.6%). The majority of patients (74.9%) used one or two

respiratory drugs. Comorbidities occurring most frequently were cardiovascular disease (26.8%) and rhinitis (26.2%, Table 1).

Table 2 Incidence of exacerbations in cohort of ICS users (n=5327)

Type of exacerbation	Number of patients n (%)	Number of exacerbations n (%)	Incidence per 1000 persons years (95%CI)
All exacerbations	2332 (100%)	8635 (100%)	310.1 (303.6–316.6)
With hospitalisation	629 (27.0%)	3280 (38.0%)	117.8 (113.8–121.9)
Without hospitalisation	1703 (73.0%)	5355 (62.0%)	192.3 (187.2–197.5)

Figure 1 Plot of exacerbations with and without hospitalisation with respect to time



With time, there was a trend to treat exacerbations of obstructive lung disease more frequently out of the hospital and less frequently in the hospital, p-value 0.003.

Incidence of exacerbations

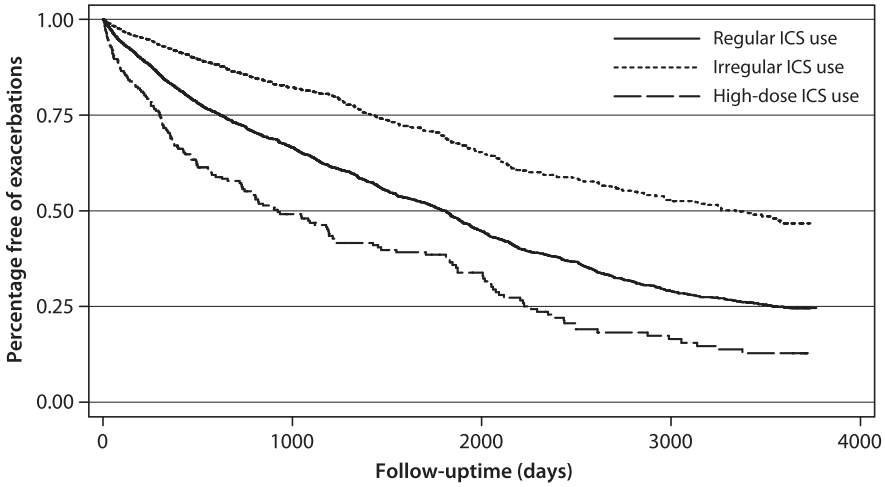
During follow-up, a total of 8635 exacerbations occurred in 2332 patients with an incidence rate of 310.1 per 1000 person years (95%CI 303.6–316.6 per 1000 person years). As shown in Table 2, 1703 (73.0%) of all patients with exacerbations were not hospitalised. There were 629 patients (27.0%) having exacerbations with hospitalisation during follow-up. Exacerbations without hospitalisation occurred more often compared with exacerbations with hospitalisation (Table 2). There was a trend in time to treating exacerbations of obstructive lung disease more frequently out of the hospital and in a decreasing proportion in the hospital (Figure 1, p-value 0.003).

Table 3 Associations between predictors and exacerbations

Characteristics	All exacerbations		With hospitalisation	Without hospitalisation
	Crude RR (95%CI)	Adjusted[a] RR (95%CI)	Adjusted[a] RR (95%CI)	Adjusted[a] RR (95%CI)
Age (years)				
18–44	0.6 (0.5–0.7)	0.7 (0.6–0.8)	0.6 (0.4–0.8)	0.7 (0.7–0.8)
45–64	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
≥ 65	1.3 (1.2–1.4)	1.1 (1.0–1.2)	1.4 (1.1–1.6)	1.1 (1.0–1.2)
ICS use				
regular	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
irregular	0.5 (0.5–0.6)	0.7 (0.6–0.7)	0.5 (0.4–0.7)	0.7 (0.6–0.8)
high-dose use	1.6 (1.4–1.9)	1.4 (1.2–1.7)	1.1 (0.8–1.4)	1.6 (1.4–1.9)
Chronic systemic corticosteroid use	3.4 (2.9–4.0)	1.9 (1.6–2.2)	2.8 (2.3–3.6)	1.5 (1.2–1.8)
Respiratory drugs				
1–2	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
3	2.0 (1.9–2.3)	1.6 (1.4–1.8)	2.2 (1.8–2.6)	1.5 (1.3–1.6)
>3	2.7 (2.4–3.1)	1.8 (1.6–2.0)	2.6 (2.1–3.2)	1.6 (1.4–1.8)
Chronic Disease Score				
≤ 3	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
> 3–6	1.6 (1.5–1.8)	1.3 (1.2–1.5)	1.3 (1.0–1.6)	1.3 (1.2–1.5)
> 6	2.5 (2.2–2.8)	1.5 (1.4–1.7)	1.7 (1.4–2.2)	1.5 (1.3–1.7)

RR = relative risk; 95%CI = 95% confidence interval; ref = reference; ICS = inhaled corticosteroids
a) Adjusted for all other variables in the model.

Figure 2 Kaplan-Meier curve showing the risk of all exacerbations of obstructive lung disease, categorized by inhaled corticosteroids (ICS) use

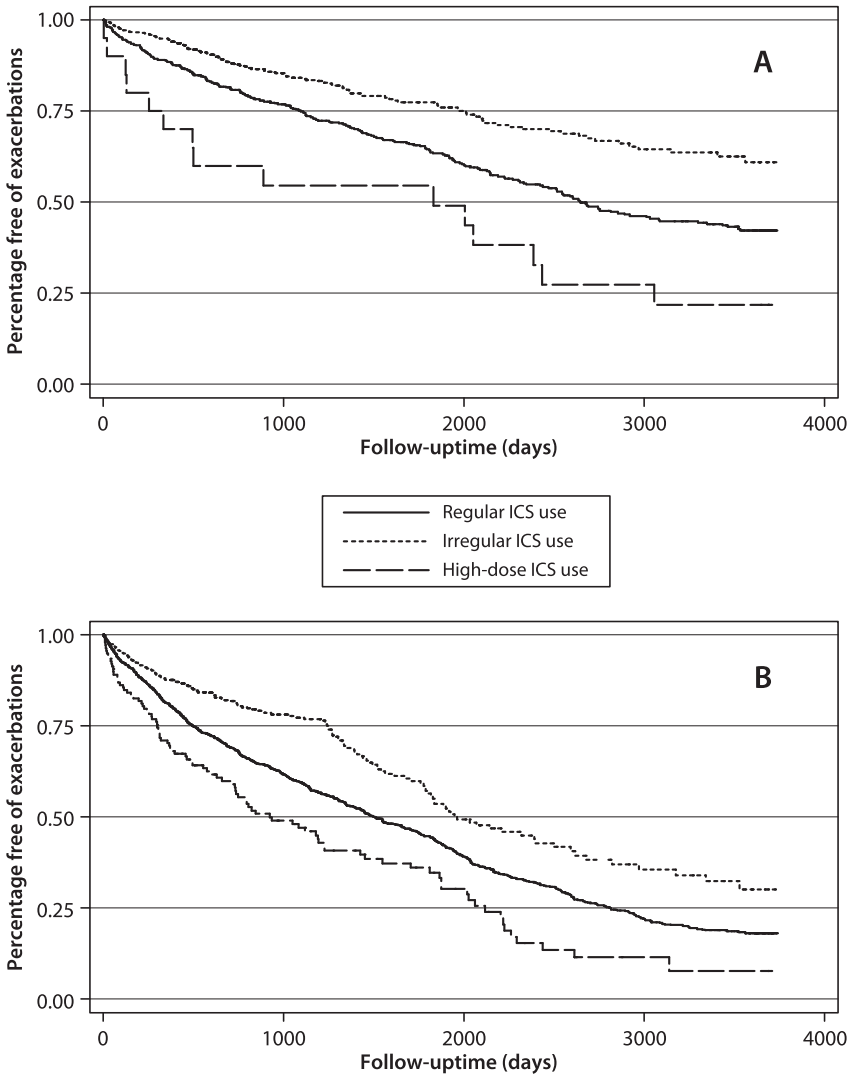


Patients with irregular ICS use were at decreased risk of an exacerbation, patients with high-dose ICS use were at increased risk of exacerbation compared with regular use.

Predictors of exacerbations

Subsequently, we created a prediction model to identify predictors for disease exacerbations (Table 3). Disease exacerbations overall were associated with high-dose ICS use (adjusted RR 1.4; 95%CI 1.2–1.7, Figure 2), chronic systemic corticosteroid use (adjusted RR 1.9; 95%CI 1.6–2.2), use of more than three respiratory drugs simultaneously (adjusted RR 1.8; 95%CI 1.6–2.0), and chronic disease severity (CDS score > 6, adjusted RR 1.5; 95%CI 1.4–1.7). Stratification on exacerbations with and without hospitalisation showed that exacerbations without hospitalisation was stronger associated with high-dose ICS use (adjusted RR 1.6; 95%CI 1.4–1.9), while exacerbations with hospitalisation was associated more pronounced with age ≥ 65 years (adjusted RR 1.4; 95%CI 1.1–1.6), chronic systemic corticosteroid use (adjusted RR 2.8; 95%CI 2.3–3.6), and use of more than three respiratory drugs (adjusted RR 2.6; 95%CI 2.1–3.2). Irregular ICS use was associated with a decreased risk of exacerbations. Stratification on age category, as proxy for asthma and COPD, showed that although older patients had more exacerbations, the effect of ICS use was similar in both age categories, compared with overall analysis (Figures 2 and 3).

Figure 3 Kaplan-Meier curves showing the risk of all exacerbations of obstructive lung disease, categorized by inhaled corticoids (ICS) use and stratified on age category



A. All exacerbations for 18-44 years old.
B. All exacerbations for 65 years or older.
Stratification on age category, as proxy for asthma (18–44 years) and COPD (≥ 65 years) showed that although older patients had more exacerbations, the effect of ICS use was similar in both age categories, compared with the overall analysis in Figure 2.

Among incident ICS users the effect of irregular ICS use (adjusted RR 0.4; 95%CI 0.3–0.5) was more pronounced (p-value < 0.001) compared with prevalent ICS users for disease exacerbation (adjusted RR 0.7; 95%CI 0.7–0.8). Stratifying on comorbidity, patients with diabetes had a more profound risk of exacerbations (p-value 0.03) when using chronic systemic corticosteroids (adjusted RR 3.4; 95%CI 1.7–7.1) compared with non-diabetic patients (adjusted RR 1.8; 95%CI 1.5–2.1).

DISCUSSION

Hospitalisation is the most stringent outcome of an exacerbation and is a major burden both on the welfare of the patients and on the health care costs for obstructive lung disease.[7,12,19] However, not all exacerbations require a hospitalisation. As shown in this study, 73% of all patients with exacerbations were not hospitalised during the study period and there was a trend in time towards treatment of exacerbations out of the hospital and less frequently with hospitalisation, in accordance to other countries.[29] In this study, exacerbations without hospitalisation occurred far more often compared with exacerbations with hospitalisation. Studying exacerbations with hospitalisation only will therefore cause underestimation of the exacerbation rate.

Asthma and COPD are distinct disease entities and there are clinical differences. However, both conditions are obstructive lung diseases and issues of confounding by disease severity apply to both conditions [30] as confounding by disease severity is an important topic in lung disease.[31,32] Moreover, stratification on age category to include primarily asthma or COPD patients in the subgroup analyses of this study did not show different results (Figure 3). Therefore, both conditions were studied together. In this study, we identified several predictors of disease exacerbations in obstructive lung disease. With respect to corticosteroid use, patients using chronic systemic corticosteroid use or high-dose ICS use were more prone to disease exacerbations, where exacerbations with hospitalisation were more profoundly associated with systemic corticosteroid use. The effect of chronic systemic corticosteroids use was also more profound among patients with diabetes mellitus. This can be explained by the fact that diabetes aggravation is a known adverse effect of corticosteroids.[33] Patients with irregular ICS use were at a decreased risk of exacerbation. This implies that more severely ill patients are at increased risk of exacerbation in obstructive lung disease and corticosteroid use can be used as a marker for disease severity.

Exacerbations in obstructive lung disease were found to be negatively associated with lung function [30,34] or with normalization of sputum eosinophil counts,[7] but such parameters are more difficult to implement in routine disease management. General practitioners need easily measurable parameters to identify patients at high risk for frequent exacerbations. Therefore, we measured exacerbations as a function of patient characteristics and medication use. Avoidance of causal factors [35] or keeping close contact with patients who are at high risk for hospitalisation, for example by telemonitoring,[7] could decrease the exacerbation rate in chronic obstructive lung disease.

Some limitations of this study may be addressed. First, there is no information on indication for ICS use. However, ICS are only indicated for obstructive lung diseases. In order to include only patients that used ICS chronically, we censored patients when discontinuing after one ICS prescription and when more than one year elapsed between subsequent ICS prescriptions, in accordance to other studies.[36]

Second, we used data covering a time period between 1993 and 2002. Because no significant changes regarding indications for corticosteroids in the guidelines of obstructive lung disease occurred, the results of this study can be extrapolated to current practice.

Last, studying one outcome event, drug use could have been defined time-varying in the time periods before outcome. However, because patients were allowed to have multiple exacerbations, drug use in this study was assessed in the period of six months prior to cohort entry and was used as time-independent covariate in the prediction model. Reason for this is that having an exacerbation could have consequences for drug use after the first exacerbation and this could have caused bias when defining drug use in a time-varying manner.

Roede et al. found that adding antibiotics to a short course of systemic corticosteroids was associated with a prolonged time to the next exacerbation and was associated with a decreased risk of developing a next exacerbation among COPD patients. In their study, hospitalisation was not included as an outcome measure.[14] Breekveldt et al. studied the medical costs of short courses of systemic corticosteroids and hospitalisation among severe asthma patients, but did not take recurrent events into account.[19] Many previous studies only studied the first exacerbation in patients [16,37] or had a relatively short patient follow-up.[9,14,19] In this study therefore, patients were followed for up to ten years and were allowed to have multiple exacerbations, with or without hospitalisations. Therefore, we are able to make a distinction between exacerbations of different severity and measure the disease burden for each patient. According to another study conducted by our group, the majority of patients were prescribed inhaled corticosteroids for asthma and COPD (64%), but for

most patients ICS were prescribed as trial medication.[38] Therefore, many patients discontinue ICS after being dispensed one prescription. Also, other studies suggest poor persistence in ICS therapy.[20,36] This is in accordance with the results of our study, 1442 patients were excluded from the cohort because of discontinuing after one ICS prescription, and 2475 (46.5%) patients were not persistent with ICS use. This finding shows that patient compliance should be carefully reviewed and that patients should be informed about the beneficial effect of ICS.

In conclusion, 73% of all patients with exacerbations were not hospitalised during the study period and there was a trend in time toward exacerbation treatment out of the hospital. Patients with chronic systemic corticosteroids or high-dose ICS use are especially at risk of exacerbations. This implies that these patients should be monitored more carefully to prevent recurrent exacerbations and the detrimental effects on quality of life and prognosis.

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2.2

Effects of corticosteroid use on readmission in obstructive lung disease

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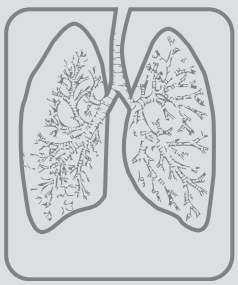


Respir Med 2009 Sep 23
[Epub ahead of print]

ABSTRACT

Background

Obstructive lung disease is a leading cause of morbidity and mortality worldwide. Some patients are readmitted, but currently predicting parameters for identifying these patients are lacking. The aim of this study was to quantify the incidence of readmission in chronic obstructive lung disease and to identify determinants for hospital readmission.

Methods

We conducted a cohort study using the PHARMO record linkage system, including demographic details and complete medication histories of more than two million community-dwelling residents in the Netherlands from 1985 onwards. Eligible patients were adult users of inhaled corticosteroids (ICS) with an admission for obstructive lung disease. The outcome parameter was readmission within a follow-up period of one year.

Results

We identified 605 ICS users with an admission for chronic obstructive lung disease, 132 of these patients were readmitted. Readmission was associated with a high Chronic Disease Score (adjusted hazard ratio [HR] 2.4; 95% confidence interval [95%CI] 1.1–5.3). Patients using short courses of systemic corticosteroids only (adjusted HR 0.5; 95%CI 0.4–0.8) or combined with antibiotics (adjusted HR 0.4; 95%CI 0.2–0.6) were at decreased risk of readmission. The effect of high-dose ICS use varied over time.

Conclusions

Treatment of exacerbations out of the hospital was associated with a decreased risk of readmission, while patients with multiple chronic diseases are at increased risk of readmission for obstructive lung disease. These patients should be educated and should be invited to consultation more often to be able to detect exacerbation in an early phase and start treatment as early as possible.

BACKGROUND

Obstructive lung disease is a leading cause of morbidity and mortality worldwide.[1,2] The prevalence of Chronic Obstructive Pulmonary disease (COPD) is 9–10% of adults aged 40 years and older, and it is estimated that COPD will be the number three in rank causes of death in 2030.[3,4] Also, approximately 5% of adults suffer from asthma,[5] although prevalence estimates vary worldwide from 1 to 18%.[2]

Hospital admission is a major burden for obstructive lung disease from several perspectives. Hospital admission is important from a clinical point of view. Uncontrolled disease may put severe constraints on patients' quality of life,[2] and severe exacerbations are associated with an increased risk of death,[6] and a more rapid decline in lung function.[7] From an economical perspective, admission contributes significantly to health care costs for chronic obstructive lung disease.[8,9]

Epidemiological studies have focused on hospital admissions as a marker for exacerbation, but most studies focus on one admission.[10-12] However, a substantial number of these patients are readmitted to the hospital.[13,14] It would be of great value to predict these readmissions, but currently predicting parameters are lacking. Therefore, the aim of this study was to quantify the incidence of readmission in patients with chronic obstructive lung disease and to identify determinants associated with readmission within one year.

METHODS

Setting

The setting of the study was the Dutch PHARMO Record Linkage System (RLS) (www.pharmo.nl). At present, the PHARMO RLS includes the demographic details and complete medication history of more than two million community-dwelling residents of more than twenty-five population-defined areas in the Netherlands from 1985 onwards.[15] This database is also linked to hospital admission records and several other health registries, including clinical laboratory pathology findings and general practitioner data. Since virtually all patients in the Netherlands are registered with a single community pharmacy, independent of prescriber, pharmacy records are virtually complete with regard to prescription drugs. For this study drug dispensing data and hospital admission data were used. The computerized drug dispensing histories contain information concerning the dispensed drug, dispensing date, the prescriber, amount dispensed, prescribed dosage regimen, and the estimated duration of use. Drugs are coded according to the Anatomical Therapeutic Chemical (ATC) classification. The hospital admission register comprises all

hospital admissions in the Netherlands, including detailed information concerning the primary and secondary discharge diagnoses, diagnostic, surgical, and treatment procedures, type and frequency of consultations with medical specialists and dates of hospital admission and discharge. All diagnoses are coded according to the International Classification of Diseases, 9th edition (ICD-9-CM).[15] All PHARMO linked research is in accordance with Dutch privacy and ethical regulation.

Study cohort

Using the PHARMO RLS we selected a postadmission cohort of patients using inhaled corticosteroids (ICS). This postadmission cohort was selected from a source population of 5327 patients using ICS in 1993. Patients were eligible for inclusion in the source population cohort when they were 18 years or older at cohort entry, had at least one-year of exposure history both prior to and after cohort-entry, and had at least two ICS prescriptions during follow-up. The discharge date of the first hospital admission marked the start of follow-up. The maximum follow-up period was one year, in accordance to other studies.[13,14,16] Follow-up ended at either the end of data collection (December 2002), censoring of the patient in the PHARMO RLS, or the maximum follow-up period, whichever came first. Asthma patients who were hospitalised for more than 60 days or patients who were hospitalised for asthma and COPD in the source population cohort were excluded from the cohort because of uncertainty of correct diagnosis.

Initial admission and readmission

Initial admission and readmission for obstructive lung disease was defined as an admission with a primary discharge diagnosis of obstructive lung disease (chronic bronchitis [ICD-9-CM code 491], emphysema [492], asthma [493], and chronic airway obstruction, not elsewhere classified [496]).[13,14] In order to avoid including an exacerbation with insufficient recovery and immediate referral to the hospital as a readmission, the time to readmission had to be longer than 21 days, in accordance with other studies.[17] When readmission occurred within 21 days, this was defined as an extended first admission and the discharge date of the early readmission was used as cohort entry date.

Exposure and covariate assessment

Drug use was ascertained every 90 days during follow-up,[13] as this is the most common length of prescriptions in the Netherlands. For ICS use, regular use implicates a dispensing rate of at least one prescription every three months.[18,19] The expected ICS prescription count was calculated as follow-up time divided by

90 days. Ratios of observed/expected ICS prescription count were calculated, and regular ICS use was defined as ratios between 0.75 and 2.0, whereas irregular ICS use was defined as ratios < 0.75 and high-dose ICS use as ratios ≥ 2.0. For systemic corticosteroids defined daily dose equivalents (DDD) per day were calculated, by dividing the DDDs of all prescriptions by follow-up time. We defined 0.5 DDD/day (equal to 5 mg prednisone per day) as chronic systemic corticosteroid use.[20] Short courses of systemic corticosteroids with a duration of less than 30 days, or combined with less than 10 day antibiotic use were considered as markers of exacerbation treatment out of the hospital.[21-24] Antibiotics only were not considered as exacerbation marker because of the uncertainty of their indication of use.[17] Because exacerbations can occur in clusters, markers of exacerbations out of the hospital were grouped together as a single exacerbation when the interval between individual exacerbation markers was 21 days or less.[17]

The number of different respiratory drugs used (ATC-code R03) was measured as a proxy for disease severity in accordance to other studies.[14,18,23,24] Considered comorbidities were rhinitis (defined as use of antihistamines and/or nasal preparations), diabetes mellitus (use of antidiabetics), cardiovascular disease (use of β-blockers, calcium antagonists, ACE inhibitors, AT2-antagonists, diuretics, and statins), dyspepsia (use of H2-receptor antagonists or proton pump inhibitors), pain or inflammation (use of NSAIDs), depression (use of antidepressants), and anxiety disorders (use of benzodiazepines), as these comorbidities frequently occur in obstructive lung disease.[17,25] Furthermore, we calculated the Chronic Disease Score in a one-year period prior to cohort entry, measuring chronic disease during a one-year period where the score increases with the number of different chronic diseases.[26]

Data analysis

The time at risk for each patient was calculated as the time from cohort entry to censoring or the end of the follow-up period or readmission, whichever came first. Kaplan-Meier survival analysis and multivariate Cox proportional hazards regression analysis was used to estimate the strength of the association between determinants and readmission, expressed as hazard ratios (HR) with 95% confidence intervals (95%CI). Possible determinants were included in the regression model if they were independently associated with the outcome (at a p-value < 0.2 level). Variables least associated with the outcome in the multivariate model were excluded in order to achieve the most simple model with sufficient predictive value (at a p-value < 0.05 level). All analyses were conducted using SPSS 14.0, and STATA 10.0.

Table 1 Baseline characteristics

Characteristics	n=605 (100%)
Age in years; mean (SD)	63.7 (13.1)
18–44	59 (9.8%)
45–64	201 (33.2%)
≥ 65	345 (57.0%)
Gender	
male	350 (57.9%)
female	255 (42.1%)
Year of inclusion	
1993–1995	336 (55.5%)
1996–1998	149 (24.6%)
1999–2002	120 (19.8%)
Death during follow-up	14 (2.3%)
ICS use	
regular	349 (57.7%)
irregular use	132 (21.8%)
high-dose use	124 (20.5%)
Chronic systemic corticosteroid use [a]	142 (23.5%)
Patients with short courses of systemic corticosteroids [b]	265 (43.8%)
Patients with combined short courses of systemic corticosteroids and antibiotics [b]	179 (29.6%)
Other respiratory drugs	
short-acting β_2-agonists	382 (63.1%)
long-acting β_2-agonists	216 (35.7%)
short-acting muscarinic antagonists	302 (49.9%)
long-acting muscarinic antagonists	6 (1.0%)
cromones	26 (4.3%)
systemic adrenergics	38 (6.3%)
xanthine derivatives	167 (27.6%)
Number of respiratory drugs	
1–2	216 (35.7%)
3	232 (38.3%)
> 3	157 (26.0%)
Drugs for comorbidities	
rhinitis	99 (16.4%)
diabetes	46 (7.6%)
cardiovascular disease	269 (44.5%)

dyspepsia	113 (18.7%)
pain or inflammation	84 (13.9%)
depression	30 (5.0%)
anxiety disorder	185 (30.6%)
Chronic Disease Score[c]	
< 6	72 (11.9%)
6–9	324 (53.6%)
> 9	209 (34.5%)

a) Cut-off value of chronic systemic corticosteroid use is 0.50 DDD (defined daily dose)/day = 5 mg/day.
b) During the follow-up period.
c) According to Von Korff method.[26]

RESULTS

Patient characteristics

We identified 605 adult ICS users with an admission for obstructive lung disease. During follow-up, 132 readmissions occurred. Time to readmission was equally distributed over follow-up time with a median of 157 days, from 23 to 365 days. The characteristics of the study population are displayed in Table 1. The mean age was 63.7 years (standard deviation [SD] 13.1 years) and 42.1% were women. There were 349 patients (57.7%) who used ICS regularly, 132 patients (21.8%) with irregular ICS use, and 124 (20.5%) with high-dose ICS use. Of all ICS, only 12 patients (2.0%) used combination products of ICS and β_2-agonists. At baseline, 142 patients (23.5%) used chronic systemic corticosteroids. During follow-up, a large proportion of patients used short courses of systemic corticosteroids only or combined with antibiotics, indicating an exacerbation for obstructive lung disease, treated out of the hospital (Table 1).

With respect to the use of other respiratory drugs, short-acting β_2-agonists were prescribed most frequently (63.1%), followed by short-acting muscarinic antagonists (49.9%) and long-acting β_2-agonists (35.7%). There were 216 patients (35.7%) who used one or two different respiratory medications, while 232 (38.3%) used three, and 157 (26.0%) used more than three different respiratory drugs simultaneously at baseline. Comorbidities occurring most frequently were cardiovascular disease (44.5%), followed by anxiety disorders (30.6%) and dyspepsia (18.7%).

For patients with a readmission, medication use preceding the first and the second admission was similar. As shown in Table 2, patients used less short-acting β_2-agonists, following initial hospital admission (p-value 0.057).

Table 2 Medication use before admission and before readmission among patients with readmission

Characteristics	90 days before admission n=132 (100%)	90 days before readmission n=132 (100%)	p-value [a]
Chronic systemic corticosteroid use [b]	45 (34.1%)	37 (28.0%)	0.268
Other respiratory drugs			
short-acting β_2-agonists	87 (65.9%)	74 (56.1%)	0.057
long-acting β_2-agonists	48 (36.4%)	56 (42.4%)	0.214
short-acting muscarinic antagonists	73 (55.3%)	84 (63.6%)	0.336
long-acting muscarinic antagonists	0	0	
cromones	5 (3.8%)	4 (3.0%)	0.755
systemic adrenergics	9 (6.8%)	5 (3.8%)	0.090
xanthine derivatives	42 (31.8%)	48 (36.4%)	0.808
Drugs for comorbidities			
rhinitis	27 (20.5%)	26 (19.7%)	0.666
diabetes	10 (7.6%)	12 (9.1%)	0.678
cardiovascular disease	61 (46.2%)	67 (50.8%)	0.518
dyspepsia	36 (27.3%)	47 (35.6%)	0.319
pain or inflammation	19 (14.4%)	18 (13.6%)	0.957
depression	7 (5.3%)	4 (3.0%)	0.656
anxiety disorder	50 (37.9%)	51 (38.6%)	0.815
Number of respiratory drugs			0.844
1–2	42 (31.8%)	42 (31.8%)	
3	51 (38.6%)	50 (37.9%)	
> 3	39 (29.5%)	40 (30.3%)	

a) χ^2 test.
b) Cut-off value of chronic systemic corticosteroid use is 0.50 DDD (defined daily dose)/day = 5 mg/day.

Predictors of readmission

Subsequently, a model was created to identify determinants for readmission (Table 3). Readmission for obstructive lung disease was associated with a high Chronic Disease Score (adjusted HR 2.4; 95%CI 1.1–5.3). Of considered comorbidities in this study, readmission was associated with dyspepsia (adjusted HR 1.8; 95%CI 1.2–2.6).

Patients using short courses of systemic corticosteroids only (adjusted HR 0.5; 95%CI 0.4–0.8) or combined with antibiotics (adjust HR 0.4; 95%CI 0.2–0.6) were at decreased risk of readmission. Readmission was not associated with high-dose

Table 3 Predictors of readmission

Characteristics (132 outcomes)	Crude HR (95%CI)	Adjusted[a] HR (95%CI)
ICS use		
regular	1.0 (reference)	1.0 (reference)
irregular use	0.7 (0.4–1.4)	0.8 (0.4–1.4)
high-dose use	1.2 (0.8–1.8)	1.3 (0.8–1.9)
Use of short courses of systemic corticosteroids	0.4 (0.3–0.6)	0.5 (0.4–0.8)
Use of combined short courses of systemic corticosteroids and antibiotics	0.3 (0.2–0.5)	0.4 (0.2–0.6)
Dyspepsia	1.9 (1.3–2.7)	1.8 (1.2–2.6)
Chronic Disease Score		
< 6	1.0 (reference)	1.0 (reference)
6–9	2.4 (1.1–5.2)	2.2 (1.0–4.8)
> 9	3.0 (1.4–6.7)	2.4 (1.1–5.3)

HR = hazard ratio; 95%CI = 95% confidence interval; ICS = inhaled corticosteroids
a) Adjusted for all other variables in the model.

ICS use for the complete follow-up period (adjusted HR 1.3; 95%CI 0.8–1.9). However, patients with high-dose ICS use were less frequently readmitted to the hospital in the first three months of follow-up (adjusted HR 0.1; 95%CI 0.01–0.6), but during 4–6 months of follow-up readmission was associated with high-dose ICS use (adjusted HR 4.0; 95%CI 2.0–8.0; Table 4 and Figure 1).

DISCUSSION

In this study, we identified determinants for readmission in obstructive lung disease. Despite regular use for a high proportion of patients, 132 (21.8%) patients were readmitted within one year. Overall, readmission was associated with a high Chronic Disease Score. Of considered comorbidities, readmission for obstructive lung disease was associated with dyspepsia, which is a known comorbidity, associated with aggravation of obstructive lung disease.[27] Treatment of exacerbations out of the hospital with short courses of systemic corticosteroids only or combined with antibiotics was associated with a decreased risk of readmission. Overall readmission was not associated with ICS use. However, during 4–6 months of follow-up readmission was associated with high-dose ICS use.

Table 4 Effect of ICS use on readmission over follow-up time

Characteristics	Crude HR (95% CI)	Adjusted [a] HR (95% CI)
0–3 months (33 outcomes)		
regular ICS use	1.0 (reference)	1.0 (reference)
irregular ICS use [b]	NA	NA
high-dose ICS use	0.1 (0.01–0.6)	0.1 (0.01–0.6)
4–6 months (36 outcomes)		
regular ICS use	1.0 (reference)	1.0 (reference)
irregular ICS use	1.1 (0.3–3.8)	1.0 (0.3–3.5)
high-dose ICS use	4.0 (2.0–7.9)	4.0 (2.0–8.0)
6–9 months (23 outcomes)		
regular ICS use	1.0 (reference)	1.0 (reference)
irregular ICS use	2.2 (0.8–6.3)	2.6 (0.9–7.6)
high-dose ICS use	1.5 (0.6–3.9)	1.6 (0.6–4.2)
9–12 months (40 outcomes)		
regular ICS use	1.0 (reference)	1.0 (reference)
irregular ICS use	1.1 (0.4–3.1)	1.2 (0.4–3.5)
high-dose ICS use	1.1 (0.5–2.4)	1.1 (0.5–2.4)

HR = hazard ratio; 95%CI = 95% confidence interval; ICS = inhaled corticosteroids; NA = not applicable

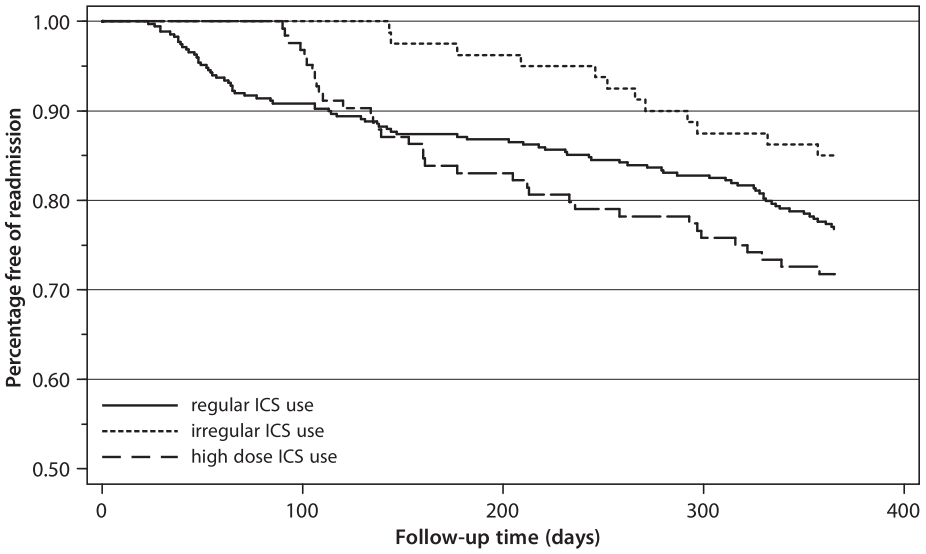
a) Adjusted for use of short courses of systemic corticosteroids, use of combined short courses of systemic corticosteroids and antibiotics, dyspepsia, and Chronic Disease Score.

b) No readmissions occurred in this group during this period.

Hospital admission is the most stringent definition of an exacerbation.[11,13,22] Studying hospital-based exacerbations leads to studying patients with most severe disease as these patients are at increased risk of admission for obstructive lung disease.[24,28] Krishnan et al. found a decrease of adherence to ICS or systemic corticosteroids to 50% after two weeks after hospital discharge.[29] Hospital discharge programs could decrease the risk of readmission on the short-term,[30] but the risk of readmission should also be decreased on the long-term as severe exacerbations are associated with an increased risk of death [6] and more rapid decline in lung function.[7] Therefore, these patients need appropriate care following admission in order to decrease the risk of readmission.

Comparison with other studies yielded differences across Europe in the incidence of readmission for obstructive lung disease. Almagro et al. found that 75 (58.5%) of 129 patients were readmitted during a one-year follow-up period in Spain.[31] The study from Gudmundsson et al. yielded a readmission rate of 60.6%, with 246 of the

Figure 1 Kaplan-Meier curve showing the risk of readmission for obstructive lung disease during the follow-up period, categorized by ICS use



ICS = inhaled corticosteroids
Patients with high-dose ICS use were less frequently readmitted to the hospital in the first three months of follow-up, but more frequently during 4-6 months of follow-up.

total 406 patients being readmitted within one year in the Scandinavian countries.[32] Our study was conducted in the Netherlands including 605 patients. Of all patients, 132 (21.8%) were readmitted within one year. This might be a consequence of differences in exacerbation treatment out of the hospital.

Exacerbations in obstructive lung disease were found to be associated with a decrease in lung function.[33,34] Other studies have found the combination of quality of life, hospitalisation for COPD in the previous year and hypercapnia at discharge,[31] and a low health status or anxiety at discharge as useful predictors for readmission.[32] General practitioners however need easily measurable parameters to identify patients at high risk for readmission. Therefore, we measured exacerbations as a function of medication use. Avoidance of causal factors [35] or keeping close contact with patients who are at high risk for readmission could decrease the frequency of readmission in obstructive lung disease.

Some limitations of this study may be addressed. First, there is no information on indication for ICS use. However, chronic treatment with ICS is only indicated

for obstructive lung disease and all patients in this study were hospitalised for obstructive lung disease. In order to include only patients that used ICS chronically, we censored patients when discontinuing after one ICS prescription, in accordance to other studies.[36]

Second, we used data covering a time period between 1993 and 2002. Because no significant changes in the guidelines in obstructive lung disease occurred for indication of ICS, the results of this study can be extrapolated to current practice.

Unlike randomised clinical trials, observational studies are prone to bias and criticized because of the occurrence of immortal time bias.[37,38] Immortal time bias is unlikely to play a role in this study as all participants were at risk of readmission immediately after hospital discharge. When readmission occurred within 21 days, this was defined as an extended first admission and the discharge date of the early readmission was used as cohort entry date.

The beneficial effects of ICS among asthma patients are well-documented, but these effects are more controversial in COPD.[34,37,39-43] Confounding by severity plays a major role in studies concerning asthma or COPD.[44,45] Therefore both conditions were studied together. The international COPD study group found a beneficial effect of ICS on the exacerbation rate requiring treatment by the general physician or in the hospital,[39] where as the ISOLDE study and the TORCH study found a beneficial ICS effect on exacerbations treated with short courses of systemic corticosteroids and/or antibiotics in randomised trials.[40,41] Sin and Tu found that ICS in postadmission patients was associated with a reduced risk of readmission,[43] but there is no firm dose-response relationship. Although low-dose ICS was inferior to medium- or high-dose ICS in protecting against mortality in COPD, high-dose was somewhat less protective than medium-dose ICS. Moreover, readmission was not an outcome parameter in this study.[42] Soriano et al. included primary care patients with COPD and studied survival. ICS were found to be associated with increased survival among these patients. However, extrapolation of these results to patients being hospitalised is uncertain.[46] Roede et al. studied exacerbations out of the hospital, but does not take admissions or readmissions into account.[17]

Blais et al. studied ICS use and the prevention of readmission for asthma among newly treated asthmatics without systemic corticosteroids in an observational study.[13] Blais concluded that regular use of ICS decreased the risk of readmission for asthma in the period of sixteen days to six months after admission. After this period, the beneficial effect of ICS use disappeared, probably because of confounding by severity.[13] These findings are in accordance with the results of this study that show that more severely ill patients use more ICS. Patients with high-dose ICS use were at decreased risk of readmission during the first three months of follow-up (adjusted

HR 0.1; 95%CI 0.001–0.6), but during 4–6 months of follow-up readmission was associated with high-dose ICS use (adjusted HR 4.0; 95%CI 2.0–8.0; Table 4 and Figure 1). Moreover, this paper studies readmission as well as exacerbations treated out of the hospital. This paper adds to the ongoing discussion about the use of ICS in patients with obstructive lung disease.

In conclusion, occurrence and successful treatment of exacerbations out of the hospital was associated with a decreased risk of readmission, while patients having multiple chronic diseases are at increased risk of readmission for obstructive lung disease. Readmission was associated with high-dose ICS use during 4–6 months follow-up. These patients should be educated and should be invited to consultation more often to be able to detect exacerbation in an early phase and start treatment as early as possible.

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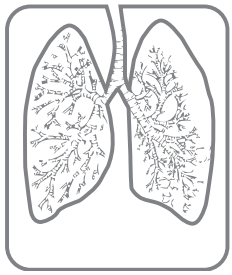

Medication changes prior to hospitalisation for obstructive lung disease: a case-crossover study

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Accepted in
Ann Pharmacother

ABSTRACT

Background

Hospitalisations have always been seen as a solid outcome parameter in pharmacoepidemiology. However, the pathway to hospitalisation and pre-hospital management is equally important. The objective of this study was to evaluate medication changes in the build-up to hospitalisation for obstructive lung disease and to quantify the association between medication use and the risk of hospitalisation.

Methods

We conducted a case-crossover study using the PHARMO record linkage system, that contains drug dispensing data from community pharmacies and hospital admission data. Patients were adults, hospitalised for obstructive lung disease in 2005 until 2007. The index date of the case period was the date of hospitalisation, control moments were set at 3, 6, 9 and 12 months before admission. For each patient, all prescriptions prior to the date of hospitalisation were identified. Medication use was ascertained in a 90 day time-window prior to each case or control moment.

Results

We identified 1481 patients with a hospitalisation for obstructive lung disease. It appeared that respiratory medication use increased in the 90 days prior to hospitalisation. Hospitalisation was associated with using three or more respiratory drugs (odds ratio [OR] 2.2; 95% confidence interval [95%CI] 1.8–2.8), use of systemic glucocorticoids (OR 4.5; 95% CI 3.8–5.4) and antibiotic use (OR 3.1; 95%CI 2.7–3.6).

Conclusions

Use of systemic glucocorticoids, antibiotics, and other respiratory drugs increased prior to hospitalisation. These results could be indicative of the development and/or treatment of an exacerbation. There is need for markers to detect exacerbations in an early phase in order to start treatment as early as possible and possibly prevent hospitalisations for obstructive lung disease.

BACKGROUND

Obstructive lung disease is a leading cause of morbidity and mortality worldwide.[1,2] The prevalence of Chronic Obstructive Pulmonary disease (COPD) is 9–10% among adults aged 40 years and older, and it is estimated that COPD will be the number three in rank causes of death in 2030.[3,4] Prevalence estimates for asthma vary worldwide from 1–18%.[2,5] Epidemiological studies among patients with obstructive lung disease have focused on hospitalisations as outcome parameter, as hospitalisation is a major burden from a clinical, social and economic point of view.[6-9] Large observational studies in Europe and the United States have shown that 30–80% of patients have uncontrolled disease, which might lead to exacerbations.[2,10] However, not all exacerbations in obstructive lung disease require hospitalisation because of a trend towards outpatient care with short courses of systemic glucocorticoids only or in combination with antibiotics [11-13] and not all patients receive equal pre-hospital management.[14] Moreover, 22–61% of all patients with a hospitalisation were readmitted to the hospital.[15-17] Therefore, the pathway to hospitalisation and pre-hospital management is equally important.

Studies on acute events often focus on the case-crossover study design.[18-20] In this design, each case serves as its own control, where each case contributed one case period and one or more control periods.[21] Using this design, confounding due to fixed factors, such as genetic factors, personality, and education are eliminated by study design.[21] This approach may also be valuable in pharmacoepidemiological studies on hospitalisation as this research field focuses on beneficial drug effects, but also on potentially harmful effects of drugs,[18] leading to hospitalisation.[5,21-25] Given the complexity of disease progression and staging, a case-crossover design could be used where each patient acts as his/her own control. The objective of this study was to evaluate medication changes in the build-up to hospitalisation for obstructive lung disease and to quantify the association between medication use and the risk of hospitalisation.

METHODS

Setting

Data were obtained from the Dutch PHARMO Record Linkage System (RLS) (www.pharmo.nl). The PHARMO RLS includes the demographic details and complete medication history based on drug dispensing data from community pharmacies of more than two million community-dwelling residents of more than twenty-five population-defined areas in the Netherlands from 1985 onwards.[26] This database is

also linked to hospitalisation records and several other health registries, including clinical laboratory and pathology findings and general practitioner data. Since virtually all patients in the Netherlands are registered with a single community pharmacy, independent of prescriber, pharmacy records are virtually complete with regard to prescription drugs. For this study drug dispensing data and hospitalisation data were used. Therefore, there was no diagnostic information to distinguish asthma and COPD patients. Also, smoking history was unavailable. The computerized drug dispensing histories contain information concerning the dispensed drug, dispensing date, the prescriber, amount dispensed, prescribed dosage regimen, and the estimated duration of use. Drugs are coded according to the Anatomical Therapeutic Chemical (ATC) classification. The hospitalisation register comprises all hospitalisations in the Netherlands, including detailed information concerning the primary and secondary discharge diagnoses, diagnostic, surgical, and treatment procedures, type and frequency of consultations with medical specialists and dates of hospitalisation and discharge. All diagnoses are coded according to the International Classification of Diseases, 9th edition (ICD-9-CM).[26] All PHARMO linked research is in accordance with Dutch privacy and ethical regulation.

Study design and study population

A case-crossover study was conducted with a sample of the PHARMO database. The study population comprised all patients with a first hospitalisation with a primary discharge diagnosis of obstructive lung disease in the period 2005 until 2007 (chronic bronchitis [ICD-9-CM code 491], emphysema [492], asthma [493], and chronic airway obstruction, not elsewhere classified [496]).[7,27] Patients who were hospitalised for both asthma and COPD (n=18) were excluded from the study population because of uncertainty of correct diagnosis. Patients were eligible for inclusion in the study population when they were 18 years or older at time of hospitalisation and had at least six months of medication history prior to hospitalisation. Each patient contributed one case period, and up to four control periods, depending on the duration of medication history available.[28] The index date of the case period was the date of hospitalisation. As prescriptions for chronic disease usually cover a period of three months in the Netherlands, control moments were set at 3, 6, 9 and 12 months before admission. Patients with a hospitalisation in these control moments were excluded from the cohort.

Exposure and covariate assessment

For each patient, all prescriptions prior to the date of hospitalisation were identified. Medication use was ascertained in a 90 day time-window prior to each case or control

moment,[7] as this is the most common length of prescriptions in the Netherlands. Use of respiratory drugs (ATC-code R03) was measured dichotomously by evaluating prescription records in accordance to other studies.[12,27,29] Drugs assessed were inhaled glucocorticoids, short-acting and long-acting β_2-agonists, short-acting and long-acting muscarinic antagonists, xanthine derivatives, and montelukast use. Usage of these drugs was summarized as the number of respiratory drugs used in each period by counting the number of drugs in different therapeutic classes. Also, usage of systemic glucocorticoids and antibiotics was assessed in each time window.

Considered comorbidities were rhinitis (defined as use of antihistamines and/or nasal preparations), diabetes mellitus (use of antidiabetics), cardiovascular disease (use of β-blockers, calcium antagonists, Angiotensin Converting Enzyme [ACE] inhibitors, Angiotensin II receptor [AT2] antagonists, diuretics and/or statins), dyspepsia (use of histamine 2 [H2]-receptor antagonists or proton pump inhibitors), pain or inflammation (use of Non-Steroidal Anti-Inflammatory Drugs [NSAIDs]), depression (use of antidepressants), and anxiety disorders (use of benzodiazepines), as these comorbidities frequently occur in obstructive lung disease.[30,31]

Data analysis

As the study had a case-crossover design, each patient represented a matched set of data for case and control exposure to medication use. For each patient, the odds of medication use during the case period was compared with the odds of medication use during the control periods. For systemic glucocorticoid use, effect of control periods on the association between use of systemic glucocorticoids and the risk of hospitalisation was evaluated by comparing the odds of glucocorticoid use in the case period with each individual control period. Conditional logistic regression analysis was used to estimate the strength of the association between medication use and the risk of hospitalisation for obstructive lung disease, expressed as odds ratios (OR) with 95% confidence intervals (95%CI). All analyses were conducted using SPSS 14.0.

RESULTS

We identified 1481 patients with a first hospitalisation for obstructive lung disease between 2005 and 2007. Of all patients, 119 (8.0%) were aged 18–44 years, 436 (29.4%) were 45–64 years, and 926 (62.5%) were aged 65 years or older, with a mean age of 66.5 (standard deviation 14.4). There were 690 (46.6%) males and 791

(53.4%) females. Of all patients, 246 (16.6%) were hospitalised for asthma, and 1235 (83.4%) for COPD.

Use of respiratory medication increased prior to hospitalisation (Table 1). Using the number of respiratory drugs during control periods, 33.1% of patients did not use respiratory drugs, 30.6% used one or two drugs for respiratory indications and 36.4% used three or more respiratory drugs. The proportion of patients using three

Table 1 Medication use among case and control periods

Characteristics	Case period n=1481 (100%)	Control periods n=5875 (100%)	OR (95%CI)
Respiratory drugs			
Inhaled glucocorticoid use	767 (51.8%)	2806 (47.8%)	1.4 (1.2–1.7)
Short-acting β_2-agonists	583 (39.4%)	1914 (32.6%)	2.0 (1.7–2.4)
Long-acting β_2-agonists	718 (48.5%)	2623 (44.6%)	1.5 (1.2–1.8)
Short-acting muscarinic antagonists	236 (15.9%)	915 (15.6%)	1.1 (0.8–1.4)
Long-acting muscarinic antagonists	363 (24.5%)	1244 (21.2%)	1.7 (1.4–2.2)
Xanthine derivatives	107 (7.2%)	421 (7.2%)	1.1 (0.7–1.6)
Montelukast	54 (3.6%)	188 (3.2%)	1.5 (0.9–2.5)
Number of respiratory drugs [a]			
0	422 (28.5%)	1944 (33.1%)	1.0 (reference)
1–2	447 (30.2%)	1795 (30.6%)	1.6 (1.3–2.0)
3 or more	612 (41.3%)	2136 (36.4%)	2.2 (1.8–2.8)
Systemic glucocorticoid use	741 (50.0%)	1772 (30.2%)	4.5 (3.8–5.4)
Antibiotics	786 (53.1%)	1941 (33.0%)	3.1 (2.7–3.6)
Drugs for comorbidities			
Cardiovascular disease	833 (56.2%)	3194 (54.4%)	1.4 (1.1–1.8)
Anxiety disorder	601 (40.6%)	2304 (39.2%)	1.3 (1.0–1.7)
Dyspepsia	535 (36.1%)	1820 (31.0%)	1.9 (1.6–2.3)
Depression	357 (24.1%)	1421 (24.2%)	1.0 (0.7–1.3)
Diabetes mellitus	236 (15.9%)	917 (15.6%)	1.2 (0.8–1.9)
Rhinitis	215 (14.5%)	808 (13.8%)	1.1 (0.9–1.4)
Pain or inflammation	204 (13.8%)	793 (13.5%)	1.0 (0.8–1.3)

OR = odds ratio; 95%CI = 95% confidence interval

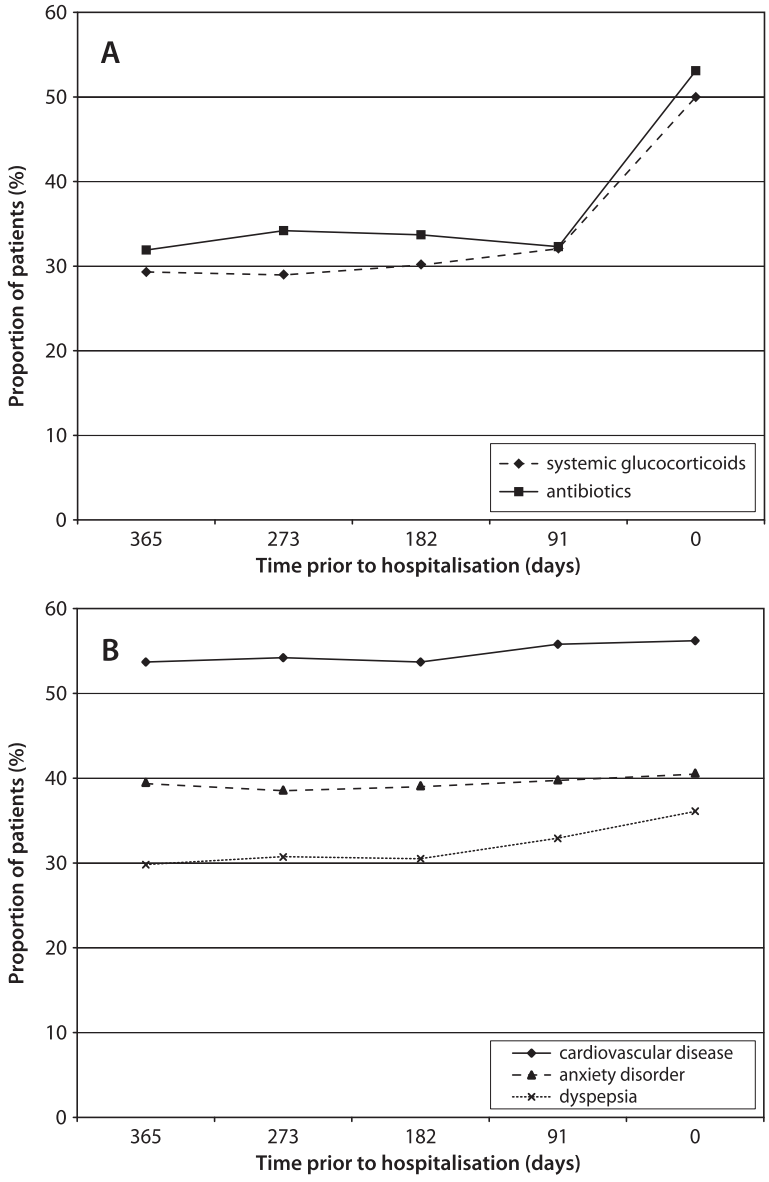
a) Respiratory drugs included are: inhaled glucocorticoids, β_2-agonists, muscarinic antagonists, xanthine derivatives, and montelukast use.

or more respiratory drugs increased prior to hospitalisation and was associated with a twofold increased risk of hospitalisation (OR 2.2; 95%CI 1.8–2.8, Table 1).
During the control periods, 30.2% of patients used systemic glucocorticoids, but this proportion increased to 50.0% in the case period (Figure 1A). Glucocorticoid use was associated with a fourfold increased risk of hospitalisation (OR 4.5; 95%CI 3.8–5.4, Table 1). Furthermore, antibiotic use was associated with a threefold increased risk of hospitalisation (OR 3.1; 95%CI 2.7–3.6).
Of the considered comorbidities in this study, only cardiovascular disease (OR 1.4; 95%CI 1.1–1.8) and dyspepsia (OR 1.9; 95%CI 1.6–2.3, Table 1) were associated with an increased risk of hospitalisation. The prevalence of medication use for other comorbidities was relatively constant in the control periods and yielded no large differences between case and control periods (Figure 1B).

DISCUSSION

In this study among patients with a hospitalisation for obstructive lung disease, it was shown that respiratory medication use increased prior to hospitalisation. The increased use of short-acting β_2-agonists prior to hospitalisation (adjusted OR 2.0; 95%CI 1.7–2.4) could reflect a decreased disease control. The use of systemic glucocorticoids, antibiotics, and other respiratory drugs (including inhaled glucocorticoids, short-acting and long-acting β_2-agonists, short-acting and long-acting muscarinic antagonists, xanthine derivatives, and montelukast use) increased prior to hospitalisation. We also found that hospitalisation for obstructive lung disease was associated with the presence of cardiovascular disease and dyspepsia, conditions both known to be associated with aggravation of obstructive lung disease.[5,32,33] These results could be indicative of the development and/or treatment of an exacerbation. There is need for markers to detect exacerbations in an early phase in order to start treatment as early as possible and possibly prevent hospitalisations for obstructive lung disease.
Because of the heterogeneity in the patient population with obstructive lung disease, it remains challenging to predict hospitalisations. Predicting and possibly preventing hospitalisations is of considerable clinical relevance. Exacerbations in obstructive lung disease were found to be negatively associated with lung function[34-36] or with normalization of sputum eosinophil counts,[10] but such parameters are more difficult to implement in routine disease management. General practitioners and pharmacists need easily measurable parameters to identify patients at high risk for hospitalisation. Therefore, we measured the risk

Figure 1 Medication use in case and control periods



In the case period (prior to hospitalisation), systemic glucocorticoids and antibiotics were used more frequently (A). Of all drugs for comorbidities, medication use for cardiovascular disease and for dyspepsia was increased prior to hospitalisation (B).

of hospitalisation as a function of medication use. In this study, we found that medication use is increased prior to hospitalisation. Irrespective of the cause of this increase, patients needed more medication prior to hospitalisation. Therefore, this medication use could be used as a marker for disease severity in obstructive lung diseases. Whether these medications could play a part in causing hospitalisation, whether patients refilled their medication but were not compliant, or whether these medications could postpone but not prevent hospitalisation should be studied in further research. For example, glucocorticoids and antibiotics could be subdivided based on active component and the most commonly used components could be identified and compared with the guidelines. Changes in medication use could reflect disease deterioration in this heterogeneous population. The results of this study are consistent with other studies that found that medication changes were associated with hospitalisation.[21,37] An important recommendation for clinical practice is that medication use of patients with severe obstructive lung disease should be continuously evaluated. Pharmacists could play a central role in this evaluation process as they have an overview on all prescribed medication used by the patient, with prescriptions of lung physicians and general physicians. Moreover, pharmacists could evaluate whether control medication is sufficiently used and whether or not rescue medication is being overused.

Physicians and pharmacists should be alert with increasing numbers of medication changes. Keeping close contact with patients who are at high risk for hospitalisation, for example by telemonitoring[10] or measurement of lung function,[38] could identify exacerbations in an early phase. Successful treatment of these exacerbations could decrease the hospitalisation rate in chronic obstructive lung disease. Upon hospitalisation, physicians should be informed about the patients' medication history as hospitalisation is associated with discontinuing of outpatient medication.[37] Upon discharge, patients should be included in a hospital discharge program, to decrease risk of emergency department visits and rehospitalisation.[39,40] Therefore, we should focus more on the continuity of care upon transitions of health care settings.

The results of this study should be interpreted in context of its limitations. First, each patient contributed one case period and up to four control periods. Of all patients included in the study, 1455 (98.2%) patients contributed four control periods. Therefore, missing a minority of control periods will contribute minimally to bias in this study. Second, patients' behaviour like smoking habits and compliance to medication might vary from day to day which could introduce confounding when comparing case periods with control periods. However, this seems unlikely to be an explanation for the results in this study. Third, the case-crossover design is designed to study effects of transient exposures on the risk of outcome events. It can

be argued that medication use for obstructive lung disease is not a transient, but a chronic exposure. However, we used the case-crossover design to evaluate trends in medication changes at several time points prior to hospitalisation. These changes are transient in nature.

In this study, multiple control periods for each patient were used to increase statistical power and to evaluate the effect of choice of control periods. Evaluating the effect of using certain control periods, use of glucocorticoids in the case period was compared with each control period separately. As glucocorticoid use was constant in each control period, the odds of glucocorticoid use in the case period compared with the control period was constant. Therefore, choice on control periods did not have major influence on the results.

In conclusion, changes in respiratory medication use prior to hospitalisation indicate development of an exacerbation in an early phase. There is need for markers to detect exacerbations in an early phase in order to start treatment as early as possible, possibly preventing hospitalisations. Moreover, we should focus more on the continuity of care upon transitions of health care settings, like hospitalisation and discharge from the hospital.

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chapter 5

Laboratory biomarkers as marker for disease severity in obstructive lung disease

3.1

Identification of exacerbations in obstructive lung disease through biomarkers

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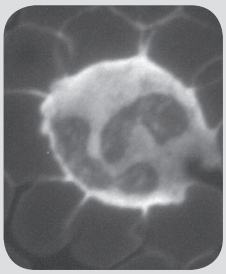


Biomarkers
2009;14(7):523-8

ABSTRACT

Background

Inflammation has been identified as an important factor for disease exacerbation in obstructive lung disease. In this study, we used neutrophil and eosinophil counts as biomarkers for exacerbation in obstructive lung disease.

Methods

We conducted a case-control study within a cohort of patients frequenting an outpatient clinic of Respiratory Medicine using data from the Utrecht Patient Oriented Database (UPOD). Cases were patients with a hospital admission for obstructive lung disease in 2005. For each case, one control patient was sampled from the same study base.

Results

We identified 143 cases (118 patients with chronic obstructive pulmonary disease and 25 asthma patients) and 143 controls. Admission was associated with both neutrophilia (adjusted odds ratio [OR] 4.3; 95% confidence interval [95%CI] 2.2–8.5), and eosinophilia (adjusted OR 2.6; 95%CI 1.1–6.2). The association with eosinophilia was only seen in asthma patients.

Conclusions

Neutrophil and eosinophil counts seem to be useful biomarkers for identifying exacerbations in pharmacoepidemiological studies on obstructive lung disease.

BACKGROUND

Worldwide, obstructive lung diseases are leading causes of morbidity and mortality.[1,2] Bronchodilators and inhaled glucocorticoids are central in the treatment of obstructive lung disease. However, response to pharmacotherapy varies and a minority of these patients continue to have symptoms and exacerbations in spite of being treated according to guidelines.[1-3] While various factors (e.g. environment, genetic factors, lifestyle) can contribute to exacerbation in obstructive lung disease,[4,5] so far no valid biomarkers have been made available to identify such exacerbations in pharmacoepidemiological studies.

Exacerbations in obstructive lung disease can be subdivided in difficult-to-treat asthma and non-respondent chronic obstructive pulmonary disease (COPD) and have been studied in pharmacoepidemiology so far mainly through identifying short courses of glucocorticoids and hospital admissions.[6-8] Although hospital admissions are widely acknowledged as a useful parameter for this purpose, they also carry a number of limitations, e.g. indication for hospital admission may vary significantly in different populations and countries, a trend towards outpatient care, underestimation of disease exacerbations not leading to a hospital admission.[7-9]

Molecular epidemiology and biomarkers are gaining importance in this research field.[10-14] Neutrophils and eosinophils play an important role in many of the inflammatory processes in obstructive lung diseases and are important factors for disease exacerbations in obstructive lung disease, also seen in many other studies.[15-18] However, these studies mostly deal with invasive methods like lung biopsies or induced sputum. Measuring inflammation parameters in peripheral blood would provide a simpler and more easily accessible biomarker.

In this study we investigated the possible association between neutrophils and eosinophils and hospital admission for disease exacerbation in a cohort of patients visiting an outpatient clinic of the for Respiratory Medicine Department on a regular basis.

METHODS

Setting

Data were obtained from the Utrecht Patient Oriented Database (UPOD). UPOD is an infrastructure of relational databases comprising administrative data on patient characteristics, laboratory test results, medication orders, discharge diagnoses and medical procedures for all patients treated at the University Medical Centre Utrecht, a 1042-bed tertiary teaching hospital in the centre of the Netherlands. All

UPOD research is in accordance with current Dutch privacy and ethical regulation. A more complete description of UPOD has been published elsewhere.[19]

Study population

From UPOD, we identified all adult (aged ≥ 18 years) patients, who were treated in the outpatient clinic of the Respiratory Medicine Department (n=3088). Of those patients, 903 had at least one haematological blood test for any clinical reason in 2005 and had no history of chemotherapy.

Hospital admission was used as an outcome measure for exacerbation in this study. Within the ICD-9-CM code group of diseases of the respiratory system (ICD-9-CM codes 460–519) admissions for otorhinolaryngological diseases (ICD-9-CM codes 460–478), (non-chronic) infectious disorders (ICD-9-CM codes 480–488, 490, 494 and 510–519) and respiratory diseases due to external agents (ICD-9-CM codes 495, 500–508) were excluded. Therefore, cases were defined as patients with an admission for obstructive lung disease (ICD-9-CM code 491, 492, 493 and 496). The date of the first admission in 2005 was defined as the index date. During this admission, the first blood measurement during admission was included in the analyses.

For each case, one control patient was sampled from the same study base. Controls had a blood test requested by the outpatient department of Respiratory Medicine within a period of one month around the index date of a case patient, and had no history of hospital admissions for obstructive lung disease. This control patient could have asthma, COPD, or respiratory related diagnoses, other than asthma or COPD. For all patients, the diagnosis was retrieved from the clinical records.

Exposure and covariate assessment

Cases and controls were compared with respect to absolute eosinophil and neutrophil counts. The absolute eosinophil and neutrophil count, on the one hand, and hospital admission, on the other, are associated through a non-linear relationship. Therefore, the absolute count was dichotomised according to the upper limit of our lab references, using our lab references of $1.6–8.3\times10^9$/l for neutrophils and $< 0.4\times10^9$/l for eosinophils.

Data on outpatient medication use at the time of the blood test was retrieved from the clinical records of both cases and controls. Glucocorticoid use at time of the blood test could be chronic use or a short course and daily dose exposure was expressed as nasal and inhaled beclomethasone equivalents or systemic prednisone equivalents, using defined daily dosages.[20]

Table 1 Basic characteristics of the study population

Characteristic	Cases n=143 (100%)	Controls n=143 (100%)	p-value) (two-sided)
Age in years; mean (SD)	65.6 (14.2)	49.9 (18.2)	< 0.001 [a]
Gender			0.722 [b]
male	78 (54.5%)	75 (52.4%)	
female	65 (45.5%)	68 (47.6%)	
Lung function; median (IQR)			
FEV_1 (l)	1.3 (0.9–1.9)	2.4 (1.6–3.3)	< 0.001 [a]
FEV_1 (% predicted)	52.6 (36.1–67.5)	79.5 (57.4–98.5)	< 0.001 [a]
PEF (l/sec)	3.8 (2.7–6.0)	6.5 (4.7–8.5)	< 0.001 [a]
FEV_1/FVC (%)	57.6 (44.7–69.2)	75.2 (62.2–81.0)	< 0.001 [a]
unknown	37 (25.9%)	17 (11.9%)	
Drug use			
short-acting β_2-agonist	73 (51.0%)	33 (23.1%)	< 0.001 [b]
long-acting β_2-agonist	91 (63.6%)	33 (23.1%)	< 0.001 [b]
nasal glucocorticoid [c]	6 (4.2%)	19 (13.3%)	0.006 [b]
≤ 200	3 (50.0%)	3 (15.8%)	1.000 [d]
> 200	1 (16.7%)	1 (5.3%)	
dose unknown	2 (33.3%)	15 (78.9%)	
inhaled glucocorticoid [c]	108 (75.5%)	52 (36.4%)	< 0.001 [b]
< 800	25 (23.1%)	10 (19.2%)	0.530 [d]
800–1336	13 (12.0%)	9 (17.3%)	
≥ 1336	47 (43.5%)	15 (28.8%)	
dose unknown	23 (21.3%)	18 (34.6%)	
systemic glucocorticoid [e]	76 (53.1%)	32 (22.4%)	< 0.001 [b]
< 10	17 (22.4%)	8 (25.0%)	0.091 [d]
10–25	18 (23.7%)	17 (53.1%)	
≥ 25	27 (35.5%)	4 (12.5%)	
dose unknown	14 (18.4%)	3 (9.4%)	

SD = standard deviation; IQR = interquartile range; FEV_1 = forced expiratory volume in 1 second; PEF = peak expiratory flow; FVC = forced vital capacity

a) Mann-Whitney test.
b) χ^2 test.
c) In µg beclomethasone equivalents. Percentage of dose categories is with respect to the number of patients using this drug.
d) χ^2 test for trend analysis.
e) In mg prednisone equivalents. Percentage of dose categories is with respect to the number of patients using this drug.

For each patient the lung function at time of the blood test was retrieved from the department of Respiratory Medicine. We used forced expiratory volume in 1 second (FEV_1), peak expiratory flow (PEF), and the FEV_1/Forced Vital Capacity (FVC) ratio as indication parameters for the lung function.[1,2]

Data analysis

Mann-Whitney tests and χ^2-tests were used as appropriate. Inhaled beclomethasone equivalents, systemic prednisone equivalents and the lung function measurements were categorised into tertiles, and missing equivalents were grouped in a separate category. Nasal beclomethasone equivalents were categorised into two groups for known equivalents and a third for missing doses. Unconditional multivariate logistic regression analysis was used to estimate the strength of the association between eosinophil and neutrophil counts and hospital admission in separate models, expressed as odds ratios (ORs) with 95% confidence intervals (CIs).

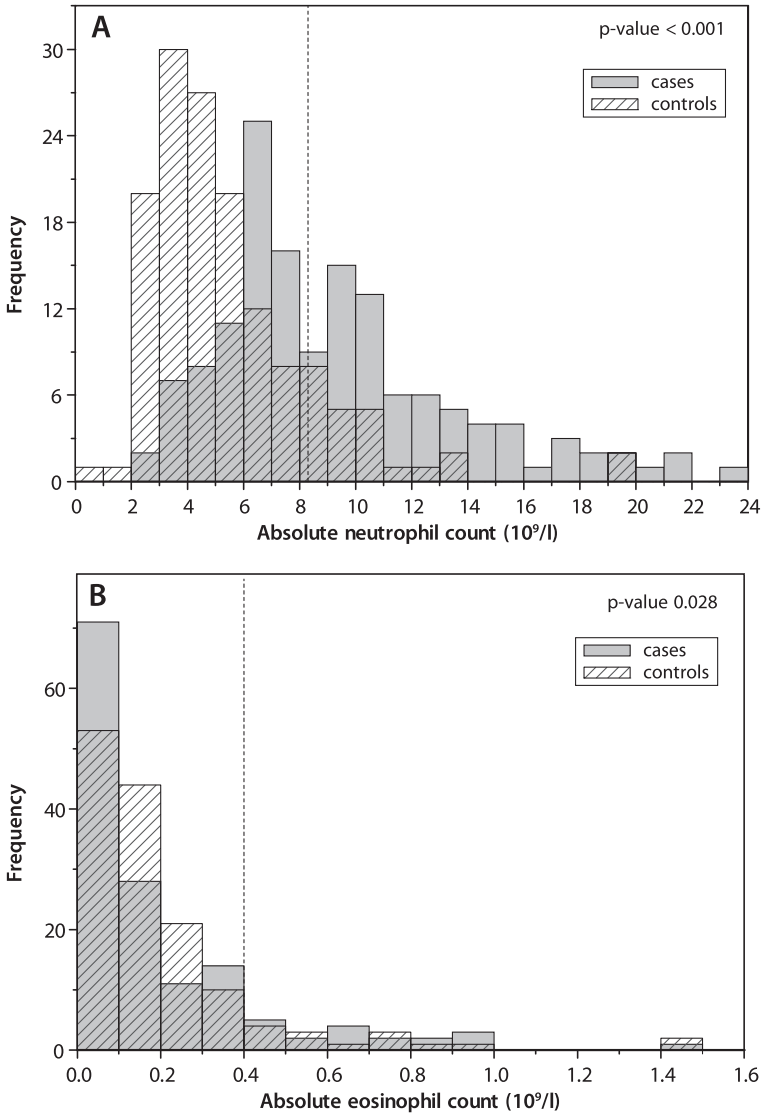
RESULTS

A total of 143 (4.6%) patients from the study cohort could be qualified as cases, including 118 patients with COPD and 25 asthma patients; in addition 143 controls were sampled from the study base. As shown in Table 1, the gender distribution was equal, but cases were older compared with controls. The lung function parameters FEV_1, PEF and the FEV_1/FVC ratio for cases were lower compared with controls ($p < 0.001$; Table 1). Also, cases used β_2-agonists and glucocorticoids more frequently at time of blood sampling compared with controls.

The distributions of the absolute neutrophil and eosinophil count are depicted in Figures 1A and 1B. No cases and one control had neutropenia ($< 1.6\times10^9$/l). As shown in Table 2, 19 (13.3%) cases had eosinophilia and 73 (51.0%) had neutrophilia. Of all controls, 15 (10.5%) patients had eosinophilia and 21 (14.7%) had neutrophilia, respectively. Overall, Table 2 shows that eosinophilia was associated with a more than twofold increased risk and neutrophilia was associated with a fourfold increased risk of hospital admission. Stratifying on diagnosis, neutrophilic COPD cases had a fourfold increased risk of admission, and admission was not associated with eosinophilia for COPD patients. With regard to asthma cases, admission was associated with eosinophilia (adjusted OR 15.3: 95%CI 3.9–60.0) and with neutrophilia (adjusted OR 5.9; 95%CI 2.0–17.5).

In clinical practice, positive predictive values (PPV) and negative predictive values (NPV) are valuable as these indicate the risk of hospital admission for patients with

Figure 1 Distribution of neutrophil (A) and eosinophil (B) counts for cases and controls



Cases have an increased absolute neutrophil count compared with controls (A), with a median (interquartile range) of 8.4×10⁹/l (6.2–11.4×10⁹/l) for cases and 4.6×10⁹/l (3.6–6.6×10⁹/l) for controls. This was statistically significant with a p-value < 0.001. The histogram for the eosinophil count is more similar for cases and controls (B) with median (interquartile range) of 0.10×10⁹/l (0.03–0.28×10⁹/l) for cases and 0.13×10⁹/l (0.07–0.26×10⁹/l) for controls. This was statistically significant with a p-value of 0.028. The vertical reference lines represent the upper limits of our lab references of 8.3×10⁹/l for neutrophils (A), and 0.4×10⁹/l for eosinophils (B).

Table 2 Associations between hospital admission and eosinophilia and neutrophilia

	Cases n (%)	Controls n (%)	Crude OR (95%CI)	Adjusted[a] OR (95%CI)
All cases	143 (100%)	143 (100%)		
eosinophilia[b]	19 (13.3%)	15 (10.5%)	1.3 (0.6–2.7)	2.6 (1.1–6.2)
neutrophilia[c]	73 (51.0%)	21 (14.7%)	6.0 (3.4–10.6)	4.3 (2.2–8.5)
COPD	118 (100%)	143 (100%)		
eosinophilia[b]	10 (8.5%)	15 (10.5%)	0.8 (0.3–1.8)	0.9 (0.3–2.4)
neutrophilia[c]	60 (50.8%)	21 (14.7%)	6.0 (3.3–10.7)	3.8 (1.8–8.0)
Asthma	25 (100%)	143 (100%)		
eosinophilia[b]	9 (36.0%)	15 (10.5%)	4.8 (1.8–12.7)	15.3 (3.9–60.0)
neutrophilia[c]	13 (52.0%)	21 (14.7%)	6.2 (2.5–15.5)	5.9 (2.0–17.5)

OR = odds ratio; 95%CI = 95% confidence interval; COPD = chronic obstructive pulmonary disease
a) Adjusted for age, gender, nasal glucocorticoid use, inhalation glucocorticoid use, systemic glucocorticoid use and lung function (FEV_1, PEF and FEV_1/FVC).
b) Eosinophilia is defined as eosinophils > 0.4×10^9/l vs. ≤ 0.4×10^9/l as reference range.
c) Neutrophilia is defined as neutrophils > 8.3×10^9/l vs. 1.6–8.3×10^9/l as reference range.

neutrophilia or eosinophilia. Overall, neutrophilia had a positive predictive value of 77.7%. After stratification on diagnosis, similar results were found for COPD cases (PPV=74.1%). For asthma patients the neutrophilia NPV (93.1%) and eosinophilia NPV (90.8%) were most informative, implying that asthma patients without neutrophilia and without eosinophilia are not likely to be hospitalised.

Of all patients, 36 (25.2%) cases and 11 (7.7%) controls had immature granulocytes. Subgroup analyses showed an adjusted OR of 3.5 (95%CI 1.6–7.6) including only patients without immature granulocytes compared with an adjusted OR of 4.3 (95%CI 2.2–8.5; Table 2) including all cases and controls. Nine of the 143 (6.3%) cases used glucocorticoids during admission before the first blood test was conducted. However, this glucocorticoid use did not have major effects on the associations found with an adjusted OR of 4.6 (95%CI 2.3–9.3) for neutrophilia when including only pre-treatment blood tests in the case group compared with an adjusted OR of 4.3 (95%CI 2.2–8.5; Table 2) including all cases and controls. For eosinophilia, the adjusted ORs were 2.7 (95%CI 1.1–6.6) and 2.6 (95%CI 1.1–6.2; Table 2), respectively. Moreover, excluding respiratory-related diagnoses, other than asthma or COPD, among controls did not have major influence on the results (data not shown).

DISCUSSION

In this study, we found an association between hospital admission and neutrophil and eosinophil counts. Stratified on diagnosis, we found a sixfold increased risk of admission for neutrophilic asthma patients with a negative predictive value of 93.1%. The results showing that admission was associated with neutrophilia for COPD patients and that hospital admission was associated with eosinophilia for asthma patients, but not for COPD patients are reassuring as these latter results are in line with current knowledge about asthma and COPD [1,2] and were the positive and negative controls in this study.

As hospital admission was associated in asthma but not in COPD patients, there seem to be pathophysiologically different phenotypes. Phenotyping is needed for understanding the molecular mechanisms of diseases, and therefore better prediction of outcomes in patients with these phenotypes, new therapeutic innovations and for phenotype-based treatment.

Our results support the hypothesis that biomarkers, such as neutrophil and eosinophil counts, may be useful as markers for disease exacerbation. However, using hospital admission as an outcome measure for exacerbation could lead to an underestimation of exacerbation frequency, because not all exacerbations result in admission and not all admissions are coded correctly.[21,22] Timely detection and identification of exacerbations without hospital admission is warranted in pharmacoepidemiological studies of lung diseases to avoid misclassification, but also in order to study severity pathways in patients with an exacerbation without hospital admission. Also from a clinical point of view these results might be interesting. Timely identification of disease deterioration might prevent admissions for obstructive lung disease in the future. Molecular epidemiology and biomarkers are promising in this research field.[12,23-25] Because of the transversal time perspective of this case-control study we cannot draw conclusions about the time relationship of the association between hospital admission and neutrophil and eosinophil counts. A replicate study should be conducted in a prospective, blinded fashion and the accuracy of the neutrophil and eosinophil count as early exacerbation marker for early identification of exacerbations should be confirmed.

It appears from previous studies that many authors struggle with the issue of whether or not the observed neutrophilia is primarily a characteristic of asthma severity or secondary to the treatment with glucocorticoids.[17,26-28] Glucocorticoids have a complex mechanism of action.[29,30] These drugs reduce the absolute count of many inflammatory cells, like T-lymphocytes, mast cells and eosinophils, but neutrophils were found to be less responsive to glucocorticoids.[17,30] Some studies have shown that glucocorticoids increase the absolute neutrophil count, by

inhibiting apoptosis.[29,31] However, these findings were done in in-vitro experiments, and in-vivo studies mainly focus on healthy volunteers and short-term effects of glucocorticoids on the neutrophil count.[32,33] Green et al. concluded that, in some asthma subjects at least, neutrophilia is not due to the glucocorticoid treatment.[27] Also Louis et al. found in their study that severe asthmatics, treated with systemic glucocorticoids, had a lower absolute neutrophil count in sputum compared to severe asthmatics who did not use systemic glucocorticoids.[28] In this study we found a strong association between hospital admission and neutrophilia for asthma patients. These results persisted after adjustment for glucocorticoid use and lung function and add to the evidence that neutrophilia among difficult-to-treat asthma patients is not solely caused by glucocorticoid treatment, but is an inflammatory characteristic of this asthma phenotype.

There are some methodological issues. First, controls sampled for this study visited the outpatient clinic of the Department of Respiratory Medicine, but their diagnosis could be different from asthma or COPD. However, excluding respiratory-related diagnoses other than asthma or COPD among controls did not have major influence on the results.

Furthermore, as UPOD is a database containing routinely collected data, there is always a clinical indication for a blood test. A possible consequence of this diagnostic suspicion bias is that it causes an over-representation of more severely ill patients. Yet, this issue applies more for controls, as admitted patients always have blood tests because of their health status. As a result, potential controls with a mild underlying disease might be underrepresented among our controls. This will lead to bias towards the null.

In conclusion, we were able to use neutrophil and eosinophil counts as a biomarker to identify exacerbation in obstructive lung disease. This shows promising possibilities in exploring the use of biomarkers in pharmacoepidemiology using routinely collected data.

Acknowledgements - The authors are grateful to Pieter Zanen, for providing the data on the lung function measurements.

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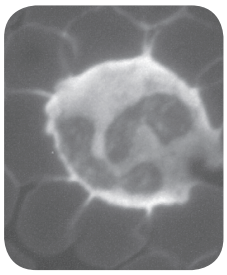

Haematocytometry analysis as discriminative marker for asthma phenotypes

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Clin Chem Lab Med
2009; 47(5):573-8

ABSTRACT

Background

There is an increasing demand for easy to measure biomarkers in clinical practice. We created the relational database Utrecht Patient Oriented Database (UPOD) to develop tools for identifying new biomarkers for disease. In this study, we used UPOD to identify better biomarkers for discriminating different asthma phenotypes.

Methods

We performed a prospective study at the University Medical Centre (UMC) Utrecht using blood from patients with asthma and a healthy reference group. Since asthma is an inflammatory disease, absolute leukocyte counts and leukocyte differential parameters were analysed using raw data files and a logistic regression model.

Results

We compared 17 difficult-to-treat asthma (DTA) cases, 13 non-difficult-to-treat asthma cases, and 19 healthy volunteers. Absolute leukocyte counts and differential parameters for leukocytes were able to discriminate asthma patients from healthy volunteers. However, among patients with asthma, difficult-to-treat cases could be more accurately defined with a neutrophil morphology change (odds ratio [OR] 8.0; 95% confidence interval [95%CI] 1.5-42.0), compared to the absolute neutrophil count (OR 4.0; 95%CI 0.8-21.0).

Conclusions

In this asthma patient population, we were able to define asthma phenotypes more precisely using neutrophil morphology parameters, compared to absolute counts. Using UPOD with differential parameters, it is possible to conduct larger scale biomarker studies, combining clinical, laboratory medicine, and epidemiological techniques.

BACKGROUND

The innovative role of the clinical chemistry and haematology laboratory is increasingly focused on the identification and development of new markers for disease. New biomarkers can help gain better understanding about the mechanism of disease for research purposes.[1] In addition, new biomarkers can be used in clinical practice to identify risk of disease, diagnosis of disease, and to monitor patient responses to therapy.[2,3] The Biomarkers Definitions Working Group has defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention".[1] Most biomarkers were discovered only after extensive data collection to answer a specific research question. However, advances in information technology have made it possible to store clinical data in an accessible electronic format. Therefore, it is now possible to conduct large-scale studies with clinical data.[4-7] These large-scale studies can be used to develop predictive models based on a patient's molecular profile and phenotype.[8,9]

The Utrecht Patient Oriented Database (UPOD), comprises all clinical data for all patients treated at the University Medical Centre (UMC) Utrecht since January 2004.[10] Because routine haematocytometry analysers generate vast amounts of data on circulating blood cells that may be of value as biomarkers, the raw data from these analysers were incorporated in UPOD. Data mining techniques can be useful for identifying markers of importance in the management of certain patient groups and to facilitate better discriminative power for clinicians.

Asthma is a common, complex multiphenotypic disease, in which underlying inflammation plays an important role. Asthma is recognized as a heterogeneous condition and can be subdivided into several phenotypes. However, multiple subphenotypes have been defined that show considerable overlap.[11,12] One phenotype is difficult-to-treat asthma (DTA), characterized by poor response to glucocorticoid therapy.[13] Early detection of DTA is warranted to prevent side effects from high doses of systemic glucocorticoids and allow more effective implementation of pharmacotherapy. At present, no easily accessible routine biomarker that discriminates between asthma phenotypes has been discovered. There is a need for an inflammatory biomarker since asthma is an inflammatory disease.

The aim of this study was to use leukocyte haematocytometry parameters as biomarker for discriminating between asthma phenotypes. In addition, this new tool was used as a model to evaluate the added value of morphological differential parameters to UPOD.

METHODS

Setting

Cell-Dyn Sapphire automated haematocytometers (Abbott Diagnostics, St. Clara, CA, USA) were used as the routine haematology analysers at the UMC Utrecht, a 1042-bed tertiary teaching hospital in the centre of the Netherlands. Five morphological differential parameters provided information about the size, complexity, lobularity, depolarisation, and cell damage. These morphological differential parameters were used as input for the default Cell-Dyn algorithm to classify leukocytes.

Study population

We conducted a prospective study using a well-defined study population and incorporated in UPOD. From January 2006 to September 2007, asthma patients without acute infection and having a haematological blood test for any clinical reason were identified from the outpatient clinic of the Department of Respiratory Medicine. These asthma patients were categorized as DTA patients or non-DTA patients, according to the American Thoracic Society (ATS) criteria, which includes the use of medication.[14] In addition to haematological parameters, age, gender, and information on allergies was obtained from the patient's medical record. Reference blood samples were collected from healthy volunteers. These volunteers did not have asthma or allergies, and did not use glucocorticoids. This study was in accordance with current Dutch privacy and ethical regulations.

Data analysis

Mann-Whitney and χ^2-tests were used, as appropriate. The absolute leukocyte counts and morphological differential parameters were measured with the Cell-Dyn Sapphire (Abbott Diagnostics, St. Clara, CA, USA) and analysed with FCS Express, version 3 (De Novo Software, Los Angeles, CA, USA) and SPSS for Windows, version 14.0 (SPSS Inc., Chicago, IL, USA).

The absolute leukocyte count was determined according to the default Cell-Dyn algorithm. To detect changes in morphology, a fixed morphological reference gate was defined using FCS Express and the data from the 19 healthy volunteers. Leukocyte subpopulations from healthy volunteers were selected based on size, granularity, lobularity and depolarisation to create gating formulas. These gating formulas were applied to compute the absolute leukocyte count, accepted by these gating formulas, for every participant. Next, the discordance between the absolute leukocyte count, accepted by the gating formula, and that calculated with the default Cell-Dyn algorithm was determined. Discordance between gating formula

and the default Cell-Dyn algorithm indicated a shift away from the reference and thus a morphological change in the leukocyte subpopulation. This discordance was used as continuous variable input to compute Receiver Operator Characteristic (ROC) curves. The optimal cut-off value was used in a predictive logistic regression model.

RESULTS

Patient characteristics

We compared three groups comprised of 17 DTA patients, 13 non-DTA patients, and 19 healthy volunteers. Asthma patients and healthy volunteers were comparable with respect to age and gender, but DTA patients were younger than non-DTA patients ($p = 0.006$, Table 1). Among patients with asthma, there was no difference in the frequency of an allergic constitution between the DTA and non-DTA group. The gating formula captured all neutrophils for healthy volunteers, but not for non-DTA or DTA patients (Figure 1). None of the study subjects were flagged by the haematocytometers for immature granulocytes.

Using leukocyte markers to identify asthma patients

Asthma patients had higher absolute neutrophil counts compared with healthy volunteers ($p < 0.001$, Table 1). Participants with neutrophils more than 4.0×10^9 neutrophils/l were nine times more likely to have asthma (odds ratio [OR] 8.8; 95% confidence interval [95%CI] 2.3–33.8). This test yielded a positive predictive value (PPV) of 84%. The negative predictive value (NPV) was 63% (Table 2).

The optimal cut-off value for morphological change was a loss of 0.1×10^9 neutrophils/l, meaning that the reference gating formula could not accept all neutrophils present in the blood sample. Stated differently, a neutrophil loss of 0.1×10^9/l or more from the gating formula was defined as a morphological change causing a shift outside the fixed reference gate.

Morphological changes occurred more frequently in asthma patients compared with healthy volunteers (Figure 2, Table 2). A morphological change was associated with a more than eight times higher risk of having asthma (OR 8.5; 95%CI 1.7–43.4, Table 2). This test yielded a PPV of 88% and a NPV of 53% (Table 2). Discordance in the absolute monocyte count tended to have some discriminative ability using univariate analysis (OR 0.3; 95% CI 0.1–1.1), but did not add any value in multivariate analyses.

Table 1 Baseline characteristics of asthma patients and healthy volunteers [a]

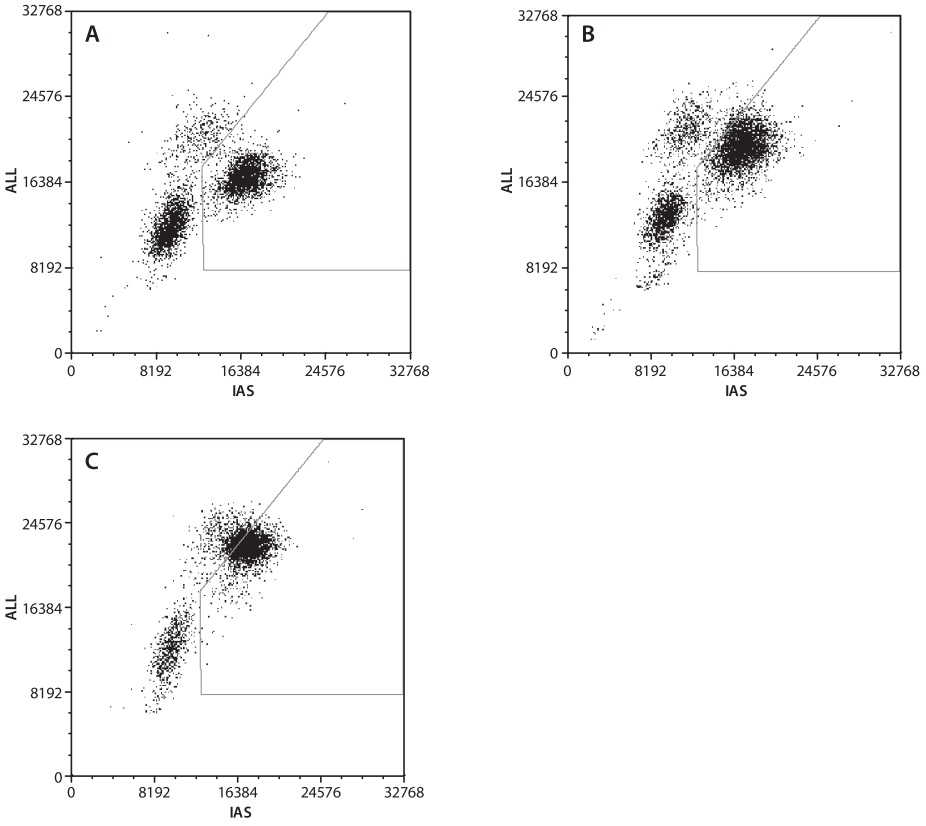
Characteristic	Asthma patients (n=30)	Healthy volunteers (n=19)	p-value (two-sided)
Age in years	44.4 ± 14.8	44.2 ± 10.6	0.944 [b]
(range)	(18-74)	(25-64)	
Gender			0.961 [c]
male	9 (33.3%)	6 (31.6%)	
female	21 (67.7%)	13 (68.4%)	
Absolute neutrophil count, $\times 10^9$/l	6.1 ± 3.5	3.5 ± 0.8	< 0.001 [b]
Absolute lymphocyte count, $\times 10^9$/l	2.4 ± 1.2	2.4 ± 0.8	0.897 [b]
Absolute monocyte count, $\times 10^9$/l	0.50 ± 0.2	0.54 ± 0.1	0.267 [b]
Absolute eosinophil count, $\times 10^9$/l	0.39 ± 0.4	0.21 ± 0.2	0.129 [b]
	DTA patients (n=17)	**Non-DTA patients (n=13)**	**p-value (two-sided)**
Age in years	38.0 ± 11.3	53.2 ± 14.9	0.006 [b]
(range)	(18-63)	(29-74)	
Gender			0.166 [c]
male	3 (22.2%)	6 (46.2%)	
female	14 (77.8%)	7 (53.8%)	
Allergic constitution	15 (83.3%)	10 (76.9%)	1.000 [c]
Absolute neutrophil count, $\times 10^9$/l	7.1 ± 4.2	4.7 ± 1.7	0.079 [b]
Absolute lymphocyte count, $\times 10^9$/l	2.6 ± 1.4	2.2 ± 0.7	0.567 [b]
Absolute monocyte count, $\times 10^9$/l	0.50 ± 0.2	0.50 ± 0.2	0.828 [b]
Absolute eosinophil count, $\times 10^9$/l	0.46 ± 0.4	0.29 ± 0.3	0.457 [b]

DTA = difficult-to-treat asthma
a) Data are presented as n (%) or mean ± standard deviation.
b) Mann-Whitney test.
c) χ^2 test (without continuity correction).

Using leukocyte markers for distinguishing DTA among asthma patients

To identify asthma patients classified as DTA, the absolute neutrophil count had less discriminative value (p = 0.079, Table 1). A high neutrophil count was not discriminative for DTA (OR 4.0; 95%CI 0.8–21.0). However, morphological changes in neutrophils were able to discriminate DTA from non-DTA patients. As shown in Table 2, 12 of 15 (80%) discordant asthma patients were DTA patients.

Figure 1 Morphological differential parameters, measured on 16-bit scales



ALL = leukocyte size (0 degrees); IAS = leukocyte complexity as a proxy for the granule count in the cytoplasm (7 degrees)
The gating formula was able to gate all neutrophils of the healthy volunteers (**A**), but not for the non-DTA (**B**) and DTA patient (**C**).

A morphological change in neutrophils was associated with DTA (OR 8.0; 95%CI 1.5–42.0) with a PPV of 80% and a NPV of 67% (Table 2).

Change in neutrophil morphology

Neutrophil morphology parameters are subject to large intra-individual variation. However, we found DTA patients and non-DTA patients to have much more variation in the size of neutrophils compared to healthy volunteers (Figure 3). Using complexity as a proxy measure for the granule count in the cytoplasm,

Table 2 Discriminative value of the absolute neutrophil count and the morphological change

			Absolute neutrophil count		
	≤ 4.0 [a]	> 4.0 [a]	OR (95%CI)	PPV (%)	NPV (%)
Healthy volunteers (n=19)	16 (84.2%)	3 (15.8%)	1.0 (reference)	reference	reference
All asthma patients (n=30)	9 (30.0%)	21 (70.0%)	8.8 (2.3–33.8)	84%	63%
Non-DTA patients (n=13)	6 (46.2%)	7 (53.8%)	1.0 (reference)	reference	reference
DTA patients (n=17)	3 (17.6%)	14 (82.4%)	4.0 (0.8–21.0)	67%	67%
			Neutrophil morphological change		
	< 0.1 [b]	≥ 0.1 [b]	OR (95%CI)	PPV (%)	NPV (%)
Healthy volunteers (n=19)	17 (89.5%)	2 (10.5%)	1.0 (reference)	reference	reference
All asthma patients (n=30)	15 (50.0%)	15 (50.0%)	8.5 (1.7–43.4)	88%	53%
Non-DTA patients (n=13)	10 (76.9%)	3 (23.1%)	1.0 (reference)	reference	reference
DTA patients (n=17)	5 (29.4%)	12 (70.6%)	8.0 (1.5–42.0)	80%	67%

OR = odds ratio; 95%CI = 95% confidence interval; PPV = positive predictive value; NPV = negative predictive value; DTA = difficult-to-treat asthma

a) Cut-off value 4.0×10^9/l for absolute neutrophil count.

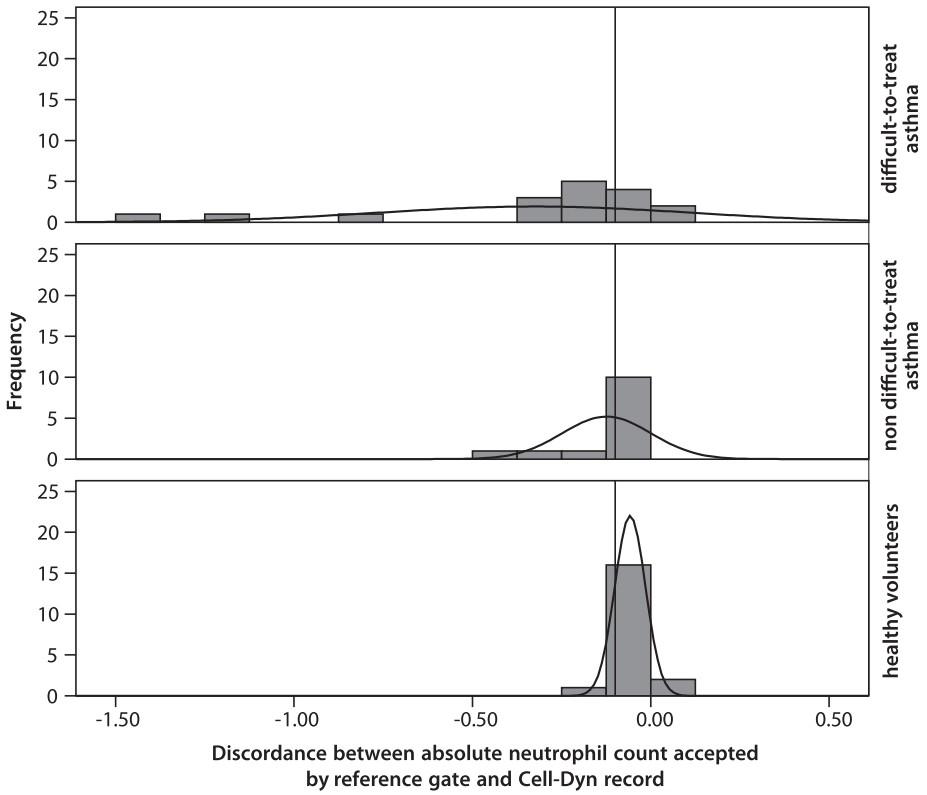
b) Cut-off value for the morphological change: loss of 0.1×10^9 neutrophils/l or more from the gating formula.

asthma patients had less granules than healthy volunteers ($p < 0.001$). There was no difference in neutrophil lobularity, depolarisation and cell damage (data not shown).

DISCUSSION

In this study, we found that absolute neutrophil counts and morphological changes were, independently, of sufficient discriminative value for distinguishing asthma patients from healthy volunteers. However, amongst asthma patients only, the absolute counts alone could not be used to distinguish DTA patients from non-DTA patients. Changes in the neutrophil morphology can have many causes. However, in this pre-selected population of patients with asthma, phenotypes could be more accurately defined using neutrophil morphology parameters, in contrast to the absolute neutrophil count. The use of fixed gating provides more detail about the neutrophilic inflammation compared to the default output of the Cell-Dyn Sapphire.

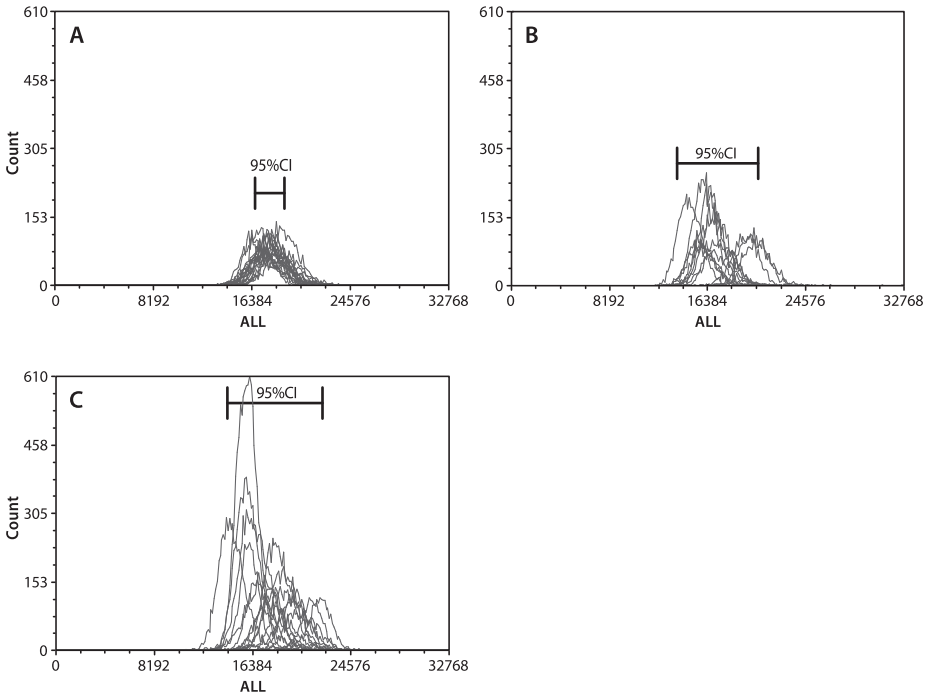
Figure 2 Histogram of the difference between the absolute neutrophil count accepted by reference gating formula and Cell-Dyn record



Asthma patients more frequently had morphological changes of circulating neutrophils than healthy volunteers. The large differences were mainly caused by DTA (difficult-to-treat asthma) patients. The vertical line represents the cut-off value of a loss of 0.1×10^9/l or more by the gating formula, used in the logistic regression model.

The finding that there was more variation in neutrophil size in asthma patients compared with healthy volunteers suggests that neutrophil morphological parameters can be used as a biomarker for asthma. Also, neutrophils of asthma patients had fewer granules than those of healthy volunteers. This might imply that neutrophils in the peripheral blood of asthma patients were less activated, or that activated neutrophils are no longer present in the blood but have migrated into tissue. Another possibility is that neutrophils had fewer granules because of

Figure 3 Histograms representing the distribution of the neutrophil size (ALL) in every participant, measured on a 16-bit scale



Healthy volunteers (**A**) had less variation in the neutrophil size, expressed as a 95% confidence interval, compared to non-DTA (difficult-to-treat asthma) patients (**B**) and DTA patients (**C**).

activation and exocytosis. The mechanisms causing these morphological changes need to be studied in more detail. In addition, this study needs to be replicated in a prospective and blinded fashion to confirm the accuracy of the test. The results of this study show that neutrophil morphological differential parameters could be used as a biomarker for discrimination between distinct asthma phenotypes.

To our knowledge this is the first study using leukocyte morphological differential parameters, collected with routine haematocytometry analysis, as markers for identifying disease phenotypes. Other studies only used absolute counts [15-17] or flow cytometry following in vitro preparation.[18-21] Some studies have been conducted with eosinophils,[22] to identify acute infections [23,24] or have evaluated an immunoplatelet method vs. flow cytometry in thrombocytopenia.[25] No studies have been conducted

in the framework of identifying asthma phenotypes in routine clinical practice by combining clinical, laboratory medicine and epidemiological techniques.
Morphology changes can be measured in automatically generated samples by incorporating a fixed morphological reference gate in the haematocytometer software. This method is a novel technique for studying asthma phenotypes, and may possibly be extended to other diseases. Although we performed this study with Cell-Dyn Sapphires, all haematocytometry analysers provide similar functionality regarding neutrophil subpopulation detection.
Because of positive results seen with this study, we now collect and store the raw morphological data files of all blood samples measured in our clinical chemistry and haematology laboratory for research purposes. With morphological differential parameters added to UPOD, this makes it feasible to use the raw morphological data as a diagnostic marker on a larger scale.
In conclusion, we were able to define asthma phenotypes more precisely using neutrophil morphological differential parameters, compared to absolute neutrophil counts. Using UPOD with morphological differential parameters, it is possible to study leukocyte morphological parameters and facilitate large-scale biomarker research, combining clinical, laboratory medicine, and epidemiological techniques.

Acknowledgements - The authors are grateful to Albert Huisman and Jolanda Kolvers for measurement of the blood samples, to all healthy volunteers for providing reference blood samples, to Abbott Diagnostics for providing the program FCS Express, and to all colleagues involved in the UPOD initiative.

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3.3

Montelukast use as marker for disease severity among asthma patients

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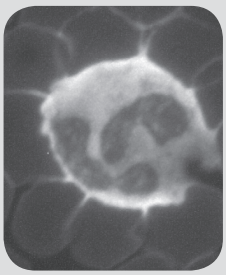

Submitted

ABSTRACT

Background

There is an increasing demand for valid markers in clinical practice. There are several ways to study disease severity in databases, depending on regional guidelines. In most European countries, montelukast is used in asthma patients not controllable with regular asthma medication. The objective of this study was to evaluate montelukast use as a marker for disease severity among asthma patients in automated databases.

Methods

This study was conducted within the Utrecht Patient Oriented Database (UPOD). Since asthma is an inflammatory disease and difficult-to-treat asthma (DTA) can be distinguished by studying neutrophil inflammation, the absolute neutrophil counts and neutrophil morphology were studied in montelukast users and non-montelukast users. Moreover, patients were categorized into DTA and non-DTA by clinical review and the predictive value of montelukast use for DTA was calculated.

Results

We compared 20 montelukast users and 29 montelukast non-users. Both absolute neutrophil counts (odds ratio [OR] 1.5; 95% confidence interval [95%CI] 0.4–6.0) and a change in neutrophil morphology (OR 2.1; 95%CI 0.6–7.4) were not discriminative for montelukast use versus non-use among asthma patients. The positive predictive value of montelukast use for DTA was 85.0%.

Conclusions

Montelukast use is an indication, but imperfect marker for disease severity among asthma patients in automated databases.

BACKGROUND

The innovative role of pharmacoepidemiology is increasingly focused on the identification and development of new markers for disease, which can be used in clinical practice.[1] Biomarkers already provided medical innovations, for example to predict adverse events like heparin-induced thrombocytopenia.[2] In recent years, large health care databases are increasingly used for research. These databases provide important tools in pharmacoepidemiological research.[3] Data mining techniques can be useful for identifying markers of importance in the management of certain patient groups and to facilitate better discriminative power for clinicians. One of the key issues in pharmacoepidemiology remains confounding by disease severity. In this study, asthma was used as a learning model to study markers for disease severity. Asthma is a common, complex multiphenotypic disease, in which the underlying inflammation plays an important role.[4,5] Difficult-to-treat asthma (DTA) is one phenotype occurring in 5–10% of asthma patients and is characterized by poor response to inhaled glucocorticoid therapy.[4,5] Early detection of DTA is warranted, possibly preventing side effects from high doses of systemic glucocorticoids and fine-tuning of pharmacotherapy could be achieved more easily. However, markers for DTA in automated databases are currently lacking.

There are several ways to study disease severity in asthma,[6,7] depending on regional guidelines. One possible marker for asthma disease severity is montelukast use. According to the GINA guideline, montelukast has two distinct roles: as an alternative for inhaled glucocorticoids in mild persistent asthma patients, although less effective; and as add-on therapy in more severe asthma patients whose asthma is not controllable with inhaled glucocorticoids.[4] In the USA, montelukast is used as an alternative for inhaled glucocorticoids in mild asthma.[8] On the contrary, in most European countries, including the Netherlands, montelukast is prescribed for asthma patients not controllable with regular asthma medication.[8] Since registration in 1998 in the Netherlands, montelukast use increased with 3.5% asthma patients in 2003 to 7.1% asthma patients in 2007 (www.gipdatabank.nl, visited on 03-02-2009). The objective of this study was to evaluate montelukast use as a marker for disease severity among asthma patients in automated databases.

METHODS

Study design and setting

We conducted a cross-sectional study using data from the Utrecht Patient Oriented Database (UPOD). UPOD is an infrastructure of relational databases comprising

administrative data on patient characteristics, laboratory test results, medication orders, discharge diagnoses and medical procedures for all patients treated at the University Medical Centre Utrecht, a 1042-bed tertiary teaching hospital in the centre of the Netherlands. Each year, around 900 adult asthma patients visit the outpatient clinic of Respiratory Medicine, comprising the study base. All UPOD research is in accordance with current Dutch privacy and ethical regulation. A more complete description of UPOD has been published elsewhere.[9]

Study population

From January 2006 until December 2007, asthma patients without acute infection and having a haematological blood test for any clinical reason were identified. We categorized asthma patients in montelukast users, and patients that did not use montelukast at time of blood sampling. Because DTA was found to be associated with a change in neutrophil morphology in literature,[10] the absolute neutrophil count and neutrophil morphology was characterized for all patients. Moreover, asthma patients were categorized in DTA and non-DTA by clinical review, according to the American Thoracic Society (ATS) criteria, which includes the use of medication.[5]
Next to the haematological parameters, age, gender, and glucocorticoid use was obtained from the UPOD database. Glucocorticoid use at time of the blood sample could be chronic use or a short course and daily dose exposure was expressed as nasal and inhaled beclomethasone equivalents or systemic prednisone equivalents, using defined daily dosages.

Data analysis

Mann-Whitney and χ^2-tests were used, as appropriate. The absolute neutrophil counts and morphology were measured with the Cell-Dyn Sapphire (Abbott Diagnostics, St. Clara, California, USA) and analysed with FCS Express, version 3 (De Novo Software, Los Angeles, California, USA) and SPSS for Windows, version 14.0 (SPSS Inc., Chicago, Illinois, USA). The cut-off value used in the predictive logistic regression model was 4.0×10^9/l for the absolute neutrophil count and normal neutrophil morphology was based on our laboratory reference, based on size, granularity, lobularity and depolarization, in accordance to other studies.[10] This method is described in more detail elsewhere.[10]

RESULTS

We identified 20 asthma patients using montelukast and 29 asthma patients without montelukast use. Montelukast users were comparable with non-montelukast users regarding age, gender, proportion hospitalisations, and leukocyte counts (Table 1). None of the study subjects were flagged by the haematocytometers for immature granulocytes. Montelukast users used more often nasal glucocorticoids, compared to non-montelukast users. Usage of inhaled glucocorticoids and systemic glucocorticoids was similar in both groups (Table 1).

Of all patients, 16 (80.0%) montelukast users and 21 (72.4%) non-montelukast users had an absolute neutrophil count of more than 4.0×10^9/l. For abnormal neutrophil morphology these numbers were 15 (75.0%) and 17 (58.6%), respectively. Using

Table 1 Baseline characteristics

Characteristic	Montelukast users (n=20)	Non-montelukast users (n=29)	p-value (two-sided)
Age in years, mean (SD)	44.2 (15.1)	50.1 (16.6)	0.254 [a]
Gender, n (%)			0.479 [b]
male	5 (25.0%)	10 (34.5%)	
female	15 (75.0%)	19 (65.5%)	
Outpatient, n (%)	13 (65.0%)	17 (58.6%)	0.652 [b]
Hospital admission, n (%)	7 (35.0%)	12 (41.4%)	
Absolute neutrophil count $\times10^9$/l, mean (SD)	7.9 (4.5)	6.2 (3.3)	0.132 [a]
Absolute lymphocyte count $\times10^9$/l, mean (SD)	2.2 (1.2)	2.6 (1.2)	0.281 [a]
Absolute monocyte count $\times10^9$/l, mean (SD)	0.54 (0.20)	0.58 (0.27)	0.669 [a]
Absolute eosinophil count $\times10^9$/l, mean (SD)	0.37 (0.42)	0.31 (0.28)	0.729 [a]
GC use			
Nasal GC, n (%)	13 (65.0%)	10 (34.5%)	0.046 [b]
dose, median (IQR) [c]	200 (100–700)	300 (200–400)	
Inhaled GC, n (%)	18 (90.0%)	20 (69.0%)	0.162 [b]
dose, median (IQR) [c]	1480 (1342–2000)	1336 (658–1360)	
Systemic GC, n (%)	14 (70.0%)	15 (51.7%)	0.246 [b]
dose, median (IQR) [d]	40 (10–62.5)	40 (30–50)	

SD = standard deviation; GC = glucocorticoid; IQR = interquartile range
a) Mann-Whitney test.
b) χ^2 test.
c) Dose in µg beclomethasone equivalents.
d) Dose in mg prednisone equivalents.

montelukast use as marker for asthma disease severity, neither the absolute neutrophil count (odds ratio [OR] 1.5; 95% confidence interval [95%CI] 0.4–6.0) nor a change in neutrophil morphology (OR 2.1; 95%CI 0.6–7.4) were discriminative for montelukast use. Subgroup analyses with outpatients and hospitalised patients did not yield different results (data not shown).

Moreover, all patients were categorized into DTA and non-DTA by clinical review and montelukast use was evaluated as marker for difficult-to-treat asthma. Of all montelukast users, 17 patients were categorized as DTA, yielding a positive predictive value of 85.0%. For non-montelukast users, 18 patients had non-DTA with a negative predictive value of 62.1%. Difficult-to-treat asthma was not associated with the absolute neutrophil count (OR 2.3; 95%CI 0.6–8.7). The neutrophil morphology was discriminative for DTA among asthma patients with an OR of 4.0 (95%CI 1.2–14.0).

DISCUSSION

In this study we evaluated montelukast use as a marker for disease severity among asthma patients in two ways. First, the association between montelukast use and the absolute neutrophil count and a change in neutrophil morphology was evaluated as this association was found for difficult-to-treat asthma among asthma patients.[10] Second, we clinically reviewed all patients. Montelukast use had a positive predictive value of 85.0% for DTA. However, the absolute neutrophil counts and morphological change were not of sufficient discriminative value for distinguishing montelukast users from non-users among asthma patients. Therefore, we conclude that montelukast use is an indication, but imperfect marker for disease severity among asthma patients in automated databases.

Several factors might contribute to the imperfect marking of montelukast use for difficult-to-treat asthma. These factors are addressed one by one. The number of participants included in this study was low, because of low montelukast use and rare blood sampling among asthma patients in clinical practice. The fact that blood samples were drawn for any clinical reason could lead to selection bias with overrepresentation of more severely ill patients, leading to bias towards the null. However, difficult-to-treat asthma is associated with a change in neutrophil morphology in this and an earlier study.[10]

There is some discussion regarding the anti-inflammatory effects of montelukast. It has been reported that montelukast reduces the bronchoalveolar lavage neutrophil count in rabbits,[11] but others found no influence of montelukast on neutrophils.[12]

This will be of minor importance, because difficult-to-treat asthma is associated with a change in neutrophil morphology in this and an earlier study.[10]
As shown in this study, 17 montelukast users were categorized as DTA, and three were not. This misclassification leads to bias towards to null. Two factors could explain the occurrence of this misclassification. First, guidelines are not fully implemented in clinical practice. Therefore, DTA patients could be undertreated by not using montelukast. However, montelukast is also used in three non-DTA patients in this study. Otherwise, there is a high non-response rate to montelukast in severe asthma patients. Results from randomised clinical trials show conflicting results regarding the effectiveness of leukotriene receptor antagonists in patients receiving moderate to high doses inhaled glucocorticoids.[13,14] For non-respondent patients, montelukast treatment is stopped, and the patient is categorized as non-user as time of blood sampling. Because of low power, there could not be discriminated between current and past montelukast users.
There are multiple ways to study disease severity in any disease,[6,7] depending on regional guidelines. In rheumatic arthritis, TNF-α antagonists are often used as marker for disease severity[15] because these drugs are prescribed to severe patients only. In the Netherlands, montelukast is prescribed to asthma patients not controllable with regular asthma medication[8] and could therefore be studied as a marker for disease severity.
In conclusion, montelukast use has a positive predictive value of 85.0% for DTA. However, the absolute neutrophil counts and morphological change were not of sufficient discriminative value for distinguishing montelukast users from non-users among asthma patients. Therefore, we conclude that montelukast use is an indication, but imperfect marker for disease severity among asthma patients in automated databases.

Acknowledgements - The authors are grateful to Albert Huisman and Jolanda Kolvers for measurement of the blood samples; to Hanneke den Breeijen for data collection; and to Abbott Diagnostics for providing the program FCS Express.

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chapter 4

Methodological aspects in biomarker studies

4.1

Disease severity in clinical practice among patients with respiratory disease

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ABSTRACT

Background

Large observational studies in Europe and the United States have shown that 30–80% of patients with respiratory disease have uncontrolled disease activity. The aim of this study was to evaluate disease severity and to assess current practice in testing disease severity in a cohort of respiratory patients. In addition, associations between potential biomarkers and hospitalisation in obstructive lung disease were studied.

Methods

A cohort study was conducted using data from the Utrecht Patient Oriented Database (UPOD). All adult patients visiting the outpatient clinic of Respiratory Medicine in 2005–2007 were included in the cohort. Parameters concerning exacerbations, age, gender, diagnosis, contacts with the outpatient clinic, laboratory tests, lung function tests, and other procedures were collected for each patient. Associations between biomarkers and hospitalisation were studied by logistic regression analysis.

Results

Approximately 3000 patients visited the outpatient clinic each year, with a total of 5356 individual patients in 2005–2007. Of all patients, 12.2% visited the emergency department and 3.6% were hospitalised annually. Haematological blood testing and lung function testing occurred more often prior to hospitalisation with a fivefold increased risk of hospitalisation in the period of 15 days prior to the index date (adjusted odds ratio [OR] 5.5; 95% confidence interval [95%CI] 3.2–9.3 and adjusted OR 5.4; 95%CI 2.9–10.0 respectively).

Conclusions

Most patients were well-controlled with regard to disease severity. Testing was conducted for a biased sample of more severely ill patients. Testing bias should therefore be taken into account in the conduct of biomarker studies using data from routine clinical databases.

BACKGROUND

Obstructive lung diseases are leading causes of morbidity and mortality.[1,2] Large observational studies in Europe and the United States have shown that 30–80% of patients with respiratory disease have uncontrolled disease activity. Patients with uncontrolled disease activity are often referred to secondary health care, which has substantial implications with respect to quality of life, health care utilization and economic costs.[1,3-6] Therefore, evaluation of disease severity in respiratory disease is essential.

Although important progress has been made in respiratory research in recent years, there is ongoing discussion about confounding in epidemiological studies introduced by differences in disease severity between comparator groups.[7-9] More severely ill patients are likely to use more medication and are at increased risk of disease exacerbations because of disease severity at the same time. Therefore, effects of disease severity and drug exposure are mixed-up, leading to spurious associations. This is illustrated with the discussion about use of β_2-agonists and the risk of asthma death, where eventually confounding by disease severity was indicated as the most probable cause of the association between use of β_2-agonists and asthma death.[10-16] It is essential to minimize this confounding in order to get a true risk estimate in epidemiological studies.

Although disease severity could be measured by symptoms or questionnaires, we need more objective markers to measure disease severity in clinical practice. The aim of this study was twofold. The first aim was to evaluate disease severity in a cohort of patients visiting the outpatient clinic of Respiratory Medicine and to assess current practice in testing disease severity. In addition, we investigated associations between potential biomarkers measured in clinical practice and hospitalisation for obstructive lung disease, using hospitalisation as a measure for disease severity.

METHODS

Setting

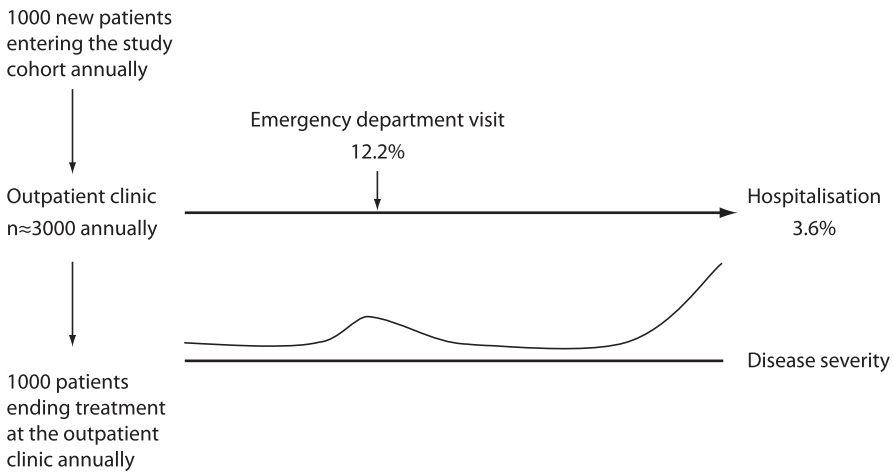
Data were obtained from the Utrecht Patient Oriented Database (UPOD). UPOD is an infrastructure of relational databases comprising administrative data on patient characteristics, laboratory test results, medication orders, discharge diagnoses and medical procedures for all patients treated at the University Medical Centre (UMC) Utrecht, a 1042-bed tertiary teaching hospital in the centre of the Netherlands. Each year, approximately 165 000 patients are treated during more than 28 000 hospitalisations, 15 000 day-care treatments, and 334 000 outpatient visits. A more

complete description of UPOD has been published elsewhere.[17] All UPOD and research is in accordance with Dutch privacy and ethical regulation.

Study cohort

The study cohort comprised all patients visiting the outpatient clinic of Respiratory Medicine in 2005–2007. The date of the first contact with the UMC Utrecht since 2005 marked the start of follow-up. Patients were eligible for inclusion in the cohort when they were 18 years or older at cohort entry. Follow-up ended at the end of data collection (December 2007) or lost-to-follow up of the patient, whichever came first. We defined two markers of disease severity: I) hospitalisation for obstructive lung disease, defined as a primary diagnosis with ICD-9-CM code 491, 492, 493, or 496,[18] and II) visiting the emergency department, in accordance to other studies.[19-21] Next to these parameters, information concerning age, gender, diagnosis, and all contacts with the outpatient clinic was collected for each patient. Performed lung imaging events, lung function tests, haematological blood tests, C-reactive protein (CRP), and allergy tests were evaluated as potential biomarkers for disease

Figure 1 Disease severity in a cohort of patients frequenting an outpatient clinic of Respiratory Medicine



The study cohort comprised all patients visiting the outpatient clinic of Respiratory Medicine in 2005–2007. Markers of disease severity were hospitalisation for obstructive lung disease and visiting the emergency department.

severity. Patients who were hospitalised for both asthma and Chronic Obstructive Pulmonary Disease (COPD) were excluded from the cohort because of uncertainty of correct diagnosis.

Data analysis

T-tests, Kruskal-Wallis and χ^2-tests were used, as appropriate. To evaluate associations of potential biomarkers measured in clinical practice with disease severity in obstructive lung disease, a nested case control analysis was conducted where patients with a hospitalisation (cases) were compared with patients without hospitalisation for obstructive lung disease (controls) during follow-up. In this case-control study, cases and controls had at least 182 days of exposure history prior to the index date, which was the first hospitalisation following cohort entry for cases and a random generated date between cohort entry and end-of-follow up for controls. Prior to the index date, haematological blood tests and lung function tests were counted in different time windows. The effect of time on the association between haematological blood testing and conducting lung function tests and the risk of hospitalisation was evaluated by comparing the odds of hospitalisation with the odds of non-hospitalisation in each time window individually. Associations between haematological blood testing and lung function tests and hospitalisation were studied by logistic regression analysis and expressed as odds ratios (ORs) with 95% confidence intervals (95%CIs). All variables that changed the regression coefficient of testing with less than ten percent were excluded from the model. All analyses were conducted using SPSS 14.0.

RESULTS

Each year, around 3000 patients visited the outpatient clinic, with 1000 patients entering the study cohort and 1000 patients leaving the cohort (Figure 1). Overall, a cohort of 5356 adult individual patients visited the outpatient clinic of Respiratory Medicine in the period 2005–2007, of which 2310 (43.1%) had the obstructive lung diseases asthma or COPD. The mean age of all patients was 54.7 years (standard deviation 16.9) and 2703 (50.5%) were male (Table 1). The characteristics of the patients visiting the outpatient clinic of Respiratory Medicine each year fluctuated slightly in terms of age, gender and diagnosis, without large differences between study years (Table 1). Of all 5356 patients, 12.2% visited the emergency department and 3.6% were hospitalised for obstructive lung disease each year (Table 1). In the

Table 1 Baseline characteristics of the study cohort and events during follow-up

Characteristics	Overall n=5356 (100%)	2005 n=3122 (100%)	2006 n=3049 (100%)	2007 n=3200 (100%)	p-value
Age in years; mean (SD)	54.7 (16.9)	55.8 (16.6)	54.6 (16.8)	53.8 (17.0)	< 0.001 [a]
18–44	1452 (27.1%)	780 (25.0%)	826 (27.1%)	919 (28.7%)	
45–64	2204 (41.2%)	1273 (40.8%)	1257 (41.2%)	1302 (40.7%)	
≥ 65	1700 (31.7%)	1069 (34.2%)	966 (31.7%)	979 (30.6%)	
Gender					
male	2703 (50.5%)	1614 (51.7%)	1543 (50.6%)	1551 (48.5%)	0.033 [b]
female	2653 (49.5%)	1508 (48.3%)	1506 (49.4%)	1649 (51.5%)	
Asthma [c]	1182 (22.1%)	731 (23.4%)	761 (25.0%)	858 (26.8%)	0.008 [b]
COPD [d]	1128 (21.1%)	778 (24.9%)	795 (26.1%)	765 (23.9%)	0.141 [b]
Events during follow-up	*Proportion each year (%)* [e]				
Visit to emergency department	12.2	334 (10.7%)	396 (13.0%)	416 (13.0%)	0.006 [b]
Hospitalisation for obstructive lung disease	3.6	107 (3.4%)	104 (3.4%)	126 (3.9%)	0.442 [b]
1	2.6	80 (2.6%)	67 (2.2%)	94 (2.9%)	
2	0.8	19 (0.6%)	30 (1.0%)	22 (0.7%)	
≥ 3	0.8	8 (0.3%)	7 (0.2%)	10 (0.3%)	

SD = standard deviation; COPD = Chronic Obstructive Pulmonary Disease
a) Kruskal-Wallis test.
b) χ^2-test.
c) ICD-9-CM code for asthma is 493.[18]
d) ICD-9-CM codes for COPD are 491, 492, or 496.[18]
e) Proportions of each year were averaged to obtain a mean proportion each year.

period 2005–2007, a total of 283 (5.3%) patients were hospitalised during follow-up, 63 for asthma and 220 for COPD.

Of the annual population of around 3000 patients, 66.5% of the patients had lung imaging events like chest x-rays, 57.8% had a lung function test, 46.1% had a haematological blood test, 21.4% had a CRP test and only a small proportion of patients was tested for allergic constitution. The proportion of patients having tests was relatively stable during the study period (Table 2). Comparing patients with and without the three most frequently conducted procedures (lung imaging events, lung function tests, and haematological blood tests) showed that these procedures were not randomly requested in the study cohort.

Table 2 Procedures during outpatient visits each year for the study cohort

Procedures	Overall[a]	2005 n=3122 (100%)	2006 n=3049 (100%)	2007 n=3200 (100%)
Lung imaging events	66.5%	2076 (66.5%)	2054 (67.4%)	2096 (65.5%)
Lung function test	57.8%	1797 (57.6%)	1720 (56.4%)	1898 (59.3%)
Haematological blood test	46.1%	1486 (47.6%)	1417 (46.5%)	1415 (44.2%)
CRP	21.4%	681 (21.8%)	562 (18.4%)	762 (23.8%)
Allergy test (blood)	9.8%	326 (10.4%)	293 (9.6%)	295 (9.2%)
Allergy test (dermatologic)	4.9%	156 (5.0%)	150 (4.9%)	149 (4.7%)

CRP = C-reactive protein

a) Proportions of each year were averaged to obtain an overall mean proportion each year.

Patients with lung imaging events were older, more frequently male and had less frequently asthma. Furthermore, these patients visited the emergency department more often, but were hospitalised in an equal proportion compared with patients without lung imaging events (Table 3). Lung function tests were more frequently conducted among asthma and COPD patients in comparison to other respiratory diseases (Table 4). In contrast, haematological blood tests were less often conducted for asthma and COPD patients. Patients with haematological blood tests visited the emergency department more often en were hospitalised for obstructive lung disease more frequently compared with patients without haematological blood tests (Table 5).

Comparing patients with and without hospitalisation during follow-up indicated that hospitalised patients were older (Table 6). Lung function tests and haematological

Table 3 Baseline characteristics of patients with and without lung imaging events in each of the follow-up years

Characteristics	2005 yes n=2076 (100%)	2005 no n=1046 (100%)	2006 yes n=2054 (100%)	2006 no n=995 (100%)	2007 yes n=2096 (100%)	2007 no n=1104 (100%)
Age in years; mean (SD)	56.8 (16.4)	53.7 (17.0)***	56.6 (16.0)	50.4 (17.5)***	56.0 (16.4)	49.7 (17.2)***
Gender; male	1131 (54.5%)	483 (46.2%)***	1116 (54.3%)	427 (42.9%)***	1128 (53.8%)	423 (38.3%)***
Asthma [a]	349 (16.8%)	382 (36.5%)***	363 (17.7%)	398 (40.0%)***	388 (18.5%)	47 (42.6%)***
COPD [b]	532 (25.6%)	246 (23.5%)	570 (27.8%)	225 (22.6%)**	542 (25.9%)	223 (20.2%)***
Events during follow-up						
Emergency department visit	260 (12.5%)	74 (7.1%)***	309 (15.0%)	87 (8.7%)***	319 (15.2%)	97 (8.8%)***
Hospitalisation [c]	72 (3.5%)	35 (3.3%)	70 (3.4%)	34 (3.4%)	85 (4.1%)	41 (3.7%)

yes = patient had lung imaging events; no = patient had no lung imaging events; SD = standard deviation; COPD = Chronic Obstructive Pulmonary Disease
a) ICD-9-CM code for asthma is 493.[18]
b) ICD-9-CM codes for COPD are 491, 492, or 496.[18]
c) Hospitalisation for obstructive lung disease.
* p-value <0.05; ** p-value < 0.01; *** p-value < 0.001

Table 4 Baseline characteristics of patients with and without lung function tests in each of the follow-up years

Characteristics	2005 yes n=1797 (100%)	2005 no n=1325 (100%)	2006 yes n=1720 (100%)	2006 no n=1329 (100%)	2007 yes n=1898 (100%)	2007 no n=1302 (100%)
Age in years; mean (SD)	55.2 (16.6)	56.6 (16.7)*	54.6 (16.6)	54.6 (17.1)	53.8 (16.8)	54.0 (17.1%)
Gender; male	907 (50.5%)	707 (53.4%)	861 (50.1%)	682 (51.3%)	907 (47.8%)	644 (49.5%)
Asthma [a]	475 (26.4%)	256 (19.3%)***	434 (25.2%)	327 (24.6%)	581 (30.6%)	277 (21.3%)***
COPD [b]	489 (27.2%)	289 (21.8%)**	511 (29.7%)	284 (21.4%)***	487 (25.7%)	278 (21.4%)**
Events during follow-up						
Emergency department visit	169 (9.4%)	165 (12.5%)**	204 (11.9%)	192 (14.4%)*	239 (12.6%)	177 (13.6%)
Hospitalisation [c]	53 (2.9%)	54 (4.1%)	64 (3.7%)	40 (3.0%)	85 (4.5%)	41 (3.1%)

yes = patient had a lung function test; no = patient had no lung function test; SD = standard deviation; COPD = Chronic Obstructive Pulmonary Disease
a) ICD-9-CM code for asthma is 493.[18]
b) ICD-9-CM codes for COPD are 491, 492, or 496.[18]
c) Hospitalisation for obstructive lung disease.
* p-value <0.05; ** p-value < 0.01; *** p-value < 0.001

Table 5 Baseline characteristics of patients with and without haematological blood tests in each of the follow-up years

Characteristics	2005 yes n=1486 (100%)	2005 no n=1636 (100%)	2006 yes n=1417 (100%)	2006 no n=1632 (100%)	2007 yes n=1415 (100%)	2007 no n=1785 (100%)
Age in years; mean (SD)	55.3 (16.6)	56.2 (16.7)	54.5 (16.4)	54.7 (17.1)	53.7 (16.8)	53.9 (17.1)
Gender; male	792 (53.3%)	822 (50.2%)	727 (51.3%)	816 (50.0%)	727 (51.4%)	824 (46.2%)**
Asthma [a]	252 (17.0%)	479 (29.3%)***	269 (19.0%)	492 (30.1%)***	302 (21.3%)	556 (31.1%)***
COPD [b]	328 (22.1%)	450 (27.5%)***	332 (23.4%)	463 (28.4%)**	307 (21.7%)	458 (25.7%)**
Events during follow-up						
Emergency department visit	237 (15.9%)	97 (5.9%)***	273 (19.3%)	123 (7.5%)***	288 (20.4%)	128 (7.2%)***
Hospitalisation [c]	73 (4.9%)	34 (2.1%)***	72 (5.1%)	32 (2.0%)***	81 (5.7%)	45 (2.5%)***

yes = patient had a haematological blood test; no = patient had no haematological blood test; SD = standard deviation; COPD = Chronic Obstructive Pulmonary Disease
a) ICD-9-CM code for asthma is 493.[18]
b) ICD-9-CM codes for COPD are 491, 492, or 496.[18]
c) Hospitalisation for obstructive lung disease.
* p-value <0.05; ** p-value < 0.01; *** p-value < 0.001

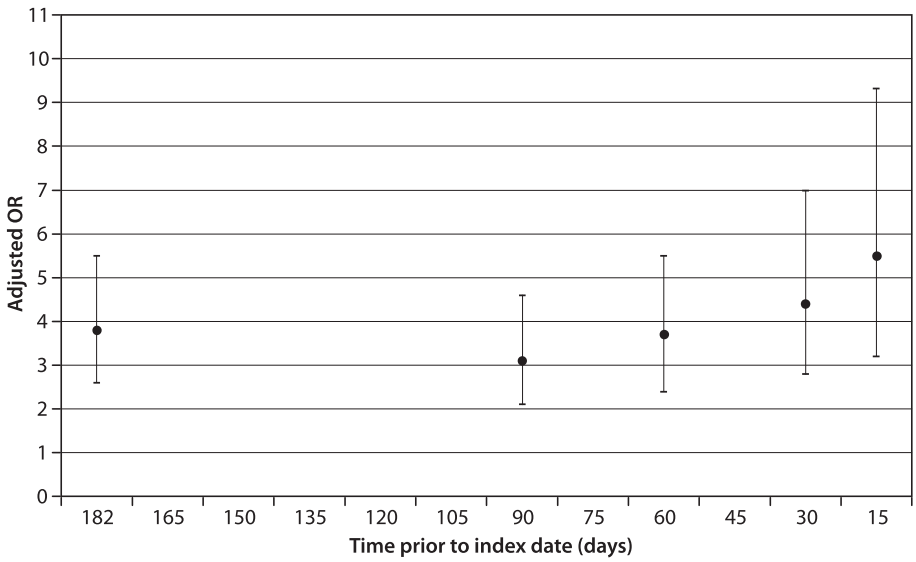
Table 6 Baseline characteristics of patients hospitalised for obstructive lung disease (cases) and patients not hospitalised for obstructive lung disease (controls)

Characteristics	Cases n=134 (100%)	Controls n=1595 (100%)	p-value
Age in years; mean (SD)	60.8 (14.6)	54.4 (16.9)	< 0.001 [a]
18–44	18 (13.4)	440 (27.6%)	
45–64	52 (38.8)	651 (40.8%)	
≥ 65	64 (47.8)	504 (31.6%)	
Gender			0.723 [b]
male	66 (49.3)	811 (50.8%)	
female	68 (50.7)	784 (49.2%)	
During outpatient visits within 182 days before index date			
Lung imaging events	56 (41.8%)	639 (40.1%)	0.695 [b]
Lung function test	62 (46.3%)	501 (31.4%)	< 0.001 [b]
Haematological blood test	56 (41.8%)	278 (17.4%)	< 0.001 [b]
CRP	18 (13.4%)	154 (9.7%)	0.161 [b]
Allergy test (blood)	5 (3.7%)	60 (3.8%)	0.986 [b]
Allergy test (dermatologic)	2 (1.5%)	19 (1.2%)	0.760 [b]

SD = standard deviation; CRP = C-reactive protein
a) t-test.
b) χ^2-test.

blood testing was conducted more often prior to the index date for hospitalised patients compared with non-hospitalised patients. With decreasing time prior to the index date, haematological blood testing was increasingly associated with hospitalisation with an adjusted OR of 5.5 (95%CI 3.2–9.3) in the period of 15 days prior to the index date (Figure 2). Also, lung function testing was associated with an increased risk of hospitalisation more profoundly with decreasing time prior to the index date. In the period of 15 days prior to the index date, lung function testing was associated with a fivefold increased risk of hospitalisation (adjusted OR 5.4; 95%CI 2.9–10.0; Figure 3). Of all cases and controls visiting the outpatient clinic of Respiratory Medicine, 724 (45.4%) controls had a respiratory diagnosis other than obstructive lung disease. However, excluding other respiratory related diagnoses among controls did not have major influence on the results with an adjusted OR of 6.0 (95%CI 3.0–12.1) when including only controls with asthma or COPD compared with an adjusted OR of 5.5 (95%CI 3.2–9.3) when all controls were included in the analysis regarding haematological testing.

Figure 2 Risk function of the relationship between haematological blood testing in the outpatient clinic of Respiratory Medicine and hospitalisation, as function of time prior to index date



Proportion of patients with haematological blood testing

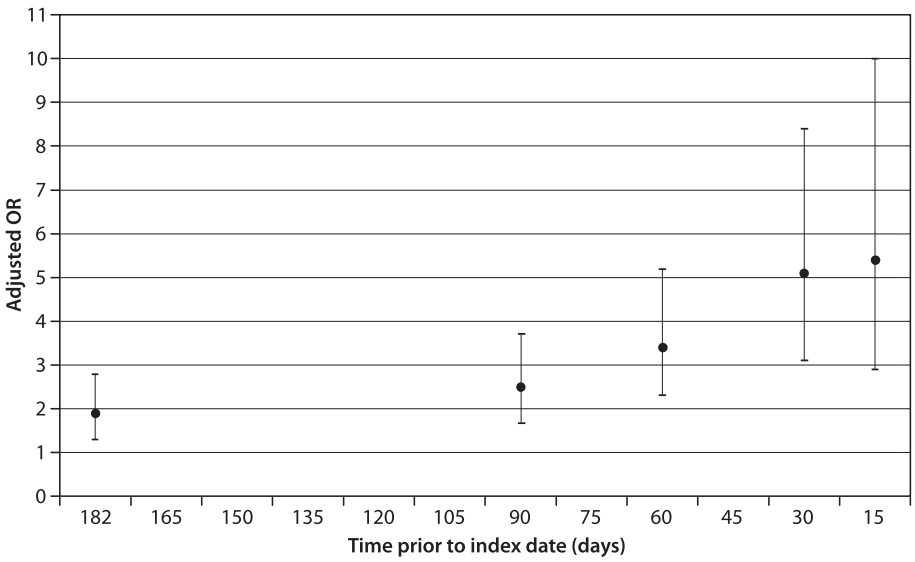
Days prior to index date	182	90	60	30	15
Cases; n=134 (100%)	56 (41.8%)	47 (35.1%)	40 (29.9%)	30 (22.4%)	22 (16.4%)
Controls; n=1595 (100%)	278 (17.4%)	258 (16.2%)	187 (11.7%)	106 (6.6%)	59 (5.6%)

The odds ratios were adjusted for age and gender.

DISCUSSION

In this study, it was shown that most patients visiting the outpatient clinic of Respiratory Medicine were well-controlled with regard to disease severity as measured by visits to the emergency department and hospitalisation for obstructive lung disease. Of all 5356 patients, 12.2% visited the emergency department and 3.6% were hospitalised for obstructive lung disease each year. Only a proportion of patients visiting the outpatient clinic of Respiratory Medicine underwent procedures to test disease severity, like lung function testing or laboratory tests to evaluate

Figure 3 Risk function of the relationship between lung function testing in the outpatient clinic of Respiratory Medicine and hospitalisation, as function of time prior to index date



Proportion of patients with lung function testing

Days prior to index date	182	90	60	30	15
Cases; n=134 (100%)	62 (46.3%)	42 (31.3%)	38 (28.4%)	25 (18.7%)	16 (11.9%)
Controls; n=1595 (100%)	501 (31.4%)	259 (16.2%)	176 (11.0%)	73 (4.6%)	42 (2.6%)

The odds ratios were adjusted for age and gender.

systemic inflammation underlying obstructive lung disease. In epidemiological studies, this would be of no consequence when the proportion of patients was a random sample from the total population. However, in routine clinical practice, there is always a clinical indication for testing. The results of this study show that lung physicians conduct lung function testing and laboratory tests selectively for more severely ill patients. Lung functions were conducted in 58% of patients and haematological blood tests were drawn from 46% of the study population. When comparing patients with and without hospitalisation during follow-up, it appeared

that lung function and haematological blood testing was associated with a fivefold increased risk of hospitalisation in the period of 15 days prior to the index date.

A biased sample is included in studies using test results in routine clinical practice with an overrepresentation of more severely ill patients. This leads to testing bias in epidemiological studies. Although testing guidelines might vary between hospitals, testing bias is an issue in all hospitals and should be evaluated to be able to get a true risk estimate in the conduct of biomarker studies. There are two ways of dealing with biased samples in epidemiological studies. One method is measurement of the parameters of interest for the total study population in accordance to large cohort studies as the Rotterdam study and the SMART study in Utrecht.[22,23] This method leads to structurally performed testing for all patients with obstructive lung disease in this study, less missing values in clinical practice and minimization of testing bias. However, structurally testing the total patient population affects the health care costs for obstructive lung disease. Moreover, conducting procedures and blood testing will decrease the patient well-being by causing stress and increases disease severity from a patient perspective. Another method is to evaluate and quantify the magnitude of testing bias in order to be able to adjust for this factor in the analysis of biomarker studies. In this study, testing bias was evaluated by comparing patient with and without hospitalisation for obstructive lung disease.

The results of this study should be interpreted in context of its limitations. All cases and controls in the nested case-control study visited the outpatient clinic of Respiratory Medicine. All cases and 871 (54.6%) controls had obstructive lung disease. Of controls, 724 (45.4%) had a respiratory diagnosis different from obstructive lung disease. However, excluding respiratory related diagnoses, other than obstructive lung disease, among controls did not have major influence on the results.

In this study lung function testing and haematological blood testing was evaluated without regard to the test result. The association found for haematological testing on 182 days prior to the index date, fitted the trend line less than the other risk estimates. This might be caused by preceding exacerbations for example. Haematological parameters reflecting inflammation like the absolute neutrophil or eosinophil count play an important role in many of the inflammatory processes in obstructive lung diseases and are important factors for disease severity in obstructive lung disease, as seen in many other studies.[24-26] Therefore, measurement of these parameters is essential in obstructive lung disease management and were found to be promising biomarkers for disease severity in obstructive lung disease.[27]

In conclusion, most patients visiting the outpatient clinic of Respiratory Medicine were well-controlled with regard to disease severity. Testing was not conducted

for all patients in the study population, but in a biased sample of more severely ill patients. Testing bias should therefore, be taken into account in the conduct of biomarker studies using data from routine clinical databases.

Acknowledgements - The authors are grateful to Pieter Zanen, for providing the data on the lung function measurements, to Hanneke den Breeijen and Leslie Beks for providing data on the source population, and to all colleagues involved in the UPOD initiative.

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4.2

Effects of glucocorticoids on the neutrophil count: a cohort study among hospitalised patients

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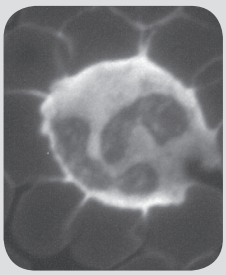


Pulm Pharmacol Ther
2009 Oct 29 [Epub ahead of print]

ABSTRACT

Background

Systemic glucocorticoids are often used in clinical practice for a large variety of indications. Clinical observations have shown that patients using glucocorticoids often have higher neutrophil counts. Debate remains whether this observed neutrophilia is associated with glucocorticoid use or that other factors, like disease and severity of disease, should be considered. The objective of this study was to investigate the effect of systemic glucocorticoids on the absolute neutrophil count in hospitalised patients.

Methods

A cohort study was conducted using data from the Utrecht Patient Oriented Database which comprises clinical data of patients of the University Medical Centre Utrecht. We identified all adult patients, hospitalised in 2005 with at least two blood samples for haematological testing during admission and compared in-hospital glucocorticoid use with non-use.

Results

A total of 809 glucocorticoid users and 2658 non-users were included in the study with comparable neutrophil counts at admission (8.2×10^9/l for glucocorticoid users and 8.0×10^9/l for non-users). Overall analysis showed a slight association between glucocorticoid use and an increase in neutrophil count (relative risk [RR] 1.3; 95% confidence interval [95%CI] 1.1–1.5). However, within diagnostic subgroups there was no increase in neutrophil count in glucocorticoid users. Furthermore, among all no dose response relationship, no effect of time between the two samples, and no effect of anti-inflammatory/sodium retaining potency was found.

Conclusions

Observed neutrophilia in users of systemic glucocorticoids is probably associated with underlying disease, rather than glucocorticoid use itself.

BACKGROUND

Glucocorticoids (GCs) have a widespread and complex mechanism of action. They have many effects, depending on disease and cell type.[1,2] Glucocorticoids reduce the number of many inflammatory cells, in particular increase the rate of apoptosis of eosinophils.[1-5] Neutrophils were found to be less responsive to these drugs.[6-8] In vitro studies have shown that glucocorticoids inhibit neutrophil apoptosis.[1,2,9-11] However, in vivo studies mainly have focused on healthy volunteers and short-term effects of glucocorticoids on the neutrophil count and showed normalization of the neutrophil count within 24 hours.[12,13] Therefore, the clinical effect of glucocorticoids on the neutrophil count is controversial.

Clinical observations have shown that patients using GCs often have higher neutrophil counts, particularly in respiratory disease. It is well known that granulocytes play an important role in asthma and chronic obstructive pulmonary disease (COPD) [14,15] but although neutrophilia has been associated with asthma severity, debate remains whether neutrophilia is a characteristic of asthma severity or results from glucocorticoid treatment.[5,8,16-24] More severely ill asthma patients receive often higher doses of glucocorticoids and glucocorticoids are highly used among hospitalised asthma patients. Therefore, in this observational study, we compared neutrophil counts in a variety of diseases in patients using GCs with non-users, taking into account several approaches to adjust for disease bias on these counts.

METHODS

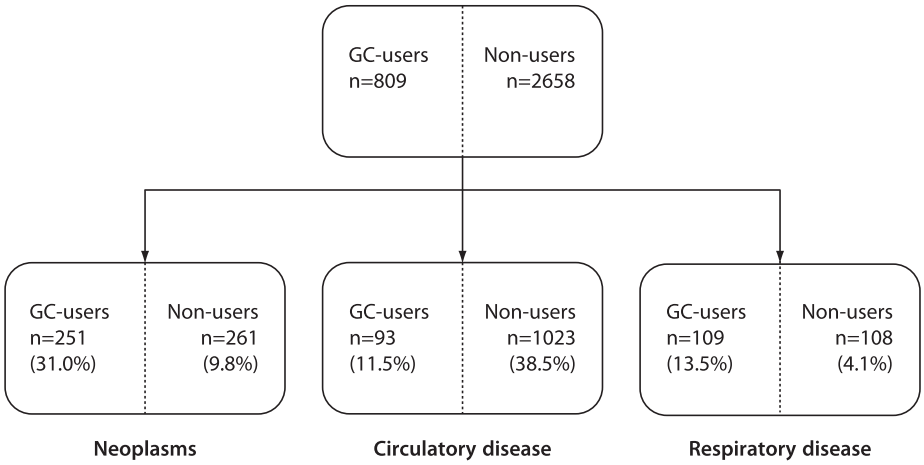
Study design and setting

A cohort study was conducted using data from the Utrecht Patient Oriented Database (UPOD). UPOD is an infrastructure of relational databases comprising administrative data on patient characteristics, laboratory test results, medication orders, discharge diagnoses and medical procedures for all patients treated at the University Medical Centre Utrecht, a 1042 bed tertiary teaching hospital in the centre of the Netherlands. Each year, approximately 165 000 patients are treated during more than 28 000 hospitalisations, 15 000 day care treatments, and 334 000 outpatient visits. UPOD data acquisition and data management is in accordance with current Dutch privacy and ethical regulations. A more complete description of UPOD has been published elsewhere.[25]

We identified all adult patients (≥ 18 years) who were hospitalised in the University Medical Centre Utrecht in 2005 and had at least two haematological blood tests during hospitalisation. Exposed patients used systemic glucocorticoids during

admission, where glucocorticoid use started before or after withdrawal of the first blood test; unexposed patients did not use systemic glucocorticoids during admission. The study design is summarized in Figure 1. For all participants the discharge diagnose was defined according to the ICD-9-CM code.[26]

Figure 1 Study design



GC = glucocorticoid
Overall analysis included all patients in the study, where subgroup analysis were conducted within the major diagnostic groups and included only patients with neoplasms, circulatory disease or respiratory diseases.

Neutrophil testing

For each glucocorticoid user, the first blood test during admission and the last blood measurement during in-hospital glucocorticoid use were selected for analysis, where these samples should cover at least a one day period. Up to four unexposed patients were sampled to each glucocorticoid user according to calendar time (with a maximum of 15 days before or after the test date of the user), neutrophil count at time of admission (max 2×10^9 neutrophils/l around the neutrophil count of the user) and days between the two blood samples (max two days around the number of days for the glucocorticoid user). We used data on eosinophil counts in the same patients measured on the same moments in time as a reference procedure as the effect of glucocorticoids on the absolute eosinophil count is widely known.[1-5] Data on glucocorticoid use was retrieved from the medication file from UPOD and daily

dose exposure was expressed as systemic prednisone equivalents, using defined daily dosages.[27] Moreover, glucocorticoids were categorized according to their anti-inflammatory/sodium retaining potency for subgroup analyses.[28]

Data analysis

Student t-tests, Mann-Whitney tests, and χ^2-tests were used, as appropriate. Glucocorticoid dose and change in neutrophil and eosinophil counts were associated through a non-linear relationship. Therefore, the glucocorticoid dose was categorized into tertiles. The change in neutrophil and eosinophil count was also categorized into tertiles to obtain three equally sized groups. These three groups were defined as an increase, decrease or no change in neutrophil or eosinophil count. Potential confounders included in the analysis were age, gender, diagnosis, duration of hospitalisation, length of period between two blood samples, C-reactive protein (CRP) levels, and death during hospitalisation.

Subsequently, unconditional multivariate Cox proportional hazards analysis was used to estimate the strength of the association between glucocorticoid exposure and either an increase versus no change, or a decrease compared to no change in the absolute neutrophil or eosinophil count, expressed as relative risk (RR) with 95% confidence intervals (95%CI). All variables that changed the regression coefficient of glucocorticoid use with less than ten percent were excluded from the model. All analyses were conducted using SPSS for Windows, version 14.0 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

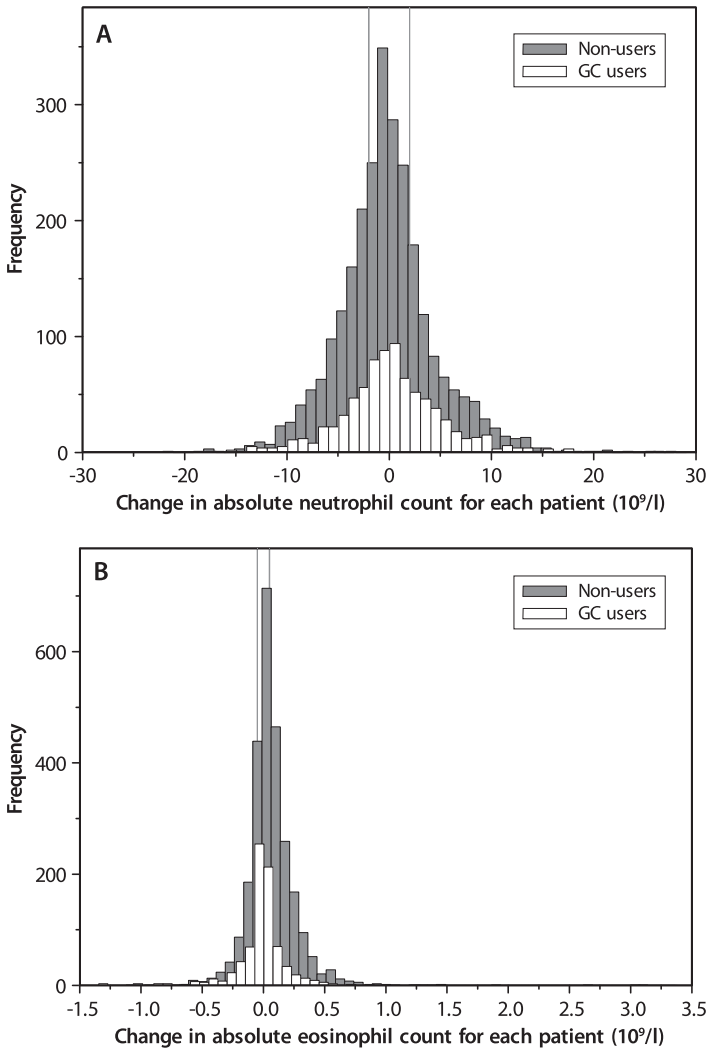
From the UPOD database, we identified 809 glucocorticoid users and 2658 non-users. The age and gender distributions of the glucocorticoid users were comparable to patients not using systemic glucocorticoids, as well as the duration of hospitalisation and the number of deaths during hospitalisation. CRP levels were comparable for GC users and non-users at time of admission, but CRP levels were lower for GC users in the second blood sample compared to non-users (Table 1). Glucocorticoid users had a lower eosinophil count than non-users, and non-users had an increase in eosinophil count during hospitalisation (Table 1, Figure 2). The absolute neutrophil count at admission was comparable for users and non-users. During hospitalisation, glucocorticoid users had an overall minor increase of 0.3×10^9 neutrophils/l, where non-users had an overall slight decrease of 0.3×10^9/l (Table 1). However, differences in neutrophil count between the two blood samples

Table 1 Baseline characteristics of glucocorticoid (GC) users and non-users [a]

Characteristic	GC users (n=809)	Non-users (n=2658)	p-value (two-sided)
Age (years)	57.4 ± 17.8	58.4 ± 18.3	0.171 [b]
Sex			0.092 [c]
male	411 (50.8%)	1440 (54.2%)	
female	398 (49.2%)	1218 (45.8%)	
Days between blood samples	6 (3–12)	4 (2–8)	< 0.001 [d]
Duration of hospitalisation	10 (7–19)	10 (7–18)	0.332 [d]
Death during hospitalisation	54 (6.8%)	157 (5.9%)	0.360 [c]
Absolute eosinophil count first blood sample (10^9/l)	0.07 (0.03–0.17)	0.09 (0.04–0.18)	< 0.001 [d]
Absolute eosinophil count second blood sample (10^9/l)	0.07 (0.03–0.15)	0.15 (0.06–0.27)	< 0.001 [d]
Absolute neutrophil count first blood sample (10^9/l)	8.2 ± 4.7	8.0 ± 4.2	0.214 [b]
Absolute neutrophil count second blood sample (10^9/l)	8.5 ± 5.1	7.7 ± 4.1	< 0.001 [b]
CRP first blood sample measured	311 (38.4%)	1196 (45.0%)	
CRP (mg/l)	29.0 (7.0–75.0)	21.0 (7.0–68.8)	0.094 [d]
CRP second blood sample measured	236 (29.2%)	285 (10.7%)	
CRP (mg/l)	23.5 (9.0–62.0)	33.0 (13.0–99.0)	0.001 [d]
Diagnosis			< 0.001 [c]
neoplasms	251 (31.0%)	261 (9.8%)	
diseases of the circulatory system	93 (11.5%)	1023 (38.5%)	
diseases of the respiratory system	109 (13.5%)	108 (4.1%)	
infectious and parasitic diseases	30 (3.7%)	63 (2.4%)	
endocrine, nutritional and metabolic diseases, and immunity disorders	22 (2.7%)	53 (2.0%)	
diseases of the nervous system and sense organs	30 (3.7%)	65 (2.4%)	
diseases of the digestive system	44 (5.4%)	198 (7.4%)	
diseases of the genitourinary system	73 (9.0%)	95 (3.6%)	
diseases of the skin, subcutaneous tissue, musculoskeletal system, and connective tissue	36 (4.4%)	148 (5.6%)	
other	121 (14.9%)	644 (24.2%)	

a) Data are presented as n (%), or mean ± standard deviation, or median (interquartile range).
b) Student t-test. c) χ^2 test. d) Mann-Whitney test.

Figure 2 Distribution of the difference of absolute neutrophil and eosinophil counts between the two samples for glucocorticoid users and non-users



A. Neutrophils. The vertical lines represent the cut-off value of 2×10^9/l difference between the two blood samples.
B. Eosinophils. The vertical lines represent the cut-off value of 0.05×10^9/l difference between the two blood samples.

for each patient were equally distributed among glucocorticoid users and non-users, with means close to zero (Figure 2). As shown in Table 1, glucocorticoid use was not randomly distributed among the diagnosis groups.

As shown in Table 2, 28.1% of users and 32.5% of non-users had a decrease of more than 2×10^9 neutrophils/l, 40.7% of users and 42.4% of non-users remained unchanged and 31.3% of users and 25.1% of non-users had an increase of more

Table 2 Association between glucocorticoid (GC) use and a change in absolute neutrophil count

Change in absolute neutrophil count	GC users n (%)	Non-user n (%)	Crude RR (95%CI)	Adjusted [a] RR (95%CI)
	Overall analysis n=809 (100%)	n=2658 (100%)		
No change [b]	329 (40.7%)	1127 (42.4%)	1.0 (reference)	1.0 (reference)
Increase [c]	253 (31.3%)	667 (25.1%)	1.2 (1.0–1.4)	1.3 (1.1–1.5)
Decrease [d]	227 (28.1%)	864 (32.5%)	0.9 (0.8–1.1)	0.9 (0.8–1.1)
	Neoplasms [e] n=251 (31.0%)	n= 261 (9.8%)		
No change [b]	114 (45.4%)	119 (45.6%)	1.0 (reference)	
Increase [c]	86 (34.3%)	71 (27.2%)	1.2 (0.8–1.6)	
Decrease [d]	51 (27.2%)	71 (27.2%)	0.8 (0.6–1.2)	
	Circulatory disease [e] n= 93 (11.5%)	n=1023 (38.5%)		
No change [b]	29 (31.2%)	399 (39.0%)	1.0 (reference)	
Increase [c]	38 (40.9%)	376 (36.8%)	1.2 (0.8–1.6)	
Decrease [d]	26 (28.0%)	248 (24.2%)	1.2 (0.8–1.8)	
	Respiratory disease [e] n=109 (13.5%)	n= 108 (4.1%)		
No change [b]	47 (43.1%)	37 (34.3%)	1.0 (reference)	
Increase [c]	22 (20.2%)	15 (13.9%)	1.1 (0.6–2.1)	
Decrease [d]	40 (36.7%)	56 (51.9%)	0.8 (0.5–1.1)	

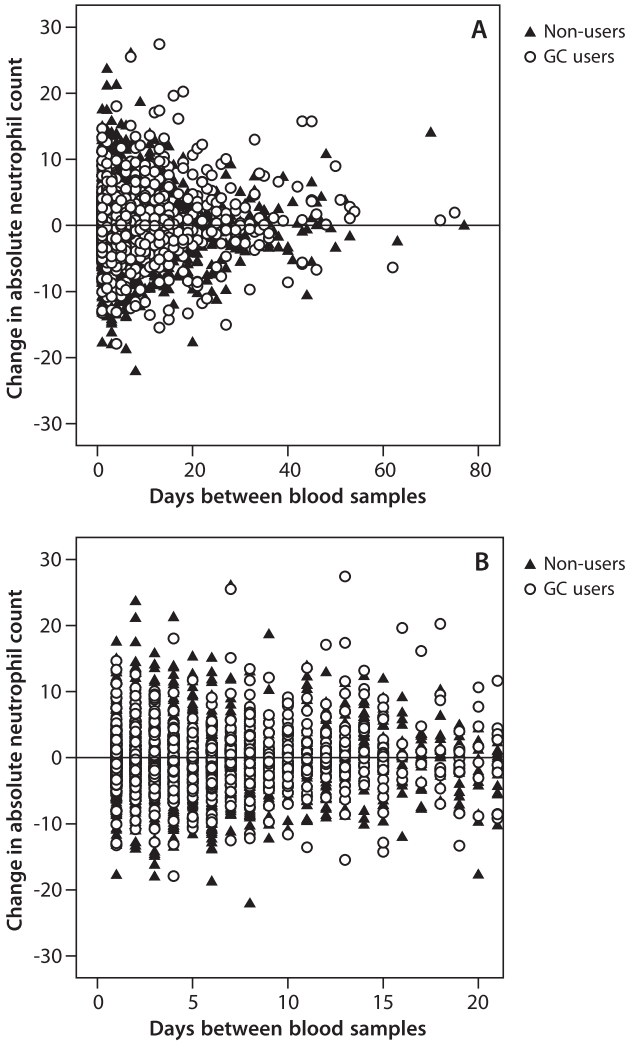
RR = relative risk; 95%CI = 95% confidence interval
a) Adjusted for diagnosis (in case of overall analysis).
b) Change in absolute neutrophil count > -2×10^9/l and < 2×10^9/l).
c) Change in absolute neutrophil count ≥ 2×10^9/l.
d) Change in absolute neutrophil count ≤ -2×10^9/l.
e) Percentages of changes in absolute neutrophil count are with respect to the subgroup.

than 2×10^9 neutrophils/l. Overall, use of systemic glucocorticoids was associated with a slight increase in the neutrophil count (crude RR 1.2; 95%CI 1.0–1.4). After adjustment for diagnosis, the adjusted RR yielded a value of 1.3 (95%CI 1.1–1.5). However, subgroup analysis for the major diagnostic groups showed that there is no association between glucocorticoid use and an increase in neutrophil count among diagnostic subgroups. The RR for neoplasms was 1.2 (95%CI 0.8–1.6), for circulatory disease 1.2 (95%CI 0.8–1.6), and for respiratory diseases 1.1 (95%CI 0.6–2.1). Glucocorticoid use was not associated with a decrease in the absolute neutrophil count in overall analysis or in subgroup analyses (Table 2). With respect to the absolute eosinophil count 220 (27.3%) of users and 534 (20.1%) of non-users had a decrease of more than 0.05×10^9/l, 366 (45.4%) of users and 893 (33.6%) of non-users remained unchanged and 220 (27.3%) of glucocorticoid users and 1227 (46.2%) of non-users had an increase of more than 0.05×10^9/l. Use of systemic glucocorticoids was inversely associated with an increase in the eosinophil count (adjusted RR 0.6; 95%CI 0.6–0.7). There was no clear association of glucocorticoid use and a decrease in eosinophil count (adjusted RR 1.2; 95%CI 1.0–1.4).

The number of days between the two blood samples for each patient varied between one and 77 days between user-non-users pairs. As shown in Figure 3, there was no effect of the time between the blood samples for each patient and the change in neutrophil count, nor were there any differences for glucocorticoid users and non-users. Regarding short-term effects, there was a wide variation in the change in neutrophil counts for glucocorticoid users as well as for glucocorticoid non-users with a one day period between the two blood samples. A sensitivity analysis revealed that limiting the maximum period between the two blood samples to seven days did not influence the results (data not shown).

Furthermore, different diagnoses require treatment with different doses of glucocorticoids, which could cause confounding in studying the relationship between glucocorticoids and the neutrophil count. Among glucocorticoid users, no dose response relationship could be found, where adjustment occurred for diagnosis (Table 3). Of the glucocorticoid users 474 (58.6%) used glucocorticoids at time of the first blood sample, 335 (41.4%) of the users started glucocorticoid treatment after withdrawal of the first blood sample. Stratification on this parameter did not influence the overall results with an RR of 1.9 (95%CI 1.4–2.5) for an increase versus no change compared with 1.3 (95%CI 1.1–1.5) when including all patients. For a decrease versus no change, the RRs were 0.7 (95%CI 0.5–1.0) for only users that started glucocorticoid use after the first blood sample compared with 0.9 (95%CI 0.8–1.1) when all patients were included in the model. Subgroup analyses for the major diagnostic groups among patients that started glucocorticoid

Figure 3 Effect of the period between the two blood samples on the change in absolute neutrophil count of each patient



There is no effect of time between blood samples and the change in absolute neutrophil count. 3B is an enlargement of 3A.

Table 3 Dose effect among glucocorticoid (GC) users, stratified according to the dose used (in mg prednisone equivalents) before the first blood sample and the mean daily dose used between the two blood samples

GC use before the first blood sample	Mean daily dose of GC use between the two blood samples	Increase[a,b] n (%)	No change[b,c] n (%)	Crude RR (95%CI)	Adjusted[d] RR (95%CI)
0	< 15	40 (30.3%)	51 (38.9%)	1.0 (reference)	1.0 (reference)
	15–40	44 (33.3%)	35 (26.7%)	1.3 (0.8–1.9)	1.3 (0.8–2.0)
	≥ 40	48 (36.4%)	45 (34.4%)	1.2 (0.8–1.8)	1.1 (0.7–1.8)
0–50	< 15	19 (51.4%)	49 (56.3%)	1.0 (reference)	1.0 (reference)
	15–40	13 (35.1%)	27 (31.0%)	1.2 (0.6–2.4)	1.8 (0.8–4.1)
	≥ 40	5 (13.5%)	11 (12.6%)	1.1 (0.4–3.0)	1.6 (0.5-4.9)
≥ 50	< 15	1 (1.2%)	7 (6.3%)	1.0 (reference)	1.0 (reference)
	15–40	27 (32.1%)	29 (26.1%)	3.9 (0.5–28.4)	4.5 (0.6–34.2)
	≥ 40	56 (66.7%)	75 (67.6%)	3.4 (0.5–24.7)	3.6 (0.5–26.4)
GC use before the first blood sample	**Mean daily dose of GC use between the two blood samples**	**Decrease[b,e] n (%)**	**No change[b,c] n (%)**	**Crude RR (95%CI)**	**Adjusted[d] RR 95%CI**
0	< 15	34 (47.2%)	51 (38.9%)	1.0 (reference)	1.0 (reference)
	15–40	21 (29.2%)	35 (26.7%)	0.9 (0.5–1.6)	1.0 (0.6–1.8)
	≥ 40	17 (23.6%)	45 (34.4%)	0.7 (0.4–1.2)	0.8 (0.5–1.6)
0–50	< 15	53 (57.0%)	49 (56.3%)	1.0 (reference)	1.0 (reference)
	15–40	33 (35.5%)	27 (31.0%)	1.1 (0.7–1.6)	1.1 (0.7–1.8)
	≥ 40	7 (7.5%)	11 (12.6%)	0.7 (0.3–1.6)	0.8 (0.4–1.9)
≥ 50	< 15	4 (6.5%)	7 (6.3%)	1.0 (reference)	1.0 (reference)
	15–40	23 (37.1%)	29 (26.1%)	1.2 (0.4–3.5)	1.5 (0.5–4.6)
	≥ 40	35 (56.5%)	75 (67.6%)	0.9 (0.3–2.5)	1.0 (0.3–2.8)

RR = relative risk; 95%CI = 95% confidence interval
a) Change in absolute neutrophil count ≥ 2×10^9/l.
b) Percentages are with respect to the subgroup.
c) Change in absolute neutrophil count > -2×10^9/l and $<2\times10^9$/l).
d) Adjusted for diagnosis.
e) Change in absolute neutrophil count ≤ -2×10^9/l.

use after the first blood sample showed no association between glucocorticoid use and an increase or decrease in neutrophil count (data not shown). Stratifying glucocorticoids according to their anti-inflammatory/sodium retaining potency did not yield different results (data not shown).

DISCUSSION

In this study, we showed that observed neutrophilia in users of systemic glucocorticoids is probably associated with underlying disease, rather than with the use of glucocorticoids itself. Overall analysis yielded a 30% increase in the risk of an increase in the neutrophil count for GC users (adjusted RR 1.3; 95%CI 1.1–1.5). However this is the summed effect of several factors, next to the possible effect of systemic glucocorticoids, e.g. diagnosis, disease severity, dose and type of glucocorticoid, and the studied time window. These factors are considered one by one.

Regarding diagnosis, subgroup analysis in the major diagnostic groups showed that there was no association between glucocorticoid use and a change in the absolute neutrophil count among diagnostic subgroups.

Concerning disease severity, glucocorticoid users have a higher absolute neutrophil count in the second blood sample, compared to non-users, and this count is above the upper limit of our laboratories normal reference range of $1.6–8.3\times10^9$ neutrophils/l, possibly indicating that glucocorticoid users were more severely ill than non-users (Table 1). However, the mean change in absolute neutrophil count for GC users, as well as for non-users was close to zero (Figure 2) and demographic parameters and disease severity markers, like duration of hospitalisation and death during hospitalisation, were comparable between GC users and non-users, CRP levels were lower in the second blood sample for GC users compared to non-users (Table 1).

Another argument showing that neutrophilia is not fully due to glucocorticoid use is that effect of dosing and anti-inflammatory potency of the glucocorticoid was not found in our study. Also, a sensitivity analysis showed that varying the cut-off value of a change in neutrophil count from 1 to 10×10^9/l did not have major effects on the associations found (data not shown). The inverse association found between glucocorticoid use and an increase in eosinophil count is reassuring as it served as an internal reference procedure of our database.

The time window of studying the effect of glucocorticoids on the neutrophil count is important. Early, non-genomic effects occur within minutes to seconds after administration, genomic mechanisms of action take 30 minutes to 18 hours.[1,29,30] In vivo studies mainly focused on healthy volunteers and short-term effects of glucocorticoids on the neutrophil count within 24 hours.[12,13] Because of the clinical relevance of a prolonged change in the neutrophil count in this study there is at least a one day period between the two blood samples of each patient. The second blood sample for users was selected during glucocorticoid use.

Our findings are in accordance with other studies that found that neutrophilia is not fully due to glucocorticoid treatment. Green et al. concluded that, in some asthma subjects at least, neutrophilia is not due to the glucocorticoid treatment.[31] Also Louis et al. found in their study that severe asthmatics, treated with systemic glucocorticoids, had a lower absolute neutrophil count in sputum compared to severe asthmatics who did not use systemic glucocorticoids.[18] Further research is needed to study inflammation in order to create more insight into the mechanistic role of neutrophils in asthma severity.

One might argue that the results of this study should be verified in a randomised controlled trial. However, such a trial will probably not be approved by any medical ethical committee, because severe asthma patients cannot be deprived of glucocorticoids since these drugs are essential in the treatment of asthma and there is no alternative treatment at this time.

There are several studies on the cellular mechanism of glucocorticoids in general.[2,6,32-35] In spite of the discussions about the glucocorticoid effect on neutrophils, this mechanism is not well-understood and should be studied in more detail. There are several mechanisms by which glucocorticoids could theoretically influence the absolute neutrophil count. By increasing the neutrophil production in the bone marrow, by demargination from the blood vessel wall, by increasing the life span, by limiting neutrophil emigration from the blood, or by a combination of these factors.[1,9,10,12,28] The bone marrow could be activated to produce more neutrophils.[12,28] However, when the bone marrow is producing granulocytes more quickly, it will also release immature granulocytes into the blood, which is not the case in our study population. Factors like stress, exercise, or infection could cause demargination of neutrophils close to the vessel wall, resulting in an increase in the neutrophil count in peripheral blood, but glucocorticoids are not likely to cause demargination.[12,28,36] Glucocorticoids were found to inhibit neutrophil apoptosis.[1,2,9-11] However, these findings were done in in-vitro experiments, and in-vivo studies only found short-term effects of glucocorticoids on the neutrophils.[12,13] Lastly, glucocorticoids could reduce the membrane CD11/CD18 appearance, causing weakened adhesion and less neutrophils leaving the blood.[12,37]

In conclusion, the overall increased risk of an increase in neutrophil count in this study is the summed effect of several factors. The observed neutrophilia in users of systemic glucocorticoids is probably associated with underlying disease, rather than the use of glucocorticoids itself.

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4.3

Testing bias in clinical databases: methodological considerations

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Submitted

ABSTRACT

Background

Laboratory testing in clinical practice is never a random process. In this study we evaluated testing bias for neutrophil counts in clinical practice by using results from requested and non-requested haematological blood tests.

Methods

This study was conducted using data from the Utrecht Patient Oriented Database, a unique clinical database as it contains physician requested data, but also data that are not requested by the physician, but measured as result of requesting other haematological parameters. We identified adult patients, hospitalised in 2005 with at least two blood tests during admission, where requests for general blood profiles and specifically for neutrophil counts were contrasted in scenario analyses. Possible effect modifiers were diagnosis and glucocorticoid use.

Results

A total of 567 patients with requested neutrophil counts and 1439 patients with non-requested neutrophil counts were analysed. The absolute neutrophil count at admission differed with a mean of 7.4×10^9/l for requested counts and 8.3×10^9/l for non-requested counts (p-value < 0.001). This difference could be explained for 83.2% by the occurrence of cardiovascular disease as underlying disease and for 4.5% by glucocorticoid use.

Conclusions

Requests for neutrophil counts in clinical databases are associated with underlying disease and with cardiovascular disease in particular. The results from our study show the importance of evaluating testing bias in epidemiological studies obtaining data from clinical databases.

BACKGROUND

In recent years, large health care databases are increasingly used and provide important tools in epidemiological research.[1,2] Advantages are that large amounts of clinical data are available at relatively low cost, and that these databases usually reflect daily practice.[3,4] However, in contrast to randomised clinical trials, where data collection is well-controlled, bias should always be considered when using routinely collected data in automated databases and methodological issues should be taken into account.[3,5-7]

Laboratory testing in clinical practice is never a random process, as the physician has reasons to perform a test. Physicians selectively request tests for patients with a high probability of abnormalities and less frequently for patients with a low probability because of patient burden and costs.[8] Such selective processes might induce testing bias in clinical database studies. There are several strategies to minimize testing bias including selection of proper patient populations, measuring outcomes for all study participants, blind testing, or using imputation techniques to deal with missing data,[8-10] but these techniques do not provide insight into size and direction of testing bias.

One example where testing bias might occur is in physicians' requests of blood tests. Neutrophil counts in peripheral blood are considered as a useful biomarker for disease severity in many conditions.[11-14] However, testing bias might occur because of underlying disease or medication use, as neutrophil counts differ in several diseases and clinical observations have shown that patients using glucocorticoids often have higher neutrophil counts. Requesting neutrophil counts specifically for certain diseases or for glucocorticoid users might cause testing bias in clinical databases. The aim of this study was to evaluate testing bias for neutrophil counts in clinical practice by using results from requested and non-requested haematological blood tests.

METHODS

Setting

This study was conducted using data from the Utrecht Patient Oriented Database (UPOD). UPOD is an infrastructure of relational databases comprising administrative data on patient characteristics, laboratory test results, medication orders, discharge diagnoses and medical procedures for all patients treated at the University Medical Centre (UMC) Utrecht, a 1042-bed tertiary teaching hospital in the centre of the Netherlands. Each year, approximately 165 000 patients are treated

during more than 28 000 hospitalisations, 15 000 day-care treatments, and 334 000 outpatient visits. UPOD data acquisition and data management is in accordance with current Dutch privacy and ethical regulations. A more complete description of UPOD has been published elsewhere.[15]

UPOD is a unique clinical database as it contains results of haematological blood tests measured with Cell-Dyn Sapphire automated blood cell analysers (Abbott Diagnostics, St. Clara, California, USA).[15] A feature of this analyser is that it measures all haematological parameters irrespective of whether these are requested or not.[15] The non-requested parameters are measured because one haematological test is technically linked to the other haematological tests and conducted automatically when one of these tests is requested. In other words, UPOD contains requested (Figure 1A) and non-requested test results (Figure 1B). Although non-requested neutrophil counts are not reported to the clinician, these neutrophil counts are collected in UPOD.

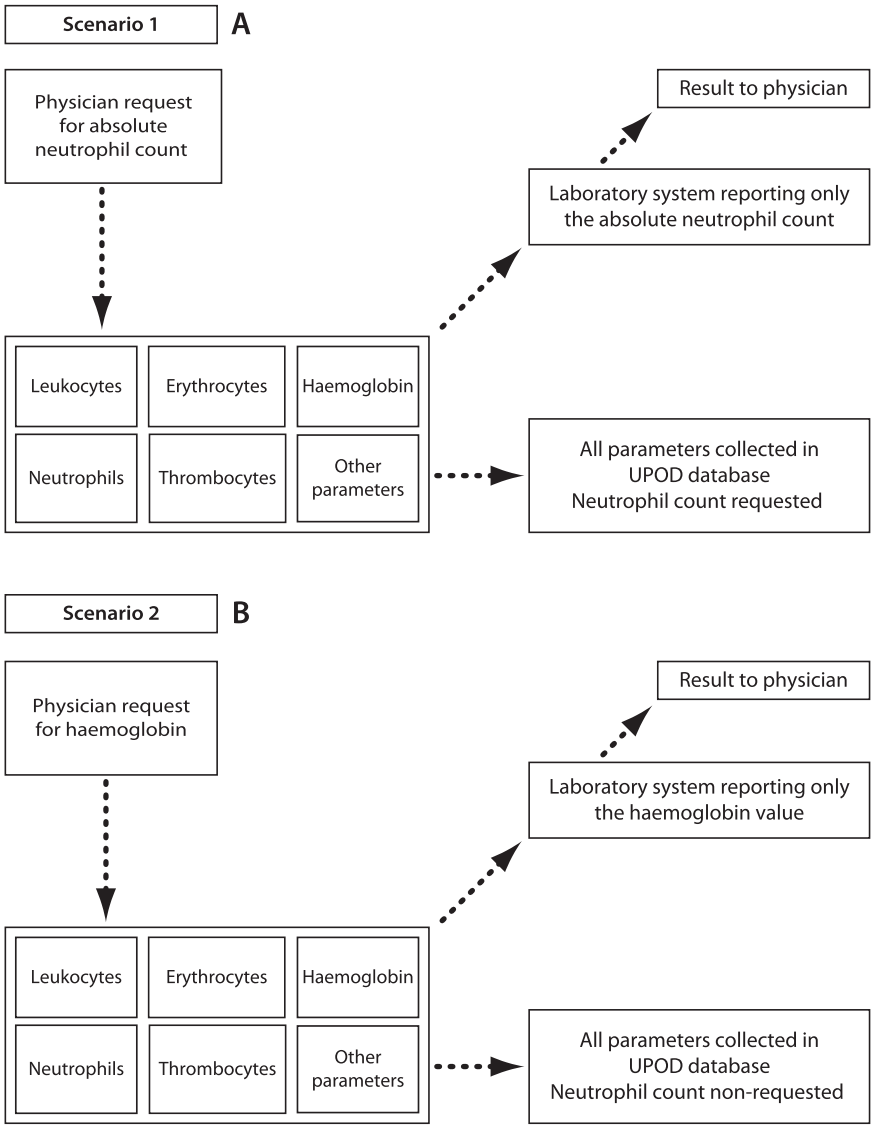
Scenarios

By comparing the measured haematological parameters with the routine hospital laboratory reporting system, reporting lab results to physicians, neutrophil counts can be categorized as requested or non-requested. Neutrophil counts appearing in the laboratory reporting system were categorized as requested; other neutrophil counts were non-requested. Using these data, we conducted two scenario analyses. Scenario 1 reflects the situation as in a typical clinical database, where all blood tests were requested. With scenario 2 we were able to study testing bias by including non-requested blood tests in our analysis.

Study population

The source population comprised 3467 adult (18 years or older) users and non-users of glucocorticoids who were hospitalised in the UMC Utrecht in 2005 and had at least two haematological blood tests, where these tests should cover at least a one-day period. According to our laboratory normal reference range for neutrophils ($1.6–8.3\times10^9$/l), there is large interindividual variation in the absolute neutrophil count. Using two blood tests, we were able to study testing bias in both blood tests separately, but also in the change in neutrophil count during hospitalisation for each patient. Within the source population, we contrasted patients with both blood tests requested and with both blood tests non-requested. Possible effect modifiers were diagnosis and glucocorticoid use. For all participants the discharge diagnose was defined according to the ICD-9-CM code.[16]

Figure 1 Illustration of scenarios



UPOD = Utrecht Patient Oriented Database
Scenario 1 (A) represents the situation in a typical clinical database where all neutrophil counts were requested by physicians. Scenario 2 (B) is unique for UPOD as this includes non-requested neutrophil counts. These non-requested neutrophil counts are measured because this test is conducted automatically when one haematological test, for example haemoglobin, is requested.

Table 1 Baseline characteristics of the study population [a]

Characteristic	Requested neutrophil count (scenario 1) n=567	Non-requested neutrophil count (scenario 2) n=1439	p-value (two-sided)
Age (years)	57.8 ± 17.8	56.9 ± 19.0	0.311 [b]
Sex			0.001 [c]
male	327 (57.7%)	715 (49.7%)	
female	240 (42.3%)	724 (50.3%)	
Length of hospitalisation	10 (7–18)	10 (6–18)	0.807 [d]
Days between blood tests	6 (3–10)	4 (2–8)	< 0.001 [d]
Death during hospitalisation	36 (6.3%)	83 (5.8%)	0.610 [c]
Haemoglobin value 1st blood test (mmol/l)	8.0 ± 1.3	7.4 ± 1.4	< 0.001 [d]
Haemoglobin value 2nd blood test (mmol/l)	7.1 ± 1.3	6.9 ± 1.2	< 0.001 [d]
Absolute neutrophil count 1st blood test (10^9/l)	7.4 ± 4.7	8.3 ± 4.0	< 0.001 [d]
Absolute neutrophil count 2nd blood test (10^9/l)	7.6 ± 4.7	7.8 ± 4.0	0.111 [d]
Change in neutrophil count for each patient (10^9/l)	0.14 ± 5.1	-0.50 ± 4.2	0.008 [b]
Glucocorticoid use	174 (30.7%)	237 (16.5%)	< 0.001 [c]
Diagnosis			< 0.001 [c]
neoplasms	71 (12.5%)	220 (15.3%)	
cardiovascular diseases	200 (35.3%)	380 (26.4%)	
respiratory diseases	53 (9.3%)	38 (2.6%)	
infectious and parasitic diseases	30 (5.3%)	21 (1.5%)	
endocrine, nutritional and metabolic diseases, and immunity disorders	30 (5.3%)	22 (1.5%)	
diseases of the digestive system	26 (4.6%)	114 (7.9%)	
diseases of the genitourinary system	33 (5.8%)	49 (3.4%)	
diseases of the skin, subcutaneous tissue, musculoskeletal system, and connective tissue	18 (3.2%)	108 (7.5%)	
other	106 (18.7%)	487 (33.8%)	

a) Data are presented as n (%), or mean ± standard deviation, or median (interquartile range).
b) Student t-test.
c) χ^2 test.
d) Mann-Whitney test.
Scenario 1 includes patients with requested neutrophil counts for both blood tests.
Scenario 2 includes patients with non-requested neutrophil counts for both blood tests.

Data analysis

Student t-tests, Mann-Whitney tests, and χ^2-tests were used to test for differences between groups, as appropriate. Linear regression analysis was used to estimate the proportion of bias associated with diagnostic subgroups and glucocorticoid use. The beta-coefficient for the contrasted scenarios was calculated for all patients in the study population as well as for only patients exposed to one factor (for example a diagnostic subgroup or glucocorticoid use). The proportion of bias explained by one factor was calculated as the weighted fraction of beta-coefficients. All analyses were conducted using SPSS for Windows, version 14.0 (SPSS Inc., Chicago, Illinois, USA).

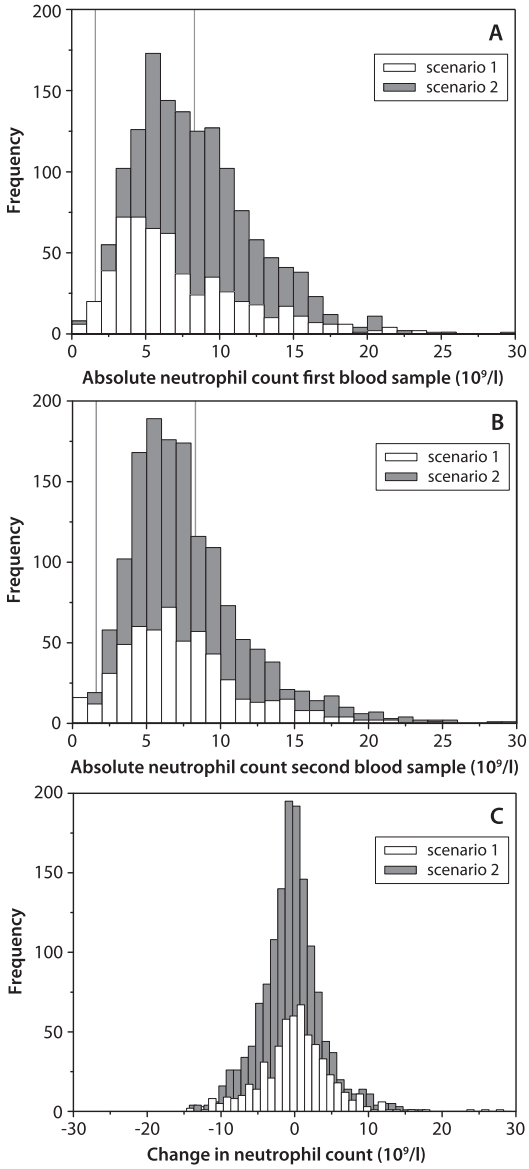
RESULTS

A total of 567 patients with requests for the absolute neutrophil count (scenario 1) and 1439 patients with non-requested neutrophil counts (scenario 2) were identified. It appeared that the absolute neutrophil count was most frequently requested in the context of a leukocyte differential request, which includes the absolute counts of neutrophils, eosinophils, lymphocytes, monocytes, and basophils (99.8% of all neutrophil count requests). Of patients with requested neutrophil counts, there was also a haemoglobin request for 97.2% of patients. For patients with non-requested neutrophil counts, 96.1% of the requests were for haemoglobin. Haemoglobin values were lower when requested compared with non-requested haemoglobin values (Table 1).

For the first blood test, lower neutrophil counts were found for patients with requested neutrophil counts compared with non-requested neutrophil counts (Table 1). Comparable neutrophil counts were found in the second blood test. Studying the change in the absolute neutrophil count during hospitalisation for each patient, patients with non-requested neutrophil counts had a mean decrease of 0.50×10^9 neutrophils/l compared with a slight increase of 0.14×10^9 neutrophils/l for patients with requested neutrophil counts (p-value 0.008, Table 1, Figure 2).

Overall, the main diagnostic subgroups were cardiovascular disease (28.9%), neoplasms (14.5%), and respiratory disease (4.5%). Requests for neutrophil counts were more often conducted for patients suffering from cardiovascular or respiratory diseases, whereas diagnoses for non-requested neutrophil counts were much more diffuse with multiple diagnoses (Table 1). There were no differences in absolute neutrophil count or change in neutrophil counts among patients with neoplasms and respiratory disease (Table 2). However, among patients with cardiovascular

Figure 2 Absolute neutrophil counts and change in neutrophil count



Distribution of the absolute neutrophil count of the first (A) and second blood test (B) and the change in neutrophil count for each patient (C) for requested neutrophil counts (scenario 1) and non-requested neutrophil counts (scenario 2). The vertical lines represent the normal reference area of 1.6–8.3×10⁹/l for the absolute neutrophil count (A and B).

Table 2 Neutrophil counts for the main diagnostic subgroups [a]

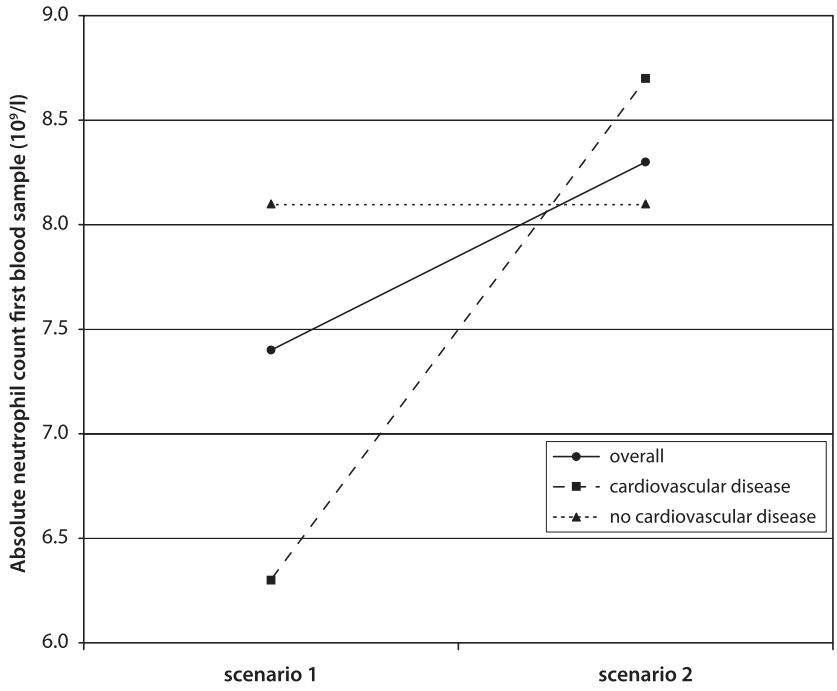
Characteristic	Requested (scenario 1)	Non-requested (scenario 2)	p-value (two-sided)
Cardiovascular disease			
N	200 (35.3%)	380 (26.4%)	
Absolute neutrophil count 1st blood test (10^9/l)	6.3 ± 3.6	8.7 ± 4.0	< 0.001 [b]
Absolute neutrophil count 2nd blood test (10^9/l)	8.4 ± 4.0	8.3 ± 4.0	0.741 [b]
Change in neutrophil count for each patient (10^9/l)	2.1 ± 4.5	-0.4 ± 4.2	< 0.001 [c]
Neoplasms			
N	71 (12.5%)	220 (15.3%)	
Absolute neutrophil count 1st blood test (10^9/l)	6.6 ± 4.1	7.1 ± 3.6	0.284 [b]
Absolute neutrophil count 2nd blood test (10^9/l)	7.7 ± 5.6	7.5 ± 4.3	0.986 [b]
Change in neutrophil count for each patient (10^9/l)	1.1 ± 5.4	0.5 ± 4.2	0.348 [c]
Respiratory disease			
N	53 (9.3%)	38 (2.6%)	
Absolute neutrophil count 1st blood test (10^9/l)	10.2 ± 6.0	9.3 ± 4.1	0.646 [b]
Absolute neutrophil count 2nd blood test (10^9/l)	8.9 ± 7.4	8.0 ± 4.0	0.778 [b]
Change in neutrophil count for each patient (10^9/l)	-1.3 ± 6.8	-1.3 ± 4.3	0.991 [c]

a) Data are presented as n (%), or mean ± standard deviation.
b) Mann-Whitney test.
c) Student t-test.
Scenario 1 includes patients with requested neutrophil counts for both blood tests.
Scenario 2 includes patients with non-requested neutrophil counts for both blood tests.

disease there was a lower absolute neutrophil count in the first blood test for requested counts compared with non-requested neutrophil counts. Excluding cardiovascular patients from analysis, the absolute neutrophil counts in the first blood test were equal with 8.1×10^9/l in both scenarios (Figure 3). The difference in absolute neutrophil count between scenarios in the first blood test could be explained for 83.2% by cardiovascular disease (p-value for effect modification 0.002). Incorporating glucocorticoid use in the linear regression model showed that diagnosis was far more important than glucocorticoid use (p-value for effect modification 0.240). Taking diagnosis into account, 4.5% of the difference in absolute neutrophil count between scenarios in the first blood test could be explained by glucocorticoid use.

With respect to the absolute neutrophil count in the second blood test, there were no differences between the scenarios neither in overall analysis nor in the

Figure 3 The effect of cardiovascular disease on the absolute neutrophil count of the first blood test



The higher neutrophil count in scenario 2 is explained by a high neutrophil count among cardiovascular patients in scenario 2. Excluding cardiovascular disease from analysis, there was no difference between the scenarios. Scenario 1 included requested neutrophil counts, scenario 2 included non-requested neutrophil counts.

main diagnostic subgroups. An increase in neutrophil count of 2.1×10^9/l was shown for requested neutrophil counts in cardiovascular patients, whereas non-requested counts revealed a decrease of 0.4×10^9 neutrophils/l (Table 2). Excluding cardiovascular disease from analysis, the change in neutrophil count was comparable in both scenarios with a mean decrease of 0.9×10^9/l for each patient with requested neutrophil counts and a mean decrease of 0.5×10^9/l for each patient including only non-requested neutrophil counts (p-value = 0.211).

DISCUSSION

In this study, we used UPOD to study bias in neutrophil testing as this database contains both requested neutrophil counts as well as non-requested neutrophil counts. Of requested neutrophil counts, haemoglobin was requested for 97% of patients as well. For non-requested neutrophil test results, 96% were generated by haemoglobin requests. Therefore, haemoglobin requests approximate random testing and can be used as comparator group. Absolute neutrophil counts differed for requested tests (scenario 1) compared with non-requested tests (scenario 2) which leads to the conclusion that testing bias was found in this study.

The bias in absolute neutrophil count in the first blood test could be explained for 83.2% by cardiovascular disease. This finding could reflect the role of neutrophils in cardiovascular disease.[13,17,18] After excluding cardiovascular disease from analysis, there were no differences in absolute neutrophil count or change in neutrophil count for each patient. This could be explained by the fact that the absolute neutrophil count was mainly requested in the context of a leukocyte differential count.

Distributions of diagnostic subgroups and testing guidelines might vary between health care institutions. As a consequence, generalizability of clinical implications, like the association with cardiovascular disease as an example in this study, might be limited. However, testing bias is an issue in all centres and should be evaluated to be able to adjust for this bias.

With development of automated machines for routine analysis, more parameters are measured than requested. When these non-requested parameters are collected, testing randomness is introduced. UPOD contains requested neutrophil counts and non-requested neutrophil counts, as well as other non-requested haematological blood tests. Therefore, the database is especially suitable to study and adjust for testing bias in clinical research questions. Conducting studies with laboratory markers in UPOD, correction factors for requested testing can be added to the statistical model to minimize testing bias in order to get a true risk estimate.

A classic example of testing bias is the association between thrombosis and use of oral contraceptives. Many studies state traditionally that the size of this association is overestimated because of diagnostic suspicion bias and referral bias, both types of testing bias.[19,20] However, a case-control study with the same referral and diagnostic strategies for cases and controls, showed that both types of bias did not play an major role in previous studies and that the risk of thrombosis while using oral contraceptives is not solely due to bias.[9] This example and the results from our study show the importance of evaluating testing bias in epidemiological studies obtaining data from clinical databases.

Acknowledgements - The authors are grateful to Maarten ten Berg for creating the Figure 1 drafts and to Svetlana Belitser for statistical advice.

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chapter 5

General discussion, conclusions and future perspectives

INTRODUCTION

This thesis describes pharmacoepidemiological studies in obstructive lung disease, used as a model for studying disease severity markers. In pharmacoepidemiological studies, measurement of disease severity is important to be able to adjust, match or select for disease severity as confounding by disease severity results in spurious associations between medication use and disease outcomes. With the expansion of the number and size of large pharmacoepidemiological databases and with recent advances in information technology, data mining techniques have been developed. These techniques can be used for the identification and evaluation of biomarkers for disease severity.

It could be stated that randomised controlled trials should be conducted to identify markers for disease severity. However, in case of using medication as a marker for disease severity, such a trial will probably not be approved by medical ethical committees, because severely ill patients with obstructive lung disease cannot be deprived of glucocorticoids since these drugs are essential in the treatment of asthma and chronic obstructive pulmonary disease (COPD) and there is no alternative treatment at this time. Therefore, especially in this patient group with a mixture of effects from disease and treatment, observational studies are essential to have more insight into several aspects of disease severity in obstructive lung disease.

The aim of this thesis was to contribute to pharmacoepidemiological research of obstructive lung disease by evaluating biomarkers that are associated with disease severity, combining (molecular) clinical, laboratory medicine and pharmacoepidemiological techniques. The purpose of this final chapter is to put the use of biomarkers for disease severity, as assessed in the studies in this thesis, into a broader perspective and to make recommendations for further research and to suggest clinical implications.

Three themes will be discussed:

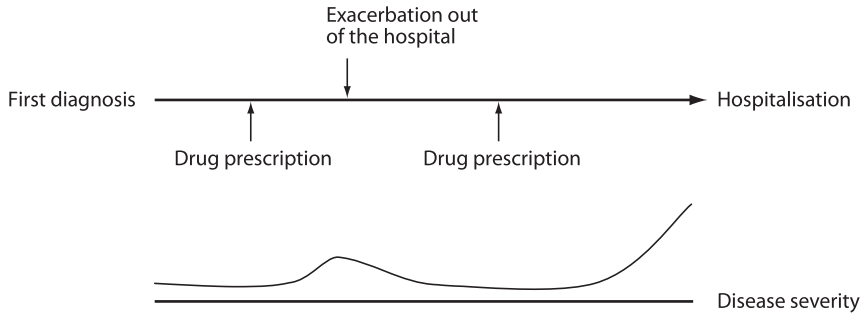
- Pathways to hospitalisation for obstructive lung disease;
- Laboratory biomarkers for disease severity in obstructive lung disease;
- Methodological aspects in biomarker studies.

PATHWAYS TO HOSPITALISATION FOR OBSTRUCTIVE LUNG DISEASE

In observational epidemiological studies, medication use and hospitalisations are the most frequently used markers for disease severity.[1-8] Hospitalisation is a major

burden on the health care costs for obstructive lung disease.[4,9-14] However, several confounding factors can affect the decision for hospitalisation as a marker and not all hospitalisations are coded correctly.[15,16] Although hospitalisation is the most stringent definition of disease severity, exacerbations out of the hospital could occur (Figure 1).[17-20] Therefore, using hospitalisation as marker for disease severity leads to underestimation of the true exacerbation rate and medication use prior to hospitalisation to identify disease deterioration is of equal importance as marker for disease severity.

Figure 1 Pathway to hospitalisation in association with disease severity



In this thesis, we have given examples of hospitalisation as marker for disease severity in **Chapter 2**. In *Chapter 2.1*, exacerbations were defined as either hospitalisation for obstructive lung disease or treatment of exacerbations out of the hospital. Of all 2332 patients with exacerbations, 1703 (73%) were not hospitalised during follow-up. In *Chapter 2.2* we studied medication use as marker for readmission to the hospital among patients with a hospitalisation for obstructive lung disease. This study showed that successful treatment of exacerbations out of the hospital was associated with a decreased risk of readmission. Comparison of the studies in *Chapters 2.1* and *2.2* showed that patients with a hospitalisation for obstructive lung disease were older, used more respiratory drugs in general and inhaled glucocorticoids and systemic glucocorticoids more specifically, and had more comorbidities. Therefore, hospitalised patients with obstructive lung disease are more severely ill compared with obstructive lung disease patients without hospitalisation. These findings imply that hospitalisation could be used as a marker

for disease severity. However, using hospitalisation as sole exacerbation marker leads to underestimation of the exacerbation rate, in accordance to studies from other countries.[21]

Patients use a spectrum of medications in the pathway prior to hospitalisation. Differences in these medication patterns between individuals could also serve as marker for disease severity. Therefore, the pathway to hospitalisation is of equal importance. In respiratory diseases, the number and dosage of medication needed to achieve a sufficient level of disease control is taken into account when evaluating disease severity.[22,23] Identification of patients at the far end of the medication spectrum will yield patients using most severe medication. These patients could be useful for the evaluation of medication markers for disease severity.[24] Some patients are insufficient responsive to glucocorticoids.[25,26] With the understanding why glucocorticoids are not effective in specific patient populations, new drugs can be developed or glucocorticoid responsiveness can be treated. The results from *Chapter 2.1* showed that patients with obstructive lung disease using chronic systemic glucocorticoids or high dose inhaled glucocorticoids are more susceptible to exacerbations, while in *Chapter 2.2* the effect of high dose inhaled glucocorticoid usage varied over time, in accordance to other studies.[4] In *Chapter 2.3*, it is shown that glucocorticoid use increased in the 90 days prior to hospitalisation and is associated with a fourfold increased risk of hospitalisation for obstructive lung disease. Taking all results together regarding medication use as marker for disease severity, glucocorticoid use, as maintenance treatment or as treatment of exacerbations, might be used as a marker for disease severity in pharmacoepidemiological studies on obstructive lung disease.

In *Chapter 3.3*, montelukast use was evaluated as a marker for disease severity among asthma patients as in most European countries, including the Netherlands, montelukast is used in asthma patients not controllable with regular asthma medication. Since registration in 1998 in the Netherlands, montelukast use increased with 3.5% asthma patients in 2003 to 7.1% asthma patients in 2007 (www.gipdatabank.nl, visited on 03-02-2009). The results showed that montelukast use is an indication of severe asthma, but imperfect marker for disease severity among asthma patients in automated databases. This is discussed more elaborately in the section on laboratory biomarkers for disease severity in obstructive lung disease.

Using medication in the pathway to hospitalisation as marker for disease severity has limitations. Medication use varies in time and is often increased just before an exacerbation. Also, patient compliance[27] and the patients' role in the decision process of medication prescription might bias the results. Moreover, medication use varies according to regional guidelines. Taking montelukast use as an example,

there are marked differences between the USA and most European countries. In the USA, montelukast is used as an alternative for inhaled glucocorticoids in mild asthma.[28] On the contrary, in most European countries, including the Netherlands, montelukast is prescribed for asthma patients not controllable with regular asthma medication.[28] But also guidelines for prescribing glucocorticoids differ due to balancing the effectiveness of glucocorticoids and side effects in asthma and discussion about the efficacy of glucocorticoids in COPD.[22,23,29,30] Therefore, medication use could be used as an indication for disease severity, but guidelines do not fully reflect routine clinical practice.[31] More objective measurable markers for disease severity are needed in pharmacoepidemiological studies.

LABORATORY BIOMARKERS FOR DISEASE SEVERITY IN OBSTRUCTIVE LUNG DISEASE

In addition to classic parameters like hospitalisation and medication use as markers for disease severity, we evaluated the use of laboratory biomarkers as marker for disease severity. As discussed in **Chapter 1**, biomarkers have several advantages over hard clinical endpoints. However, in obstructive lung disease, no biomarkers for disease severity are currently available for routine clinical practice. As asthma and COPD are heterogeneous inflammatory diseases, there is a need for biomarkers to measure disease severity and to discriminate between phenotypes, in which the type of underlying inflammation is incorporated.[22,32-35] There is increasing evidence that some difficult-to-treat asthma (DTA) patients are non-responsive to glucocorticoids and have high neutrophil counts.[32,34,36-42] The absolute neutrophil count or neutrophil morphology could be used as a biomarker for disease severity in obstructive lung disease in epidemiological studies.

In *Chapter 3.1*, we used the absolute neutrophil count as a biomarker for identification of hospitalisation in obstructive lung disease where hospitalisation was used as measure for disease severity. Hospitalisation for obstructive lung disease was associated with neutrophilia. Therefore, the absolute neutrophil count seems to be a useful biomarker for disease severity in pharmacoepidemiological studies on obstructive lung disease. Although the results persisted after adjustment for glucocorticoid use, it is controversial whether neutrophilia is a true marker of disease severity or the result of glucocorticoid treatment. This will be discussed further on in this chapter.

Although neutrophilia seems to be associated with disease severity,[3,11-15] the question remains whether these neutrophils differ in activation state and morphology in

DTA patients compared with neutrophils in mild-to-moderate asthma patients. Therefore, neutrophil morphology parameters were studied as a marker for disease severity, in addition to the absolute neutrophil count. In *Chapter 3.2*, we found that absolute neutrophil counts and morphological change were independently of sufficient discriminative value for distinguishing asthma patients from healthy volunteers. However, among asthma patients, the absolute counts alone could not be used to distinguish DTA patients from non-DTA patients. Changes in the neutrophil morphology could have many causes, but in the pre-selected asthma population in *Chapter 3.2*, we were able to define asthma phenotypes more precisely using neutrophil morphology parameters, compared with absolute neutrophil counts. An association between neutrophil morphology and DTA was also found in *Chapter 3.3*. Therefore, neutrophil morphology is a promising biomarker to identify the phenotype DTA.

To express the value of a disease severity marker, sensitivity and specificity are often used.[43] Sensitivity is defined as the proportion of patients with the disease of interest that is positively identified by the marker. Specificity is defined as the proportion of healthy patients that is found healthy by the marker. Therefore, in order to be able to calculate the sensitivity and specificity, the outcome should be already known. In clinical practice, positive predictive values (PPV) and negative predictive values (NPV) are more valuable, as these parameters indicate the risk of disease for patients given a certain biomarker test result. This is more informative from a patient perspective.[43,44] Therefore, patient outcome studies should be conducted in addition to diagnostic test evaluation.[43]

Using absolute neutrophil counts in *Chapter 3.1*, it was shown that neutrophilia had an overall PPV of 78% for hospitalisation for obstructive lung disease. Using asthma patients only in *Chapter 3.2*, the absolute neutrophil count had a PPV of 67% for distinguishing DTA among asthma patients. For neutrophil morphology, the PPV for DTA was 80%. In *Chapter 3.3*, we showed that using montelukast as a pharmacoepidemiological marker for DTA yielded a PPV of 85% for a montelukast user being a DTA patient. However, the absolute neutrophil count and morphology had PPVs of 43% and 47% respectively. Therefore, montelukast use is indicative, but an imperfect marker for DTA. The absolute neutrophil count and morphology seem promising biomarkers for disease severity in asthma. A replicate study should be conducted in a prospective, blinded fashion and the accuracy of the neutrophil count and morphology as biomarkers for disease severity in asthma should be confirmed. Moreover, the mechanistic role of the morphological changes should be studied in more detail.

The morphology parameters in the studies presented in this thesis were measured in routine analysis with automated haematocytometers. These haematocytometers provide advantages for epidemiological studies as morphological markers that need microscopic inspection or in vitro preparation are time consuming and not appropriate for routine assessment of haematological parameters. Using leukocyte morphology parameters, collected with routine haematocytometry analysis, is a new way of studying asthma phenotypes and neutrophilic inflammation in general. Using this method makes it feasible to conduct larger scale biomarker studies, combining clinical, laboratory medicine, and epidemiological techniques. Biomarkers to characterise disease severity could be used in epidemiological studies as objective measurable markers to measure and adjust for disease severity and therefore reduce this type of confounding in studies dealing with associations between medication use and outcome parameters.

METHODOLOGICAL ASPECTS IN BIOMARKERS STUDIES

Procedures and laboratory tests are conducted in all health care centres and patient populations. Clinical routine procedures and tests will not be conducted with the total patient population, but with a fraction of the population. This would be of no consequence in epidemiological studies when this proportion is a random sample from the patient population. However, laboratory testing in clinical practice is never a random process as the physician always has reasons to test. We found that lung function tests and haematological parameters were conducted selectively for more severely ill patients leading to testing bias in clinical databases (*Chapter 4.1*). Therefore, using laboratory test results in epidemiological studies to identify and evaluate biomarkers for disease severity could lead to biased results.

With the development of automated machines for routine analysis, more parameters are measured than requested. When these non-requested parameters are collected, testing randomness is introduced. This testing randomness could be used to study testing bias in clinical practice. Utrecht Patient Oriented Database (UPOD) contains non-requested haematological parameters that are measured because one haematological test is technically linked to the other haematological tests and conducted automatically when one of these tests is requested. In *Chapter 4.3* requests for general blood profiles and specifically for neutrophil counts were contrasted. Differences in absolute neutrophil count at time of admission could be explained for 83.2% by underlying cardiovascular disease and for 4.5% by glucocorticoid use. This finding implies that requests for the absolute neutrophil count in clinical

practice is associated with underlying disease and this could bias the study results. Distributions of diagnostic subgroups and testing guidelines might vary between hospitals, but testing bias is an issue in all hospitals and should be evaluated to be able to adjust for this bias.

Bias in epidemiological studies using biomarkers could also be introduced when using a biomarker for disease severity that is associated with drug treatment. Clinical observations have shown that patients using glucocorticoids often have higher absolute neutrophil counts, particularly in obstructive lung disease. It is well known that granulocytes play an important role in asthma and COPD,[45,46] but debate remains whether neutrophilia is a true marker for disease severity or results from glucocorticoid treatment.[22,32,34,37,38,47-52] In-vitro experiments found that glucocorticoids inhibit neutrophil apoptosis.[53-56] However, in-vivo studies only found short-term effects of glucocorticoids on the neutrophils and showed normalization of the neutrophil count within 24 hours.[57,58] Other studies concluded that neutrophilia is not caused by glucocorticoid treatment.[49,59,60] Therefore, the effect of glucocorticoids on the absolute neutrophil count is controversial.

To study whether systemic glucocorticoids have effect on the absolute neutrophil count, and therefore could confound in the association between disease severity and the absolute neutrophil count as found in *Chapter 3.1*, a study among hospitalised patients, irrespective of diagnosis, was conducted in *Chapter 4.2*. It was shown that diagnosis was the factor most strongly associated with the absolute neutrophil count. Therefore, neutrophilia in users of systemic glucocorticoids is probably associated with underlying disease, rather than glucocorticoid use itself and the absolute neutrophil count could be used as a marker for disease severity in obstructive lung disease as shown in *Chapter 3.1*. Further evidence suggesting that underlying disease is a major confounder in studies with regard to the neutrophil count were found in *Chapter 4.3*.

As a sensitivity analysis, we adjusted the results in *Chapter 3.1* for diagnosis, as well as for request for the absolute neutrophil count (*Chapter 4.3*). The odds ratio (OR), adjusted for diagnosis and request for the absolute neutrophil count in both blood samples was 1.5 (95% confidence interval [95%CI] 1.2–1.9) for an increase in neutrophil count with no change as a reference compared with an OR, adjusted for diagnosis only, of 1.6 (95%CI 1.3–2.0). For a decrease in neutrophil count the OR were 0.9 (95%CI 0.7–1.1) in both models. Therefore, we corrected for diagnosis only as confounding factor in *Chapter 3.1*. These results imply that testing bias as studied in *Chapters 4.1* and *4.3* is less important than the underlying diagnosis in studying the value of the absolute neutrophil count as a measure for disease severity in obstructive lung disease.

Unlike the haematological parameters that are technically linked to other haematological tests and conducted automatically when one of these tests is requested, other, non-haematological parameters, like C-reactive protein (CRP), are only measured upon request. As shown in *Chapter 4.1*, CRP is measured in less than half of all hospitalised patients. As insurance companies are gaining a more important role in the decision process of which drugs should be prescribed, it might be expected that insurance companies in the future will more strictly define which laboratory tests will be reimbursed. Such policy changes will affect which laboratory tests are requested and therefore which tests we are able to include in research datasets when using clinical databases containing data collected from daily clinical practice. Because of differences in health care centres and in insurance conditions, the situation might evolve that certain laboratory tests are requested for some patients but not for others introducing bias in databases.

A possible solution would be to implement standard procedures describing which laboratory tests are requested for a first visit to the hospital. This way, structured multidisciplinary information would be available for all patients and we will be able to conduct large cohort studies in clinical practice, in accordance to prospective cohort studies as the Rotterdam Study and the SMART study.[61,62] However, structurally testing the total patient population affects the health care costs for obstructive lung disease, will cause patient stress, decrease the patient well-being, and increases disease severity from a patient perspective. Another method is to evaluate and quantify the magnitude of testing bias in order to be able to adjust for this factor in the analysis of biomarker studies. In this thesis, testing bias was evaluated by comparing patient with and without requested laboratory tests results.

CONCLUSIONS AND FUTURE RESEARCH PERSPECTIVES

From the findings presented in this thesis it can be concluded that identification and evaluation of markers for disease severity is essential to measure and adjust for confounding by disease severity in epidemiological studies. Automated databases containing pharmacy records like the PHARMO Record Linkage System or the General Practice Research Database (GPRD) are valuable to identify markers for disease severity in the pathways to hospitalisation based on drug prescriptions, like systemic glucocorticoids in inflammatory disease. Clinical databases linking information on biomarkers to clinical data like UPOD are fundamental for biomarker research. UPOD contains requested and non-requested haematological

blood tests. Therefore, this database is especially suitable to evaluate testing bias in clinical research questions. Conducting studies with laboratory markers in UPOD, correction factors for requested testing can be added to the statistical model to minimize testing bias in order to get a true risk estimate. The absolute neutrophil count and neutrophil morphology can be considered as promising markers for disease severity in obstructive lung disease. Replicate studies should be conducted in a prospective, blinded fashion and the accuracy of biomarkers for disease severity should be confirmed. Moreover, the mechanistic role of the neutrophil count and morphological changes should be studied in more detail. Evaluation of disease severity in pharmacoepidemiological studies is warranted to avoid misclassification with respect to grading severity, to study severity pathways and to evaluate the predictive value of biomarkers for disease severity. Data mining using large clinical databases with linkage of clinical information and laboratory test results is essential to identify and evaluate potential biomarkers associated with disease severity. Data mining studies that combine (molecular) clinical, laboratory medicine and pharmacoepidemiological techniques add promising diagnostic biomarkers to the multidisciplinary health care needed for patients with severe obstructive lung disease.

In the future, more databases might be linked and extended with other types of data, improving possibilities to conduct more sophisticated (international) biomarker studies. Taking UPOD as an example, this database might also be extended with other types of data, for example outpatient medication use. Recently, a digital system for recording prescription medication by specialists had been introduced. This will provide information about medication that has been prescribed in the outpatient clinics. Moreover, all other medication use, prescribed by specialists from other hospitals or by the general practitioner, need to be registered. For this data, public pharmacy data will be needed. There are several possibilities with advances in information technology. First, linkage with the electronic patient file (in Dutch EPD) in the future will provide all information regarding health care for each patient. However, privacy issues are important here and the information will not be in an automated database format. Another option is usage of a universal method of encrypting a patients' identity. This will lead to one universal patient number in existing databases providing possibilities for data linkage and collaboration of multiple disciplines and countries.

CLINICAL IMPLICATIONS

Physicians and pharmacists should be alert with increasing numbers of medication changes. Keeping close contact with patients who are at high risk for hospitalisation, for example by telemonitoring [63] or measurement of lung function,[64] could identify exacerbations in an early phase. Early detection might prevent further disease deterioration or hospitalisation for obstructive lung disease. Upon hospitalisation, physicians should be informed about the patients' medication history as hospitalisation is associated with discontinuing of outpatient medication.[65] Upon discharge, patients should be included in a hospital discharge program, to decrease risk of emergency department visits and rehospitalisation.[66,67] Therefore, we should focus more on the continuity of care upon transitions of health care settings.

From an epidemiological perspective, there is a need for more structurally performed testing, as testing the absolute neutrophil count and CRP for all asthma and COPD patients. This will lead to less missings in databases and minimizes testing bias, as studied in *Chapters 4.1* and *4.3*. Each patient with obstructive lung disease on a first visit to the outpatient clinic of Respiratory Medicine should be structurally screened for risk factors and comorbidities, according to large cohort studies as the Rotterdam study in Rotterdam and the SMART study in Utrecht.[61,62] This way, phenotyping will be more applicable in epidemiological studies and there are more possibilities for personalised health care. Multidisciplinary health care, including epidemiological, laboratory medicine and clinical disciplines, is needed for patients with severe obstructive lung disease.

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chapter 6

Summary
Samenvatting

Summary

Physicians evaluate diagnosis and severity of disease during clinical visits according to patient characteristics, symptoms, physical examination, imaging techniques or laboratory testing. Disease severity is an important parameter in order to obtain a patient personalised treatment. In pharmacoepidemiological studies using health care databases usually incomplete clinical information is available and is mostly restricted to information regarding hospitalisation, diagnosis, drug prescriptions, and laboratory test results. Characterisation of disease severity plays an important role in pharmacoepidemiological studies, as is illustrated by the discussion regarding the association between use of β_2-agonists and the risk of asthma death. Eventually, confounding by disease severity was indicated as the most probable explanation of this observed association. More severely ill patients are likely to use more medication and are at increased risk of having disease exacerbations. Therefore, the effects of disease severity and drug exposure are mixed-up, leading to spurious associations between medication use and disease outcomes. To minimize bias, it is essential to evaluate disease severity in pharmacoepidemiological studies. Disease severity can be measured by means of symptoms and questionnaires, but these measurements could be subjective. Therefore, there is a need for more objective markers to evaluate disease severity. The studies in this thesis focused on medication use and biomarkers as marker for disease severity.

In pharmacoepidemiological studies, all medication use of a patient can be used as a marker for disease severity. Moreover, specific prescriptions can be used as marker for disease severity, for example TNF-α antagonists among rheumatoid arthritis patients or insulin in patients with diabetes mellitus type 2. In addition to medication use, biomarkers could be used to measure disease severity. The Biomarkers Definition Working Group has defined a biomarker as a characteristic

that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention. There as several advantages of biomarkers over hard clinical endpoints. Biomarkers are often cheaper and easier, more quickly and earlier to measure than clinical endpoints. Also, using biomarkers is more ethical when the biomarker is measurable before tissue damage occurs. Biomarkers can be used to monitor disease progression, but also allow for earlier identification of disease deterioration, and can be used to adjust for disease severity in pharmacoepidemiological studies.

Pharmacoepidemiology and clinical chemistry and haematology laboratories are increasingly focused on the identification and development of new markers for disease, that can be used in clinical practice. Cooperation of these three disciplines and the linkage of laboratory parameters and clinical data can provide many new opportunities to search for biomarkers in close conjunction to clinical practice.

In this thesis, obstructive lung disease was used as a tool to study markers for disease severity. Obstructive lung disease includes two complex diseases, asthma and Chronic Obstructive Lung Disease (COPD). Worldwide, the prevalence for asthma ranges from 1–18%. The prevalence of COPD is estimated at 9–10% of adults aged ≥ 40 years old. Both asthma and COPD have multiple phenotypes. A phenotype is defined as a subtype of disease, based functionally or pathologically by a molecular mechanism or by treatment response. However, the existing way of phenotyping leads to multiple (sub)phenotypes that have considerable overlap. Therefore, the classification of the heterogeneous diseases asthma and COPD needs to be re-evaluated.

Difficult-to-treat asthma (DTA) is a heterogeneous phenotype with characteristics of both asthma and COPD. This phenotype occurs in 5–10% of the asthma population and accounts for about fifty percent of the total health care costs for asthma. There is increasing evidence that some DTA patients are non-responsive to glucocorticoids and have high neutrophil counts in the peripheral blood. Patients with DTA keep having symptoms and exacerbations despite treatment according to guidelines. Therefore, a diagnosis of DTA and asthma exacerbations could be used as a measure for disease severity in obstructive lung disease.

This thesis is divided into three parts:

1) the evaluation of medication use as marker for disease severity in the pathways to hospitalisation for obstructive lung disease,

2) the evaluation of the absolute neutrophil count, neutrophil morphology and montelukast use as potential biomarkers for disease severity, and
3) the evaluation of methodological aspects that should be considered in the conduct of biomarker studies.

The evaluation of medication use as a marker for disease severity is described in **Chapter 2**. In this chapter data from the PHARMO Record Linkage System was used. This database includes the demographic details and complete medication history of more than two million community-dwelling residents of more than twenty-five population-defined areas in the Netherlands from 1985 onwards.

In *Chapter 2.1* exacerbations among users of inhaled corticosteroids (ICS) were studied by means of hospitalisation for obstructive lung disease and short courses of oral corticosteroids out of the hospital. Of all 5327 patients in the study, 2332 patients experienced 8635 exacerbations during follow-up with a trend towards treatment of exacerbations out of the hospital (p-value 0.003). Among patients with exacerbations, 73% (1703 patients) was not hospitalised during follow-up. Exacerbations were associated with high-dose ICS use (adjusted relative risk [RR] 1.5; 95% confidence interval [95%CI] 1.2–1.7) and chronic oral corticosteroid use (adjusted RR 1.9; 95%CI 1.6–2.2). Using hospitalisation only as exacerbation marker therefore leads to underestimation of the exacerbation rate, because of exacerbation treatment out of the hospital.

In *Chapter 2.2* medication use was studied as marker for readmission to the hospital among patients with a previous hospitalisation for obstructive lung disease. Of 605 ICS users with a hospital admission for obstructive lung disease, 132 were readmitted to the hospital within one year. Readmission was associated with a high Chronic Disease Score (adjusted hazard ratio [HR] 2.4; 95%CI 1.1–5.3). This study also showed that successful treatment of exacerbations out of the hospital was associated with a decreased risk of readmission. Patients using short courses of systemic corticosteroids only (adjusted HR 0.5; 95%CI 0.4–0.8) or combined with antibiotics (adjusted HR 0.4; 95%CI 0.2–0.6) were at decreased risk of readmission. Patients with multiple chronic diseases should be educated regarding their diseases and should be invited to consultation more often to be able to detect exacerbation in an early phase and start treatment as early as possible.

Chapter 2.3 describes changes in medication use prior to hospitalisation for obstructive lung disease. This case-crossover study included 1481 patients with each patient serving as their own control. The period of three months prior to hospitalisation was the case period, while control moments were on 3, 6, 9, and 12 months prior to hospitalisation. Medication use was ascertained in a 90 day time-window prior to each case or control moment. The results showed that usage of glucocorticoids, antibiotics and other respiratory drugs was relatively stable in the control periods, but increased in the 90 days prior to hospitalisation. Hospitalisation was associated with use of three or more respiratory drugs (odds ratio [OR] 2.2; 95%CI 1.8–2.8), use of oral glucocorticoids (OR 4.5; 95%CI 3.8–5.4) and antibiotic use (OR 3.1; 95%CI 2.7–3.6). These results could be indicative of the development and/or treatment of an exacerbation. There is need for markers to detect exacerbations in an early phase in order to start treatment as early as possible and possibly prevent hospitalizations for obstructive lung disease.

The second part of this thesis, described in **Chapter 3**, focuses on the use of laboratory biomarkers as marker for disease severity in obstructive lung disease using data from the Utrecht Patient Oriented Database (UPOD). UPOD is an infrastructure of relational databases comprising administrative data on patient characteristics, laboratory test results, medication orders, discharge diagnoses and medical procedures for all patients treated at the University Medical Centre (UMC) Utrecht.

Because inflammation plays an important role in disease severity among patients with obstructive lung disease, the absolute neutrophil and eosinophil count was studied in *Chapter 3.1*. In a case-control study, 143 cases were defined as patients with a hospitalisation for obstructive lung disease. The 143 controls were also patient in the outpatient clinic of Respiratory Medicine, but were not hospitalised. Hospitalisation was associated with neutrophilia (adjusted OR 4.3; 95%CI 2.2–8.5) and eosinophilia (adjusted OR 2.6; 95%CI 1.1–6.2). Stratifying on diagnosis, the association with eosinophilia was only observed in asthma patients, but not in COPD patients. This is reassuring because the associations of eosinophilia in asthma patients and neutrophilia in COPD patients are in line with current knowledge on asthma and COPD. These results suggest that the absolute neutrophil and eosinophil count can be used as a biomarker for disease severity in obstructive lung disease. Many authors struggle with the issue of whether the observed neutrophilia in asthma patients is primarily a characteristic of asthma severity or secondary

to the treatment with glucocorticoids, because of suggestions in literature that glucocorticoids could inhibit neutrophil apoptosis. The results in this study were adjusted for age, gender, glucocorticoid use and lung function. Therefore, this study adds to the evidence that neutrophilia among DTA patients is not solely caused by glucocorticoid treatment, but is an inflammatory characteristic of this asthma phenotype. The effect of glucocorticoids on the absolute neutrophil count is further studied in *Chapter 4.2*.

Although neutrophilia seems to be associated with disease severity in asthma, the question remains whether these neutrophils differ in activation state and morphology in DTA patients compared with neutrophils in mild-to-moderate asthma patients. Therefore, in *Chapter 3.2*, 17 DTA patients, 13 non-DTA patients and 19 healthy volunteers were compared with respect to the absolute neutrophil count and neutrophil morphology as possible biomarker for discriminating different asthma phenotypes. Asthma patients without acute infections and with a haematological blood test in clinical practice were included in the study. The absolute neutrophil counts and neutrophil morphology were able to discriminate asthma patients from healthy volunteers. However, among patients with asthma, DTA cases could be more accurately defined with a neutrophil morphology change (OR 8.0; 95%CI 1.5–42.0), compared to the absolute neutrophil count (OR 4.0; 95%CI 0.8–21.0). Using UPOD, biomarker studies could be conducted on a larger scale, because the morphology parameters are measured and stored automatically.

The diagnosis DTA is based upon multiple characteristics, as defined by the ATS American Thoracic Society (ATS) criteria. One of these characteristics regards medication use, but further information is needed regarding symptoms, lung function and disease control. Such characteristics are not always available in pharmacoepidemiological studies. Therefore, in *Chapter 3.3* it was studied whether montelukast use could be used as an easily measurable marker for disease severity, as montelukast is prescribed to asthma patients that are not controllable with regular asthma medication in most European countries, including the Netherlands. Asthma patients without acute infections and with a haematological blood test in clinical practice were included in the study. The study population comprised 20 montelukast users and 29 non-montelukast users and the absolute neutrophil counts and neutrophil morphology were compared between both groups. Both the absolute neutrophil counts (OR 1.5; 95%CI 0.4–6.0) and a change in neutrophil morphology (OR 2.1; 95%CI 0.6–7.4) were not discriminative for montelukast use versus non-use. Therefore, all patients were also categorised as DTA and non-DTA by clinical

review. Similar results as in *Chapter 3.2* were found. DTA was not associated with the absolute neutrophil count (OR 2.3; 95%CI 0.6–8.7) and neutrophil morphology was discriminative for DTA among asthma patients with an OR of 4.0 (95%CI 1.2–14.0). Of all 20 montelukast users, 17 patients had DTA with a positive predictive value of 85.0%. Therefore, it can be concluded that montelukast use is an indicator, but imperfect marker for disease severity among asthma patients in automated databases.

The third part of this thesis in **Chapter 4** concentrates on a number of methodological aspects that should be considered in the conduct of biomarker studies. The aim of the study in *Chapter 4.1* was to evaluate disease severity and to assess current practice in testing disease severity in a cohort of respiratory patients. To do so, all patients visiting the outpatient clinic of Respiratory Medicine of the UMC Utrecht in 2005–2007 were included in the study. Approximately 3000 patients visited the outpatient clinic each year, with a total of 5356 individual patients in 2005–2007. Of all patients, 12.2% visited the emergency department and 3.6% were hospitalised for obstructive lung disease each year. Haematological blood testing and lung function testing occurred more often prior to hospitalisation with a fivefold increased risk of hospitalisation in the period of 15 days prior to the index date (adjusted ORs 5.5; 95%CI 3.2–9.3 and 5.4; 95%CI 2.9–10.0 respectively). It can be concluded that most patients visiting the outpatient clinic of Respiratory Medicine were well-controlled with regard to disease severity. Testing for disease severity by haematological parameters and lung function tests was conducted for more severely ill patients. Testing bias should therefore be taken into account in the conduct of biomarker studies using data from routine clinical databases.

Bias could also be introduced in biomarker studies in case the marker for disease severity itself is associated with medication use. In clinical practice, patients with systemic glucocorticoid use seem to have a high absolute neutrophil count. In *Chapter 4.2*, the effect of systemic glucocorticoid use on the absolute neutrophil count in the peripheral blood was studied. In this study, all adult patients who were hospitalised in the UMC Utrecht in 2005 and had at least two blood samples during hospitalisation were included, irrespective of diagnosis. A total of 809 glucocorticoid users and 2658 non-users were compared. The absolute neutrophil counts at admission in both study groups were comparable (8.2×10^9/l for glucocorticoid users and 8.0×10^9/l for non-users). Overall analysis showed a slight association between glucocorticoid use and an increase in neutrophil count (RR 1.3; 95%CI 1.1–1.5).

However, this is the summed effect of several factors, next to the possible effect of systemic glucocorticoids. These factors include diagnosis, disease severity, dose and type of glucocorticoid, and the studied time window. Within diagnostic subgroups there was no association between glucocorticoid use and the absolute neutrophil count. Furthermore, no dose response relationship, no effect of time between the two blood samples, and no effect of anti-inflammatory/sodium retaining potency was found. Therefore, the observed neutrophilia in users of systemic glucocorticoids in clinical practice is probably associated with the underlying disease, rather than glucocorticoid use itself.

As already shown in *Chapter 4.1*, tests were more often requested for more severely ill patients. In *Chapter 4.2*, blood tests for the absolute neutrophil count were used. It is of importance whether these blood samples were requested or not. In *Chapter 4.3* testing bias in the request of blood tests for the absolute neutrophil count in routine clinical practice was evaluated by comparing requested and non-requested neutrophil counts. In the UMC Utrecht, haematological blood tests are conducted by Cell-Dyn Sapphire haematocytometers. One characteristic of these machines is that all haematological measurements are performed, irrespective of whether this measurement was requested by a physician or not. The non-requested parameters are measured because one haematological test is technically linked to the other haematological tests and conducted automatically when one of these tests is requested. Non-requested parameters are also stored in the UPOD database. Therefore, testing bias could be evaluated by comparing requested and non-requested blood tests. A total of 567 patients with requested neutrophil counts and 1439 patients with non-requested neutrophil counts were analysed. All patients were hospitalised during the study period in 2005. The absolute neutrophil count at admission differed with a mean of 7.4×10^9/l for requested counts and 8.3×10^9/l for non-requested counts (p-value < 0.001). This difference could be explained for 83.2% by the occurrence of cardiovascular disease as underlying disease and for 4.5% by glucocorticoid use. This finding implies that requests for the absolute neutrophil count in clinical practice is associated with underlying disease and this could bias the study results. Distributions of diagnostic subgroups and testing guidelines might vary between hospitals, but testing bias is an issue in all hospitals and should be evaluated to be able to adjust for this bias.

In **Chapter 5** the main findings are discussed and put in a general perspective of disease severity in pharmacoepidemiological studies. From the findings presented

in this thesis it can be concluded that identification and evaluation of markers for disease severity is essential from a clinical point of view as well as to be able to adjust for confounding by disease severity in epidemiological studies. Replicate studies should be conducted in a prospective, blinded fashion and the accuracy of biomarkers for disease severity should be confirmed. Studies that combine (molecular) clinical, laboratory medicine and pharmacoepidemiological techniques add promising diagnostic biomarkers to the multidisciplinary health care needed for patients with severe obstructive lung disease. This chapter is ended with recommendations for clinical practice and future research perspectives.

Samenvatting

Artsen stellen een diagnose en bepalen de ernst van ziekte tijdens persoonlijk contact aan de hand van patiëntkenmerken, symptomen, lichamelijk onderzoek of laboratoriumbepalingen. Ernst van ziekte is een essentiële parameter voor een op maat gemaakte behandeling. In farmaco-epidemiologische studies die gebruik maken van gegevens uit klinische databases is vaak niet alle klinische informatie voor handen en is deze vaak beperkt tot data met betrekking tot ziekenhuisopname, diagnose, recepten voor geneesmiddelen en laboratoriumuitslagen. Het bepalen van de ernst van ziekte is essentieel bij het uitvoeren van farmaco-epidemiologische studies. Dit wordt geïllustreerd door de discussie rondom de associatie tussen het gebruik van β_2-agonisten en het overlijden aan astma, waarbij uiteindelijk bleek dat deze associatie werd verstoord door de ernst van ziekte. Patiënten die ernstig ziek zijn, gebruiken veel geneesmiddelen en hebben ook een hoger risico op exacerbaties. Hierdoor raken de effecten van de ernst van ziekte en geneesmiddelgebruik met elkaar vermengd wat kan leiden tot schijnassociaties tussen geneesmiddelgebruik en ziekte-uitkomsten. De ernst van ziekte kan worden gemeten met behulp van symptomen of vragenlijsten, maar deze kunnen subjectief worden geïnterpreteerd. Daarom is er behoefte aan de ontwikkeling van objectieve maten voor de ernst van ziekte. In dit proefschrift werd de nadruk gelegd op het gebruik van geneesmiddelen en biomarkers als maat voor de ernst van ziekte.

In farmaco-epidemiologische studies kan het volledige geneesmiddelgebruik van een patiënt worden gebruikt om de ernst van ziekte in kaart te brengen. Bovendien kunnen specifieke geneesmiddelen als maat voor de ernst van ziekte fungeren. Voorbeelden hiervan zijn TNF-α antagonisten bij reumatoïde artritis of insuline bij diabetes mellitus type 2. Naast recepten voor geneesmiddelen kunnen ook biomarkers dienst doen als maat voor de ernst van ziekte. De 'Biomarkers

Definition Working Group' heeft een biomarker gedefinieerd als een parameter die objectief kan worden gemeten en een indicator is voor een normaal biologisch proces, een pathogeen proces of een farmacologische respons op een therapeutische interventie. Biomarkers hebben verschillende voordelen boven harde klinische eindpunten. Biomarkers zijn vaak goedkoper en makkelijker, sneller en vroeger te meten dan klinische eindpunten en vaak ook ethischer als een biomarker meetbaar is voordat weefselschade optreedt. Hierdoor kan het verloop van de ziekte worden gevolgd, maar kunnen ook exacerbaties sneller worden opgespoord, en kunnen biomarkers gebruikt worden om te corrigeren voor ernst van ziekte in farmaco-epidemiologische studies.

De farmaco-epidemiologie en klinisch-chemische en hematologische laboratoria zijn in toenemende mate geïnteresseerd in het ontwikkelen van markers die in de klinische praktijk kunnen worden gebruikt. Samenwerking van deze drie disciplines en het koppelen van laboratoriumgegevens aan klinische gegevens geeft veel mogelijkheden om nieuwe markers voor de ernst van ziekte te identificeren die bruikbaar zijn in de klinische praktijk.

In dit proefschrift werden obstructieve longziekten gebruikt als voorbeeld om diverse maten voor de ernst van ziekte te bestuderen. Obstructieve longziekten kunnen worden onderverdeeld in twee complexe ziektebeelden, astma en COPD (Chronic Obstructive Lung Disease). Wereldwijd komt astma in 1-18% van de volwassenen voor. COPD heeft een prevalentie van 9-10% bij volwassenen van 40 jaar of ouder. Zowel astma als COPD bestaan uit vele fenotypes. Een fenotype is een subtype van een ziekte, functioneel of pathologisch gedefinieerd op basis van het moleculaire ziektemechanisme of op basis van respons op behandeling. Echter, de bestaande manieren van fenotypering bij obstructieve longziekten leiden tot vele (sub)fenotypes die aanzienlijke overlap met elkaar vertonen. Daarom moet de classificatie van de heterogene ziekten astma en COPD opnieuw worden gemaakt.

Moeilijk-te-behandelen astma (difficult-to-treat asthma, DTA) is een heterogeen fenotype met zowel kenmerken van astma als van COPD. Dit fenotype komt voor bij 5-10% van de astmapatiënten en zorgt voor ongeveer 50% van de kosten die in de gezondheidszorg voor astma worden gemaakt. In toenemende mate zijn er aanwijzingen dat sommige DTA patiënten onvoldoende reageren op corticosteroïden en een hoog aantal neutrofielen in het perifere bloed hebben. Patiënten met DTA blijven symptomen en exacerbaties houden, ondanks behandeling volgens de

richtlijnen. Daarom kan de diagnose DTA, alsmede het hebben van exacerbaties gebruikt worden als maat voor de ernst van ziekte bij obstructieve longziekten.

Dit proefschrift is onderverdeeld in drie delen:
1) het evalueren van geneesmiddelgebruik als maat voor de ernst van ziekte in de aanloop naar ziekenhuisopname voor obstructieve longziekten,
2) het evalueren van het aantal neutrofielen, de neutrofielmorfologie en montelukastgebruik als potentiële biomarkers voor de ernst van ziekte, en
3) het evalueren van een aantal methodologische aspecten van biomarkerstudies.

Het evalueren van geneesmiddelgebruik als maat voor de ernst van ziekte wordt besproken in **Hoofdstuk 2**. Hiervoor is gebruik gemaakt van de PHARMO database. Deze database bevat demografische gegevens en de volledige medicatiehistorie van meer van twee miljoen Nederlanders sinds 1985.

In *Hoofdstuk 2.1* werden exacerbaties onder gebruikers van inhalatiecorticosteroïden (ICS) bestudeerd aan de hand van ziekenhuisopnames voor obstructieve longziekten en stootkuren met orale corticosteroïden buiten het ziekenhuis. Van alle 5.327 patiënten hadden 2.332 patiënten in totaal 8.635 exacerbaties tijdens de follow-up periode, waarbij er een trend waarneembaar was richting het behandelen van exacerbaties buiten het ziekenhuis (p-waarde 0.003). Van de patiënten met exacerbaties werd 73% niet opgenomen in het ziekenhuis. Exacerbaties waren geassocieerd met het gebruik van hoge doseringen ICS (gecorrigeerd relatief risico [RR] 1,5; 95% betrouwbaarheidsinterval [95%BI] 1,2–1,7) en chronisch oraal corticosteroïdgebruik (gecorrigeerd RR 1,9; 95%BI 1,6–2,2). Geconcludeerd kan worden dat het gebruik van ziekenhuisopname als maat voor exacerbatie leidt tot een onderschatting van het aantal exacerbaties, omdat behandeling vaak buiten het ziekenhuis plaatsvindt.

In *Hoofdstuk 2.2* werd heropname onder patiënten met een eerdere ziekenhuisopname voor obstructieve longziekten bestudeerd. Van de 605 ICS gebruikers met een ziekenhuisopname voor obstructieve longziekten, werden 132 patiënten binnen een jaar opnieuw opgenomen in het ziekenhuis. Heropname was geassocieerd met de aanwezigheid van veel comorbiditeiten (gecorrigeerde hazard ratio [HR] 2,4; 95%BI 1,1–5,3). Patiënten met stootkuren van orale corticosteroïden alleen (gecorrigeerde HR 0,4; 95%BI 0,4–0,8) of in combinatie met antibiotica (gecorrigeerde HR 0,4; 95%BI 0,2–0,6) hadden een verlaagde kans op heropname.

Patiënten met veel chronische aandoeningen moeten daarom geïnformeerd worden over hun ziekte en vaker op consult worden gevraagd om exacerbaties in een vroeg stadium te kunnen identificeren en behandeling van de exacerbatie zo snel mogelijk te kunnen starten.

Hoofdstuk 2.3 beschrijft de veranderingen in medicatie voorafgaand aan een ziekenhuisopname. Dit werd onderzocht in een zogenoemde case-crossoverstudie met 1.481 patiënten, waarbij elke patiënt als zijn of haar eigen controle fungeerde. De periode van 3 maanden voor de ziekenhuisopname was de case periode, terwijl controlemomenten op 3, 6, 9 en 12 maanden voor de ziekenhuisopname lagen. In elk tijdsvak van 90 dagen werd het geneesmiddelgebruik geëvalueerd. Het bleek dat het gebruik van orale corticosteroïden, antibiotica en andere respiratoire geneesmiddelen redelijk stabiel was in de controleperioden, maar toenam in de 90 dagen voor ziekenhuisopname. Ziekenhuisopname was geassocieerd met het gebruik van drie of meer respiratoire geneesmiddelen (odds ratio [OR] 2,2; 95%BI 1,8–2,8), gebruik van orale corticosteroïden (OR 4,5; 95%BI 3,8–5,4) en antibioticagebruik (OR 3,1; 95%BI 2,7–3,6). Dit kan duiden op de ontwikkeling of behandeling van een exacerbatie. Daarom is er behoefte aan markers om exacerbaties in een vroeg stadium te identificeren om zo snel mogelijk met de behandeling te kunnen beginnen en mogelijk ziekenhuisopnames te voorkomen.

Het tweede deel van dit proefschrift, dat wordt beschreven in **Hoofdstuk 3**, concentreert zich op laboratorium biomarkers als maat voor de ernst van ziekte in obstructieve longziekten. Voor deze studies is gebruik gemaakt van gegevens uit UPOD (Utrecht Patient Oriented Database). UPOD is een dataplatform dat elektronisch vastgelegde laboratoriumuitslagen, klinische gegevens en gegevens over medicatiegebruik bevat van alle patiënten die een behandeling hebben ondergaan in het Universitair Medisch Centrum (UMC) Utrecht.

Omdat ontsteking een belangrijke factor is bij de ernst van ziekte in obstructieve longziekten, werd in *Hoofdstuk 3.1* het aantal neutrofielen en eosinofielen in het perifere bloed bestudeerd. In een patient-controle onderzoek werden 143 patiënten met een ziekenhuisopname voor obstructieve longziekten geïdentificeerd en een zelfde aantal controles die onder poliklinische behandeling van de longarts waren, maar niet werden opgenomen in het ziekenhuis. Ziekenhuisopname was geassocieerd met zowel neutrofilie (een hoger aantal neutrofielen in het bloed dan het referentiegebied, gecorrigeerde OR 4,3; 95%BI 2,2–8,5) en eosinofilie

(gecorrigeerde OR 2,6; 95%BI 1,1–6,2). Wanneer onderscheid gemaakt werd tussen astma en COPD, was de associatie met eosinofilie alleen aanwezig bij astmapatiënten en niet bij COPD patiënten. Dat is geruststellend, omdat de associatie met eosinofilie bij astmapatiënten en neutrofilie bij COPD patiënten bekend is in de literatuur. Deze resultaten suggereren dat het neutrofielen- en eosinofielenaantal gebruikt kunnen worden als marker voor de ernst van ziekte in patiënten met obstructieve longziekten. Neutrofilie wordt bij astmapatiënten vaak geweten aan het corticosteroïdgebruik door deze patiënten, omdat gesuggereerd wordt dat corticosteroïden de apoptose van neutrofielen zouden remmen. De gevonden associaties in deze studie werden gecorrigeerd voor leeftijd, geslacht, corticosteroïdgebruik en longfunctie. Daarom draagt deze studie bij aan de reeds bestaande vermoedens dat neutrofilie onder patiënten met DTA niet alleen wordt veroorzaakt door corticosteroïdgebruik, maar een inflammatoir kenmerk is van dit fenotype. Het effect van corticosteroïden op neutrofielen wordt nader onderzocht in *Hoofdstuk 4.2*.

Alhoewel neutrofilie geassocieerd lijkt te zijn met de ernst van ziekte, blijft het de vraag of deze neutrofielen een andere activatiestatus hebben in patiënten met DTA in vergelijking met patiënten met een mildere vorm van astma. Daarom werden in *Hoofdstuk 3.2* 17 DTA patiënten, 13 non-DTA patiënten en 19 gezonde vrijwilligers vergeleken met betrekking tot het aantal en de morfologie van neutrofielen als mogelijke biomarker om astmafenotypes te kunnen onderscheiden. Astmapatiënten zonder acute infectie en met een hematologische bloedafname in de klinische praktijk werden geïncludeerd in de studie. Het neutrofielenaantal en de neutrofielenmorfologie konden astmapatiënten onderscheiden van gezonde vrijwilligers. Echter, wanneer DTA patiënten werden vergeleken met niet-DTA patiënten, bleek dat DTA beter kon worden onderscheiden van niet-DTA met behulp van neutrofielenmorfologie (OR 8,0; 95%BI 1,5–42,0) dan met het aantal neutrofielen in het bloed (OR 4,0; 95%BI 0,8–21,0). Met behulp van UPOD kunnen biomarkerstudies op grotere schaal worden uitgevoerd, omdat de morfologieparameters op een automatische manier worden gemeten en opgeslagen.

De diagnose DTA wordt op basis van een aantal kenmerken gesteld, zoals gedefinieerd door de 'American Thoracic Society' (ATS). Een van deze kenmerken is geneesmiddelgebruik, maar er is ook informatie nodig met betrekking tot symptomen, longfunctie en controle van de ziekte. Deze kenmerken zijn niet altijd voor handen in farmaco-epidemiologische studies. Daarom werd in *Hoofdstuk*

3.3 onderzocht of montelukast een makkelijk meetbare marker voor de ernst van ziekte kan zijn, aangezien montelukast is de meeste Europese landen - waaronder Nederland- wordt voorgeschreven aan astmapatiënten die onvoldoende controle van hun ziekte bereiken met de meer gangbare medicatie. Astmapatiënten zonder acute infectie en met een hematologische bloedafname in de klinische praktijk werden geïncludeerd in de studie. De studiepopulatie werd verdeeld in 20 montelukastgebruikers en 29 niet-montelukastgebruikers en wederom werd het neutrofielenaantal en de neutrofielenmorfologie bestudeerd. Zowel het neutrofielenaantal (OR 1,5; 95%BI 0,4–6,0) als de morfologie (OR 2,1; 95%BI 0,6–7,4) konden montelukastgebruikers onvoldoende onderscheiden van niet-montelukastgebruikers. Daarom zijn dezelfde patiënten ook ingedeeld in DTA en non-DTA door middel van klinische evaluatie. Hierbij werden vergelijkbare resultaten gevonden als in *Hoofdstuk 3.2* met een OR van 2,3 (95%BI 0,6–8,7) voor het neutrofielenaantal en 4,0 (95%BI 1,2–14,0) voor de neutrofielenmorfologie. Van alle 20 montelukastgebruikers hadden 17 patiënten DTA. Dit geeft een positief voorspellende waarde van 85,0% voor montelukastgebruik. Geconcludeerd kan worden dat montelukast een indicator, maar geen ideale marker is voor de ernst van ziekte onder astmapatiënten in geautomatiseerde databases.

Het derde deel van dit proefschrift beschrijft in **Hoofdstuk 4** een aantal methodologische aspecten waarmee rekening gehouden moet worden bij het uitvoeren van biomarkerstudies. Het doel van de studie in *Hoofdstuk 4.1* was om de ernst van ziekte onder patiënten met respiratoire aandoeningen te evalueren en te onderzoeken in hoeverre in de dagelijkse klinische praktijk getest wordt op de ernst van ziekte. Hiertoe zijn alle patiënten van de polikliniek longziekten in het UMC Utrecht in de periode 2005–2007 gevolgd. Het bleek dat er elk jaar ongeveer 3.000 patiënten contact hadden met de polikliniek; in totaal bezochten 5.356 individuele patiënten de polikliniek longziekten in de periode 2005–2007. Van deze patiënten kwam elk jaar gemiddeld 12.2% op de EHBO en werd gemiddeld 3.6% van de patiënten opgenomen voor obstructieve longziekten. Hematologische bloedmonsters en longfunctiemetingen werden veel vaker vlak voor opname uitgevoerd. In de 15 dagen voor opname was er een vijfvoudig verhoogde kans op hematologische bloedmonsters en longfunctiemetingen ten opzichte van patiënten zonder ziekenhuisopname (gecorrigeerde OR 5,5; 95%BI 3,2–9,3 en 5,4; 95%BI 2,9–10,0 respectievelijk). Hieruit kan worden geconcludeerd dat het merendeel van de patiënten op de polikliniek longziekten goed onder controle is. Het testen op de ernst van ziekte met bloedmonsters en longfuncties wordt selectief uitgevoerd bij de

meer ernstig ziekte patiënten. Bij het uitvoeren van biomarkerstudies met gebruik van gegevens uit de dagelijkse praktijk moet daarom rekening worden gehouden met 'testing bias'.

Bias in biomarkerstudies kan ook worden geïntroduceerd als de maat voor de ernst van ziekte zelf geassocieerd is met het medicatiegebruik. In de klinische praktijk blijkt dat patiënten met systemisch corticosteroïdgebruik vaker een hoog neutrofielenaantal hebben. Daarom werd in *Hoofdstuk 4.2* bestudeerd wat het effect is van systemisch corticosteroïdgebruik op het neutrofielenaantal in het perifere bloed. In deze studie werden alle volwassen patiënten geïncludeerd die in 2005 in het UMC Utrecht werden opgenomen en minimaal twee bloedmonsters tijdens opname hadden, onafhankelijk van de diagnose. Hierbij werden 809 corticosteroïdgebruikers vergeleken met 2.658 niet-corticosteroïdgebruikers. De neutrofielenaantallen waren vergelijkbaar ten tijde van ziekenhuisopname ($8{,}2\times10^9$/l voor corticosteroïdgebruikers en $8{,}0\times10^9$/l voor niet-gebruikers). Een overall analyse liet een zwakke associatie zien tussen corticosteroïdgebruik en een toename in het neutrofielenaantal (RR 1,3; 95%BI 1,1–1,5). Echter, dit is het gesommeerde effect van meerdere factoren naast het mogelijke effect van corticosteroïden, zoals diagnose, de ernst van ziekte, dosis en type corticosteroïd en het tijdsvlak waarin het effect is bestudeerd. Binnen diagnostische subgroepen was er geen associatie waarneembaar tussen het gebruik van corticosteroïden en het neutrofielenaantal. Er was geen dosis-responsrelatie, geen effect van de tijd tussen de twee bloedmonsters van patiënten en geen effect van de anti-inflammatoire/natriumbehoudende potentie van de verschillende corticosteroïden. De geobserveerde neutrofilie in patiënten met corticosteroïdgebruik is daarom waarschijnlijk gerelateerd aan de onderliggende ziekte, en niet aan het corticosteroïdgebruik zelf.

Zoals in *Hoofdstuk 4.1* al bleek, is het aanvragen van laboratoriumbepalingen niet willekeurig verdeeld in de patiëntenpopulatie. In *Hoofdstuk 4.2* is al gewerkt met bepalingen met betrekking tot het neutrofielenaantal. Van belang is, of deze tests wel of niet gericht zijn aangevraagd. In *Hoofdstuk 4.3* werd 'testing bias' bij het aanvragen van het neutrofielenaantal in de dagelijkse klinische praktijk bestudeerd aan de hand van aangevraagde en niet-aangevraagde laboratoriumbepalingen. In het UMC Utrecht worden de hematologische bepalingen uitgevoerd met Cell-Dyn Sapphires hematocytometers. Een kenmerk van dit apparaat is dat het automatisch alle hematologische bepalingen uitvoert bij aanvraag van een of meerdere van deze bepalingen, onafhankelijk van het feit of de bepaling door de arts is aangevraagd of niet. Ook de niet-aangevraagde bepalingen worden opgeslagen in de UPOD

database. De aanwezigheid van 'testing bias' kan zodoende worden gestudeerd door aangevraagde en niet-aangevraagde bepalingen met elkaar te vergelijken. In totaal werden 567 patiënten met aangevraagde neutrofielenaantallen vergeleken met 1.439 patiënten met niet-aangevraagde neutrofielenaantallen. Alle patiënten waren tijdens de studieperiode in 2005 opgenomen in het ziekenhuis. Het neutrofielenaantal ten tijde van ziekenhuisopname verschilde met een gemiddelde van $7{,}4\times10^9$/l voor aangevraagde en $8{,}3\times10^9$/l voor niet-aangevraagde aantallen (p-waarde < 0,001). Dit verschil kon voor 83,2% verklaard worden door de onderliggende ziekte, in dit geval cardiovasculaire ziekte, en voor 4,5% door corticosteroïdgebruik. Hieruit kan worden afgeleid dat aanvragen voor het aantal neutrofielen in de klinische praktijk geassocieerd is met de onderliggende aandoening. Dit kan de resultaten verstoren. Het aantal patiënten met een bepaalde ziekte en de richtlijn voor het aanvragen van laboratoriumbepalingen kan variëren tussen ziekenhuizen, maar 'testing bias' is in alle centra aanwezig en moet worden bestudeerd om hiervoor te kunnen corrigeren in epidemiologische studies die gebruik maken van routinematig verkregen laboratoriumbepalingen.

In **Hoofdstuk 5** wordt dit proefschrift besloten door de resultaten van de individuele studies in een breder perspectief te plaatsen. Uit de studies in dit proefschrift kan worden geconcludeerd dat de identificatie en evaluatie van maten voor de ernst van ziekte essentieel zijn, enerzijds voor de klinische praktijk, en anderzijds voor epidemiologisch onderzoek om de ernst van ziekte te kunnen meten en hiervoor te kunnen corrigeren. Prospectieve replicatiestudies zijn nodig om de toegevoegde waarde van biomarkers bij het bepalen van de ernst van ziekte in de klinische praktijk te bewijzen. Studies die de (moleculaire) klinische praktijk, klinische chemie en farmaco-epidemiologie samenbrengen, voegen diagnostische markers toe aan de multidisciplinaire gezondheidszorg die nodig is voor patiënten met ernstige obstructieve longziekten. Dit hoofdstuk wordt afgesloten met aanbevelingen voor de klinische praktijk en voor toekomstig onderzoek.

chapter 7

Dankwoord
List of co-authors
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About the author

Dankwoord

Met dit dankwoord is een einde gekomen aan mijn AIO-periode. Op zo'n moment wordt er van je gevraagd om de afgelopen jaren te omschrijven in een notendop. Als ik hierover nadenk, schiet de uitdrukking "bloed, zweet, en tranen" door mijn hoofd. Bloed voor de experimenten en voor de referentiegroep van de studies die besproken zijn in Hoofdstuk 3.2 en 3.3 waarbij ik natuurlijk controlepersoon nummer 1 was. Zweet van het harde werken en af en toe werd er ook een traantje weggepinkt na de zoveelste teleurstelling. Mijn eerste artikel werd op 28 april 2009 gepubliceerd, in de zomer van datzelfde jaar was mijn proefschrift af. Ik heb erg veel geleerd in deze periode. Mensen die niet klimmen of klettersteigen – het grootste gedeelte van de mensheid – denken vaak dat klimmers een soort adrenalinejunkies zijn. Maar tijdens de gang naar de top is er vaak weinig dat te maken heeft met een adrenalineshot. Het is vaak afzien en doorzetten (Jon Krakauer in *Into thin air*). Zo is het met promoveren eigenlijk ook. Er zijn perioden van genieten, maar er zijn ook lange perioden van afzien, tanden op elkaar en doorzetten. Maar als je dan uiteindelijk het doel hebt bereikt, geeft dat zo'n goed gevoel. Het proefschrift is daar, de top is bereikt! Ja, het was het waard!!

Een proefschrift ontstaat niet vanzelf, op deze plaats wil ik iedereen bedanken die mij, ieder op eigen wijze, heeft geholpen met het bereiken van dit resultaat. In het bijzonder wil ik de volgende mensen bedanken.

Bert Leufkens, jaren geleden was jij degene die mij heeft laten kennismaken met de epidemiologie tijdens mijn Farmacie-opleiding. Tijdens die vier weken onstond er bij mij een gevoel dat nooit meer is weggegaan. Tijdens de wetenschappelijke stage kon ik verder proeven aan de epidemiologie. Dat is dusdanig goed bevallen, dat dit me heeft doen besluiten me verder in het het onderzoek te willen verdiepen. Ik

ben trots dat jij, degene die aan de wieg heeft gestaan van mijn interesse voor de epidemiologie, nu ook mijn promotor wilde zijn.

Wouter van Solinge, je beschreef me wel eens als een jonge hond die de halsband om moest doen en netjes aan de lijn moest lopen. Vaak verschilden wij van mening en inzicht en dat gaf altijd aanleiding tot interessante en leerzame discussies. Jij gaf me de mogelijkheid om UPOD te helpen ontwikkelen en hiermee studies te doen, een database van grote waarde! Bedankt voor de prettige samenwerking.

Madelon Bracke, je gaf me de mogelijkheid om een Master in Epidemiology te combineren met mijn promotie. Jouw enthousiasme en positieve instelling heeft me altijd geboeid en gaf me veel energie. Bedankt! Jouw vergelijking van ons project met een bol wol was af en toe erg toepasselijk. Jammer dat we dit project niet samen konden afmaken, maar vanaf de zijlijn ben je me altijd blijven aanmoedigen.

René Schweizer, een arts was onmisbaar in het promotie-team. Bedankt dat jij de brug wilde zijn tussen de epidemiologie en de klinische praktijk. Daar heb ik veel van geleerd.

Patrick Souverein, altijd wilde je meedenken over alles wat de wetenschap behelst, en altijd las je vol enthousiasme mijn manuscripten, ook al was je niet altijd co-auteur. Je bent gaandeweg steeds meer betrokken geraakt bij mijn projecten en uiteindelijk ook officieel als co-promotor. Ik heb veel gehad aan onze brainstormsessies en onze discussies over de opzet en analyse van studies. Bedankt!

Jan-Willem Lammers, bedankt voor het feit dat er altijd een gaatje in jouw agenda leek te zijn, net als ik het even nodig had. Om te filosoferen, de opzet van een studie te bespreken of om mee te denken als clinicus in mijn studies.

Hanneke den Breeijen, jij begon halverwege mijn promotietijd als UPOD manager. Dat heb je geweten! Bedankt voor het data verzamelen en meedenken, maar vooral voor de coaching. Altijd had je tijd om even een hart onder de riem te steken, we hadden eindeloze discussies hoe we dit proefschrift in een goed format konden gieten.

Graag wil ik ook de leden van de beoordelingscommissie, prof. dr. Toine Egberts, prof. dr. Marcel Bouvy, prof. dr. Gert Folkerts, prof. dr. Leo Koenderman, en prof. dr. Jan Lindemans hartelijk bedanken voor het doornemen van het manuscript.

Bij het realiseren van studies met UPOD zijn altijd veel mensen betrokken. Maarten ten Berg, die betrokken was bij UPOD vanaf dag 1. Ludi van Dun van Abbott, die altijd klaar stond om te helpen bij de verzameling en analyse van de FCS files. Leslie Beks, van de directie informatievoorziening & financiën, afdeling zorginformatie & systemen in het UMC Utrecht, altijd bereid om data te leveren die nog niet in UPOD waren opgenomen maar wel mijn nieuwsgierigheid wekte. Pieter Zanen, die het altijd leuk vond als ik zijn longfunctiedatabase wilde gebruiken. Albert Huisman en Jolanda Kolvers die me altijd wilden helpen met de gekste dingen en vele anderen.

Vaak wordt vergeten hoe belangrijk goede secretariële ondersteuning is. Mijn dank gaat daarom ook uit naar alle betrokken secretariaten, Addy, Ineke, Marije en Suzanne van farmaco-epidemiologie & farmacotherapie; Carin, Irma, Joukje en Sonja van klinische chemie en hematologie; en Berry en Mariken van longziekten voor de hulp en het stroomlijnen van de (niet altijd wetenschappelijke) werkzaamheden. Berry en Mariken, bedankt voor de goede zorgen tijdens de maanden van het statusonderzoek. Jullie hebben me zeer welkom laten voelen bij de afdeling longziekten. Berry, het was altijd leuk om onze verhalen te delen en samen ontzettend veel te lachen. Ik ga onze gezellige lunches missen!

Ook wil ik alle mensen bedanken van de afdelingen longziekten en klinische chemie & hematologie van het UMC Utrecht, van de afdeling farmaco-epidemiologie & farmacotherapie, en alle andere mensen die meegewerkt hebben aan dit onderzoek, aan de zijlijn, faciliterend of als proefpersoon. Zonder jullie was dit allemaal niet gelukt. In het bijzonder veel dank aan Isabelle Hubeek voor de mooie foto's van de neutrofiel en mijn kamergenoten: Stefan, Pieter en Kim, gevolgd door Bas, Grace en Francisco.

Francis te Nijenhuis, bedankt dat jij mijn proefschrift wilde vormgeven. Dankzij jouw meedenken en creatieve inspiratie is het een fantastisch visitekaartje geworden. Ik ben er trots op!

Familie en vrienden, bedankt voor jullie steun bij hoogte- en dieptepunten tijdens de afgelopen periode.

Dana en Ido, jullie daagden me tijdens talrijke en afwisselende gezamenlijke vrije tijdsbestedingen uit om op een andere manier naar mijn studies te kijken en over

mijn proefschrift na te denken. Daarom ben ik zo blij dat jullie mij tijdens de verdediging willen bijstaan als paranimfen.

Tim en Marleen, dank voor jullie begrip dat ik met name in de laatste fase van het schrijven van dit proefschrift niet altijd mee kon doen aan de gezelligheid beneden. Ook jullie hebben me energie gegeven om dit project af te ronden.

Lieve mam, dankzij jouw inzet en toewijding kreeg ik alle vrijheid om mijzelf te ontwikkelen en ben ik zover gekomen. Jij bent er altijd voor me. Meer dan eens heb ik me verbaasd over hoe geduldig jij mijn verhalen wilde blijven aanhoren en tips bleef geven hoe ik een project zou kunnen aanpakken. Zonder jou had ik dit niet kunnen volbrengen en daar ben ik je heel dankbaar voor. Ik waardeer jouw inzet, doorzettingsvermogen en leefstijl enorm en heb er veel van geleerd voor mijn eigen leven. Mam, ontzettend bedankt!!

Lieve Robert, ik heb het beste voor het laatst bewaard. Jij bent mijn steun en toeverlaat, mijn soulmate. Altijd wil je mijn verhalen aanhoren en meedenken over hoe het anders kan. Samen deelden we de teleurstelling van manuscript afwijzingen en vierden we de acceptaties met champagne. Jouw onvoorwaardelijke steun geeft me veel energie. Je gaf me alle ruimte om keihard aan mijn proefschrift te werken, zette jezelf op een lagere prioriteit en trok me af en toe ook aan de haren mee naar buiten voor de broodnodige ontspanning. Een man die zich zo in een ander kan verplaatsen en mij zo naadloos kan aanvullen is zeer zeldzaam, ik vind het een voorrecht met jou te mogen samenleven. Dank je wel. Nu breekt *ONZE* tijd weer aan, we hebben veel in te halen...

Karin

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Karin Velthove was born on the 26th of September 1981 in Rotterdam, the Netherlands. She completed secondary school in 1999 at the Comenius College in Capelle aan den IJssel. Subsequently, she started studying pharmacy at the Utrecht University. In 2005 she obtained her Pharmacist degree with honours.

The same year she started working on the research described in this thesis at the Division of Pharmacoepidemiology and Pharmacotherapy of the Utrecht University, in affiliation with the Department of Clinical Chemistry and Haematology and the Department of Respiratory Medicine of the University Medical Centre Utrecht. During her work, she obtained her Master in Science degree in Epidemiology at the EMGO institute of the VU University in Amsterdam, with flawless results, and she participated in an international cooperation with Abbott Diagnostics in St. Clara, CA, USA.

Since October 2009 she holds a position as a medical advisor at Sanquin Blood Supply Foundation.