Design, conduct and analysis of surveys on work-related asthma

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Abstract

Surveys on work-related asthma serve public health investigation, research on exposure-response relations, screening for pre-clinical disease, and demonstrations of effectiveness of interventions. Hypotheses dictate survey design, which include cross-sectional, case-control, cohort, and intervention studies. Tools for characterizing medical risk factors and outcomes include questionnaires, spirometry, tests of bronchial hyperreactivity, exhaled indices, induced sputum, immunological tests, and nasal inflammatory indices. An important component of surveys is exposure assessment to compare a population to existing literature and other surveys with attention to exposure level, range, and variability among workers. Allergen exposures are challenging to characterize with respect to peak exposures and evolving immunochemical measurement methods. Exposure assessment strategies are developing rapidly for analysis of exposure-response relationships, whether for sensitization to allergens or for respiratory symptoms or diagnoses. Power calculations should guide decisions about whether to implement surveys. Research needs include surveys of populations with irritant or neutrophilic asthma and populations in damp buildings. The relevance of dermal exposure to sensitizers requires examination as a risk factor for asthma. New causes of work-related asthma may be identified by surveying industries with excess asthma in population-based studies that do not have recognized causes of asthma.

Introduction

Our knowledge of new asthmagens, risk factors, and primary prevention of occupational asthma (OA) depends largely on epidemiological surveys of workforces and populations. Such surveys complement clinical investigation for diagnoses in individuals, laboratory investigation for mechanisms, and animal experiments for assessing biological plausibility. We consider surveys as a research tool when sentinel cases in a workforce prompt public health investigation; when primary prevention requires understanding of exposure-response relations or work and worker risk factors; and when secondary prevention requires attempts to identify pre-clinical disease. In addition, population surveys can be used in medical surveillance to show trends and the effectiveness of interventions.

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These motivations for research surveys dictate the research questions. In the case of sentinel-event follow-back to workplace populations, the questions may be whether an excess of respiratory disease consistent with asthma exists and, if so, whether an occupational cause is apparent. In the case of primary prevention research, work-related risk factors, such as process and exposures, are examined for their relations to respiratory health and disease prevalence or incidence. In the case of secondary prevention studies, worker attributes, such as biomarkers of immunological responses, are examined for their relations to exposures or clinical outcomes either in cross-sectional or longitudinal designs. Medical surveillance for intervention effectiveness is often considered a public health endeavor, rather than research, since comparison groups are rarely feasible or even ethical. Nonetheless, intervention outcome can be powerful evidence of causality when changing a particular exposure results in lessening of asthma incidence or prevalence.

In turn, research questions dictate survey design, along with feasibility of implementation (Tab. 1). Regardless of survey design, it is important to capture exposure status at the time of disease onset. The simplest and cheapest studies are commonly cross-sectional surveys of a workforce, but the findings are limited to associations, which may or may not be causal. To answer the questions of whether excess asthma is present requires a comparison group. External comparisons might be another workforce or population-based estimates. In large workforces, internal comparisons may be possible if the workforce has a range of exposure to implicated processes or agents. If the workforce suspected of having an asthma risk is large and has many affected workers, case-control studies may be efficient in determining potential risk factors, but again, only associations are found, and their interpretation depends on biological plausibility, evaluation of potential confounders, attempts to establish work-related patterns, and replication in other exposed populations.

To address questions of incidence in relation to occupational risk, longitudinal cohort studies are advantageous, but are expensive and difficult. Longitudinal studies avoid the temporal confusion of whether environmental conditions precede the

Table 1. Different survey design options and some typical characteristics, assuming appropriate implementation

Design	Frequency of use	Strength of scientific evidence	Costs
Cross-sectional	++++	+	+
Case-control	++	++	+
Cohort	+	+++	+++
Intervention study	+	++++	++++

health outcome. However, employee turnover, particularly with the healthy worker effect commonly found in populations at risk of OA, may preclude obtaining follow-up on affected individuals. Similarly, intervention effectiveness studies are difficult unless subclinical biomarkers of health effect are available, and comparison populations for longitudinal follow-up are seldom feasible or ethical (unless interventions are being compared).

For some diseases, such as cancer, follow-back of time-space clusters in a workplace is inherently limited by power issues and bias. In contrast, work-related symptoms of asthma as an outcome warrant follow-back much of the time. Although asthma is a prevalent disease affecting about 10% of the population, adult-onset asthma has an incidence in the range of 1.2 to 4.0 per thousand person-years [1, 2] based on questionnaire responses. Obtaining incidence density estimates over employment in a workforce can show whether excess incidence is likely, despite neglecting workers who may have left because of asthma before a cross-sectional survey.

Before a study design is chosen

Any survey always starts with a research question, translated into a hypothesis. The research questions can have a wide range of backgrounds. Employers, workers or occupational health specialists may have questions related to health risks. These questions may have been initiated by observations from the scientific literature. The question then arises as to whether observations made "in a population X with exposure to agent Y, with specific health effects Z" are of relevance for another population with a similar exposure pattern. Research questions may also be triggered by sentinel cases or a series of cases occurring in a company (place) or within a narrow time window (time), which require a more thorough survey to establish whether an excess risk exists and whether determinants in the work environment may have played a role. An example is the occurrence of OA in enzyme-exposed workers in the detergent industry in the 1970s. Reports of such cases to the occupational physician were followed by systematic surveys. Sentinel case reporting in the U.S. to state health departments have resulted in new asthmagens being established, such as 3-amino-5-mercapto-1, 2, 4-triazole (AMT) in the pesticide industry [3], and new settings for asthma risk, such as damp buildings [4, 5].

Research questions refer to what epidemiologists call an occurrence relation. An occurrence relation describes disease occurrence in relation to known or suspected determinants. Occurrence relations can be simple and straightforward, as in the case of a respiratory irritant causing the reactive airways dysfunction syndrome. Here, symptoms occur immediately after exposure and the relationship manifests itself easily. In the case of immune-mediated asthma, the natural history is a com-

plex interplay of exposure and sensitization followed by inflammatory responses. These relations are modified by individual susceptibility, and the combination of all relevant variables results in complex time patterns of disease. These elements of the occurrence relation have to be considered when the research question is translated into a study design and practical study plan. The study plan contains the design of the study, what is being measured and how, a power analysis, and a description of the planned data analysis.

Design options

Cross-sectional studies

Two epidemiological designs usually lead to results relatively rapidly: cross-sectional surveys and case-control studies. Many cross-sectional surveys on asthma have been conducted. They consist of measurement of the disease (asthma) prevalence using questionnaires and sometimes medical tests or other measures of disease presence. They give a rapid and relatively cost-effective indication of the presence of asthma, although assessing the work-relatedness of symptoms and signs may require a more thorough clinical evaluation in the individual worker. Relating excess risk in subgroups to particular exposures, processes, or work indices is another approach to determining work-relatedness.

The disadvantage of this design is that cross-sectional surveys are extremely sensitive to the healthy worker effect. A survey by Peretz et al. [6] among bakers and workers from related industries with exposure to flour and α-amylase showed that strong differences existed in the prevalence of atopy (against common allergens) and atopic responses to work-environment allergens. Workers from flour milling industries were less often atopic than bakers. It is most likely that these differences are to some extent associated with differences in health-related selection in and out of the workforce. This type of selection out of the workforce may be associated with flattening of exposure-response relationships between exposure to allergens and sensitization, symptoms, and medication use [6, 7], although development of tolerance may play a role. Few studies exist that have explicitly studied selection out of the workforce for OA, but a range of studies indicate that health selection has taken place. It is expected to operate strongly in the case of immune-mediated OA because affected workers often observe a direct relationship between exposure and their symptoms. Although there is little doubt that this phenomenon plays a crucial role in creating healthier current workforces for cross-sectional studies, its magnitude has yet to be established in most situations. The healthy worker effect leads to biased results from cross-sectional studies. The bias may affect prevalence estimates, as well as measures of association between asthma, sensitization or other outcome, and determinants under study.

Case-control studies

Case-control studies are not as often applied, but they may be used within (nested) a cross-sectional study, based on prevalent cases, as a cost-effective and efficient follow-up design. Usually all cases identified in the cross-sectional survey and a sample of the controls receive a more detailed evaluation. An important role for case-control studies is in the evaluation of potential health clusters of asthma. Prevalent studies are likely to have cases and controls that do not adequately represent either group, and this is especially true for the cases, considering the healthy worker effect. Incident case-control studies can be nested within longitudinal studies.

Cohort and intervention studies

A cohort is a group of persons who are followed or traced over a period of time. The concept of a cohort comes from the Roman Empire where it was common to follow the mortality experience of cohorts (the Latin word '*cohors*' refers to one tenth of a legion) of soldiers over time to keep track of the fighting potential of the army. Cohort studies have been shown to be extremely powerful in OA research.

A simple measure to describe disease occurrence in a cohort is the incidence, the number of cases with disease divided by the number of individuals at risk at the beginning of follow-up. This measure of disease is sensitive to loss to follow-up and competing causes of disease or mortality. A better measure is the incidence rate. An incidence rate is the number of cases who developed the disease of interest during follow-up in a cohort of initially disease-free individuals, divided by the accumulated person-time (usually expressed as person-years). Cohort studies require calculation of person-time of follow-up until development of disease or loss to follow-up. This calculation can be extremely complex when different time-related variables are involved, such as age, birth cohort, and exposure duration, although most studies published so far have been relatively straight forward with regard to these aspects. The Life Table Analysis System software designed by the National Institute for Occupational Safety and Health (NIOSH), which was developed for mortality studies, exists to make these calculations. This software can be used for this type of study as well by manipulating the input structure of the data. An example of a well-conducted study in which this approach was used is the follow-up of laboratory animal workers by Elliot et al. [8]. A total of 603 workers contributed 2527 person-years over a 12.3-year period. The 12-year incidence rates of laboratory animal allergy (LAA) symptoms and LAA for all workers were 2.26 (95% CI 1.61–2.91) and 1.32 (95% CI 0.76–1.87) per 100 person-years, respectively. Higher rate ratios were seen with increasing reported hours of exposure to tasks that required working with animal cages or with many animals at one time. The most common symptoms were related to rhinitis rather than to asthma. If incidence rates are available for an

exposed and a control group, incidence rate ratios or relative risks can be calculated as a measure of association between exposure and disease.

Several approaches have been used in the design of cohort studies for OA. Most published cohort studies have been of workforces in one company, one industry, or one job category. Cohort studies among individuals naïve towards their occupational exposure, often among apprentices, have given good insight into the determinants of OA and allergy [9]. Apprentices become exposed to specific agents for the first time in their lives because some occupational exposures may be exceptionally rare in the general environment. This is true for spray painters exposed to isocyanate monomers and oligomers. Domestic or general environmental exposure to these agents is usually absent. Alternatively, some allergens are not exclusively found in the work environment, but exposure in the work environment is orders of magnitude higher than in other environments. This is likely the case for allergens like latex and wheat allergens. Studies of apprentices are extremely powerful, but studies among workers, starting with a cross-sectional survey and following disease-free workers with only a brief exposure history, have comparable potential as demonstrated in studies of the baking industry [10]. Cohort studies for non-allergic asthma in relation to irritant or bioaerosol exposures are in their infancy.

Cohort studies for asthma usually involve an extensive baseline survey and regular follow-up surveys. The incidence rate of development of sensitization is relatively high – between 1 and 10 cases per 100 person years of follow-up – and cases may occur within the first few months of exposure [11–13]. The frequency of follow-up surveys varies in available studies. A reasonable follow-up frequency is one survey per year, although studies of mechanistic aspects of development of sensitization may require more intensive follow-up [14]. Unfortunately, follow-up time in most studies has been relatively short, not more than a few years. Studies with more than 5 years of follow-up [12, 15] show that asthma or sensitization incidence remains high after the first few years of follow-up. This finding is at odds with the suggestion that risk declines rapidly after the first month of exposure. Because most studies have been relatively short, little is known about the predictive value of determinants of asthma and allergy over longer periods of time. Most published cohort studies are prospective, but retrospective studies exist [15]. Retrospective studies make use of information collected earlier, and in the example of Kruize et al. [15], pre-employment testing information was available for a population of laboratory animal workers.

Some cohort studies have not focused on incidence, but considered change in a physiological parameter like lung function over time. Few such studies have been conducted among populations with a high risk for developing OA. The study by Portengen et al. [16] showed that the decline in lung function over a 3-year follow-up period was strongest among sensitized and allergen-exposed individuals compared to non-exposed or non-sensitized individuals. This type of cohort study is often referred to as a longitudinal or repeated measurement study.

Health outcome tools

Questionnaires

Standard questions about respiratory symptoms and physician diagnoses should be selected from validated instruments, with a view to the purpose of the survey [17, 18]. If the aim is to identify possible cases, then questions of high sensitivity are needed. In an analytical epidemiological study of risk factors, questions with high specificity for asthma should be used. In emerging issues, such as asthma among occupants of damp buildings, analytical comparisons can be made with other populations if identical questions are used.

For asthma, the long-standing American Thoracic Society-Division of Lung Disease (ATS/DLD) questions [19], designed for study of chronic bronchitis and emphysema, have largely been replaced with questions used in the European Community Respiratory Health Study (ECRHS), which were derived from the International Union Against Tuberculosis and Lung Disease questionnaire [20]. A combination of symptoms or weighted score can predict airways hyperreactivity, clinical diagnosis by an expert panel, incident asthma, and other asthma outcomes in relation to risk factors for asthma [21–24]. Regardless of questions, symptoms-based definitions of asthma result in classifying a much higher proportion of a worker population as having asthma compared to the self-report of physician-diagnosed asthma. Questions aimed at establishing associations between symptom patterns and work-related exposures are an important issue in questionnaires focused on OA. Work-related symptom patterns include predominance at work, exacerbation during spills, or a delayed immunological response after working hours or during the night. Similarly, symptoms may progress over the work week or improve during holidays or weekends.

In the United States, questions from the Third National Health and Nutrition Examination Survey [25], the National Health Interview Survey [26], or the Behavioral Risk Factor Surveillance Survey [27] allow external comparisons regarding symptom and diagnosed asthma prevalences in a working population in comparison to national or state-based prevalences. In Europe, comparisons of symptom and diagnosis prevalences in workforces can be compared with ECRHS prevalences. Such comparisons can establish that excesses of asthma or chest symptoms exist in working populations suspected of occupational lung disease [5, 28, 29].

Cross-sectional questionnaire analyses can also address asthma incidence in relation to employment or process changes during employment. The ATS/DLD and ECRHS questionnaires ask persons with physician-diagnosed asthma for their age at asthma onset. This information, in conjunction with employment date and current age, allows calculation of incidence density of asthma in adulthood prior to employment, in comparison with incidence after onset of employment, both expressed as asthma diagnoses per person-months at risk. With this rate ratio comparison, employment-related incidence excesses as high as 7.5-fold have been documented in

damp buildings with high prevalences of building-related chest symptoms [5, 30]. The healthy worker effect tends to underestimate asthma incidence after employment in cross-sectional studies, so such rate ratio comparisons may be lower than would be found in a longitudinal cohort study.

All strong questionnaires need a work history module, which is usually tailored to the workforce being studied. As mentioned above, dates of employment or entry into job titles with exposure correlates can be used to calculate incidence density of asthma before and after exposure began. Work history can be linked to exposure assessment to calculate cumulative, average, or peak exposure based on job-exposure matrices. In the absence of extensive quantitative exposure assessment, questionnaire indices of exposure, such as process, job title, tasks, history of spill exposure and frequency, duration of exposure, and use of respiratory protection can be associated with respiratory health outcomes. Some exposure risks, such as spills, may never be characterized quantitatively because they are unanticipated and unmeasured events. Nonetheless, these qualitative risks may have considerable public health value in suggesting priorities for intervention and the possible importance of peak exposures. Examples include early studies of the pulp and paper industry, which has a risk of reactive airways dysfunction syndrome [31], and the isocyanate industry, in which spills were associated with asthma cases [32, 33]. In early studies within an industry, such qualitative associations may help prioritize needs for careful exposure assessment, such as in process-related risk.

An evolving area of possible exposure risk that can be partially addressed by questionnaire is dermal exposure to allergens and sensitizers. In a new waferboard factory, incident asthma-like symptoms were associated with affirmative responses to questions about skin and clothing stains from isocyanates [34]. Dermal exposure assessment is in its infancy, with attempts to estimate relative exposures with skin wipes, surface wipes, cotton glove burden of analytes [35], and adhesive tape stripping [36]. In the meantime, questions reflecting the likelihood of skin integrity, use of skin protection, stains where applicable, and dermatitis may push this field forward in relation to asthma prevention measures [37]. For another sensitizing occupational lung disease, beryllium disease, skin exposure may be an important route of sensitization [38]. The parallels with latex asthma, in which latex dermatitis may be a sensitizing event, are intriguing. Considerable animal evidence exists for sensitization *via* the dermal route for latex, beryllium, and isocyanates [39–41].

Socioeconomic implications of OA can be addressed by adding questionnaire modules pertinent to absenteeism due to respiratory disease and quality of life. Examples include SF12 and the Marks' asthma quality of life questionnaires [42, 43].

Spirometry

Although asthma attacks precipitate airways obstruction, the utility of spirometry in surveys of populations at risk of OA is limited. Between asthma attacks, asthmatics

usually have normal spirometry. Hence, cross-sectional studies of spirometry at work are seldom sensitive in identifying those with physician-diagnosed asthma or work-related asthma. In workforces at risk for asthma, bronchodilator testing of those with abnormal spirometry of an obstructive nature may differentiate asthmatic workers from those with chronic obstructive lung disease. Cross-shift and cross-week changes of spirometry are labor-intensive and also insensitive. Thus, the serial peak flow or serial spirometry that is of clinical value in documenting work-related decrements over several weeks of measurements [44] is seldom practical in a survey setting [45]. Compliance with several measurements per day on work days and days away from work is rarely obtained on a population basis, even when restricted to those with symptoms, measured bronchial hyperreactivity, or physician-diagnosed asthma.

Long-standing asthma can result in partial irreversible airflow limitation. For longitudinal cohort studies, assessment of average decrements of forced expiratory volume in one second (FEV_1) over periods of time may be of some utility, although such evaluation is more pertinent to chronic obstructive lung disease than asthma. In such studies, the detection of excess declines in FEV_1 in individuals over an interval is dependent on frequency of measurement, spirometry quality, and time of follow-up [46]. Recent NIOSH software for evaluation of longitudinal spirometry makes these analyses much easier [47].

Bronchial hyperreactivity

Methacholine or histamine challenge tests are done in field settings with abbreviated protocols. In those with chest symptoms suggestive of asthma, documented hyperreactivity is an attractive objective measure of asthma. However, in workforce populations, normal tests are frequently found in those reporting physician-diagnosed asthma. This insensitivity may reflect adequately treated asthma. One approach to objective documentation is to aggregate those with prescription asthma medication with those with documented hyperreactivity and those with bronchodilator responses on spirometry (an alternative test for reversible airways obstruction in those with abnormal spirometry) [5].

Exhaled indices

Exhaled nitric oxide (eNO) has been adopted in clinical settings as a means of monitoring control of allergic asthma. As a marker of eosinophilic inflammation, increased eNO may indicate inadequate medication or compliance. Recently, eNO has been used in general population field studies in the evaluation of asthma [48]. However, the applicability of this test among workers at risk of immunological or irritant OA in surveys of current workers has not been established [49, 50].

Some investigators have examined eNO as a marker of inflammation in relation to workplace irritant or particulate exposures, sometimes excluding known asthmatics [51, 52]. Inconsistent findings have been found for irritant and particulate exposures [53, 54] and also for biomass exposures [55, 56]. A cross-sectional study among farmers demonstrated that eNO levels were positively associated with endotoxin exposure in non-smokers and non-atopics [55]. However, in building-related asthma associated with biomass markers, eNO was not higher in those with either physician-diagnosed asthma or epidemiologically defined asthma based on symptoms [56]; however, the pathophysiology of dampness-associated asthma may not involve immunoglobulin E (IgE) and eosinophilic inflammation [5], which are associated with elevated eNO.

Exhaled breath condensate is currently being investigated in field studies as a means of identifying malondialdehyde as a biomarker of oxidative stress, inflammatory markers such as cytokines, and to establish dose for metals and solvents [57]. In a building-related asthma study, interleukin-8 in exhaled breath condensate was higher in those with physician-diagnosed asthma and with work-related respiratory symptoms compared to controls [56].

Induced sputum indices

Sputum induction for cell differential counts has been investigated in clinical settings evaluating those with OA [49]. Those with IgE-associated asthma usually have increased sputum eosinophilia with exposure. Some evidence exists for sputum neutrophilia in those with isocyanate asthma, irritant exposure as in paper mill workers [58], and microorganism exposure. Induced sputum has also been used for evaluating other inflammatory markers, such as interleukins, matrix metalloprotease-9 activity, and activated basophils by flow cytometry. Induced sputum is difficult for survey participants in field settings, particularly in those without symptoms [59]. Accordingly, survey application has been limited. An investigation of mushroom workers used spontaneous sputum induction in those with cough and no other diagnoses, rather than induced sputum in the entire population [60]. Others have induced sputum in clinical settings [61, 62].

Immunological tests

Atopy is a risk factor for asthma caused by high-molecular-weight agents in the workplace, although its predictive value is not high enough to warrant preplacement screening in the workplace. Exposure level determines the likelihood of developing asthma due to high-molecular-weight sensitizers because specific sensitization is the primary mechanism. Individual susceptibility, such as atopy, is merely

a modifying factor for exposure-response relations, but may merit characterization of atopic status in exposure-response studies. The means of assessing atopy are by questionnaire regarding history of hay fever, eczema, and asthma; by total IgE measurement in serum; by antigen-specific IgE levels for a panel of common allergens; or by skin prick tests to a similar panel of common aeroallergens.

In field surveys of populations at risk for asthma, tests of specific immunological reactivity are often an outcome variable or an intermediate variable between allergen exposure and OA. Tests for specific immunological reactivity include specific serum IgE and IgG antibody tests and skin prick tests to specific allergens. Thus, workplace surveys exist that examine reactivity to wheat allergen, α-amylase, soy antigens, rat urinary protein, snow crab antigen, latex antigens, and many others. There is no role in field studies for specific inhalation challenge to assess immunological reactivity to an inhaled allergen, since these tests must be performed in specialty clinical centers and entail risk that is unacceptable outside of a clinical diagnostic context. In other occupational lung diseases, cell-mediated immunological reactivity is an endpoint and intermediate outcome in epidemiological study. The dominant example is testing for beryllium sensitization in beryllium-exposed worker surveillance with the beryllium lymphocyte proliferation test [63]. However, such lymphocyte tests may have a role in field surveys for low-molecular-weight antigens such as isocyanates [64] if shown to play a role in pathophysiology of isocyanate asthma [65].

Nasal inflammatory indices

Work-related nasal disease is common and may be a risk factor for OA [66]. Thus, a variety of tools have been considered for characterizing nasal disease, such as nasal eNO and nasal lavage for inflammatory markers; nasal swabs for cytology; nasal inspiratory peak flow, rhinomanometry, and acoustic rhinometry for nasal resistance; and nasal morphometry. Their application in field studies has been limited because of participant reaction, invasiveness, time, and the need to establish normal values. Murgia et al. [62] demonstrated higher interleukin-6 in nasal lavage of chromium-exposed electroplating workers in comparison to controls.

Exposure assessment in occupational asthma surveys

There are several reasons to include an exposure assessment component in OA surveys. The first and most basic one is for documentation purposes. To allow meaningful comparisons with the existing literature and between different studies, it is useful to have some insight into the level, range, and variability of exposure of workers in a newly conducted survey. Results from a survey are difficult to put in context, especially when there is no background information about the exposure.

Information about specific industries or job titles is usually not sufficient because exposure levels can differ between countries for the same industry and job title. Also, the level of technological development may differ between companies, resulting in exposure differences. A basic impression of the exposure can be obtained simply by monitoring a random sample of workers and taking a limited number of repeated measurements for each worker.

Another reason to monitor exposure is the analysis of exposure-response relationships and to evaluate the effect of interventions. These two aims require more elaborate exposure assessment strategies. The analysis of exposure-response relationships for high-molecular-weight allergens and asthma has a short history and started in the early 1990s. Results from this research have shown that a range of exposure-endpoint relationships exist for OA. These studies have indicated that lowering exposure will most likely reduce the burden of disease, although a residual risk can probably not be avoided completely. There is still very little experience with intervention studies in the field of OA, and the need to improve the methodology and implement exposure studies is clearly present [67]. The most thoroughly described evidence involves latex gloves. Use of powder-free gloves is known to be associated with considerably lower exposure to latex allergens [68]. Follow-up studies in dental school student cohorts have shown that introduction of powder-free gloves is associated with disappearance of latex sensitization and asthma [69]. Some useful references exist that describe some methodological issues for intervention studies in occupational respiratory disease epidemiology. Brosseau et al. [70] describe the exposure assessment component of a pilot study of the Minnesota Wood Dust Study. In this study, an intervention was undertaken in 48 businesses (written recommendations, technical assistance, and worker training). The comparison companies received only written recommendations. Changes from baseline in dust concentration, dust control methods, and worker behavior were compared between the groups 1 year later. Workers in intervention relative to comparison businesses reported greater awareness, increases in stage of readiness, and behavioral changes consistent with dust control. The median dust concentration change in the intervention group from baseline to follow-up was 10.4% lower than the change in comparison businesses [71, 72]. Meijster et al. [73] describe results from a four-year intervention program in the baking and flour processing industry with similarly modest exposure reductions. These changes in exposure were smaller than expected and illustrate that conducting effective rigorous interventions is extremely complex. Such changes can hardly be detected without quantitative exposure measurements.

Occurrence of and variability in allergen exposure

Little information exists about how exposure to allergens in the work environment occurs. A recent study among bakery workers, who are exposed to allergens in

particulate form, has shown that workers are exposed to a high number of peaks over a work shift (Fig. 1). These peaks are usually associated with a range of different tasks. Between the peaks, exposure is extremely low. There is hardly any background exposure to particulates. More than 75% of the work shift time-weighted average exposure could be explained by peak exposures during a limited set of well-defined tasks and activities [74].

For some other high-molecular-weight allergens, like latex and rat urinary allergens in lab animal settings, detailed information is lacking, but it is likely that for allergens in particulate form, peaks are equally important. Peak patterns of exposure can easily be explained by the properties of particulates. Earlier studies among bakery workers have shown that most of the particulates are relatively large, >5 μm. Particulates of this size remain airborne for only a short period of time because of gravity. Sedimentation is rapid and occurs often in less than a minute to maximally a few minutes. Thus, exposure occurs only when bags are opened, flour is used for manual dusting of dough, and brooms or pressurized air are used for cleaning.

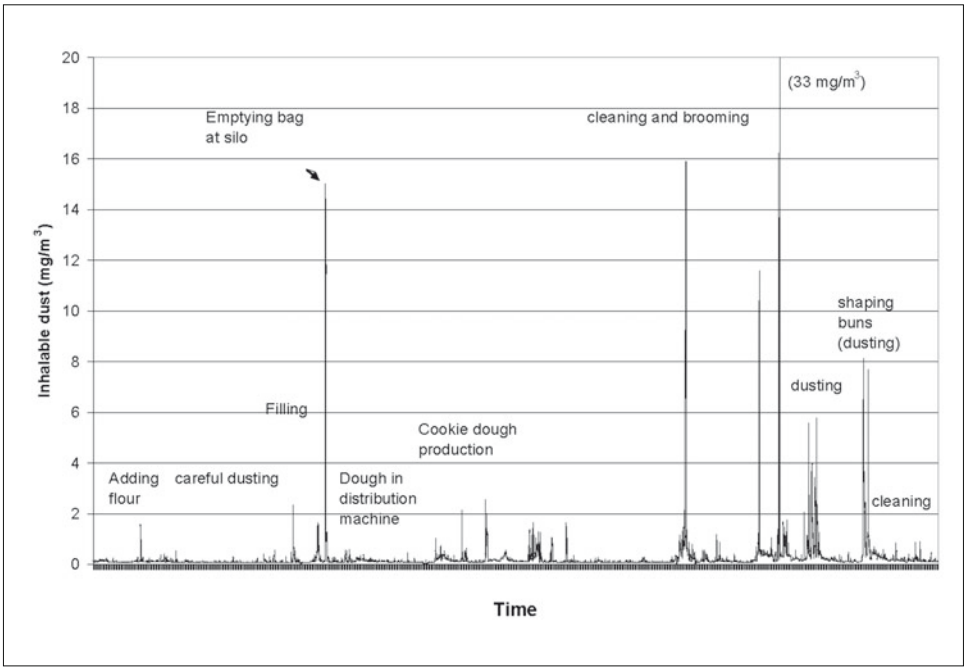

Figure 1.
Typical peak pattern of dust exposure for a bakery worker with some major tasks during the peaks described (from the study described by Meijster et al. [74], figure not published in the original paper, courtesy of the author).

For low-molecular-weight allergens, which are more often gaseous, constant low background exposure may theoretically occur, with peaks superimposed, dependent on the performance of certain tasks. However, the evidence for this is very limited. Recent studies among car spray painters, who are exposed to isocyanate monomers and oligomers in gaseous and solid (particulate) phase suggest that the exposure is often undetectably low, probably because of the presence of rigorous exposure control measures like spray-painting booths and local exhaust ventilation [75, 76]. The exposure is detectable when high exposure tasks are performed and when tasks are performed without exposure control measures: during spraying outside the booth, when spills occur, during preparation of paints, etc. So, also here, exposure occurs as a series of peaks over time.

Exposure to particulates and gases is always extremely dynamic and varies strongly over time and space. Pronk et al. [75] describe results from a large measurement series among car and industrial spray painters. For each worker, repeated measurements were performed (Fig. 2). These results show that car spray painters

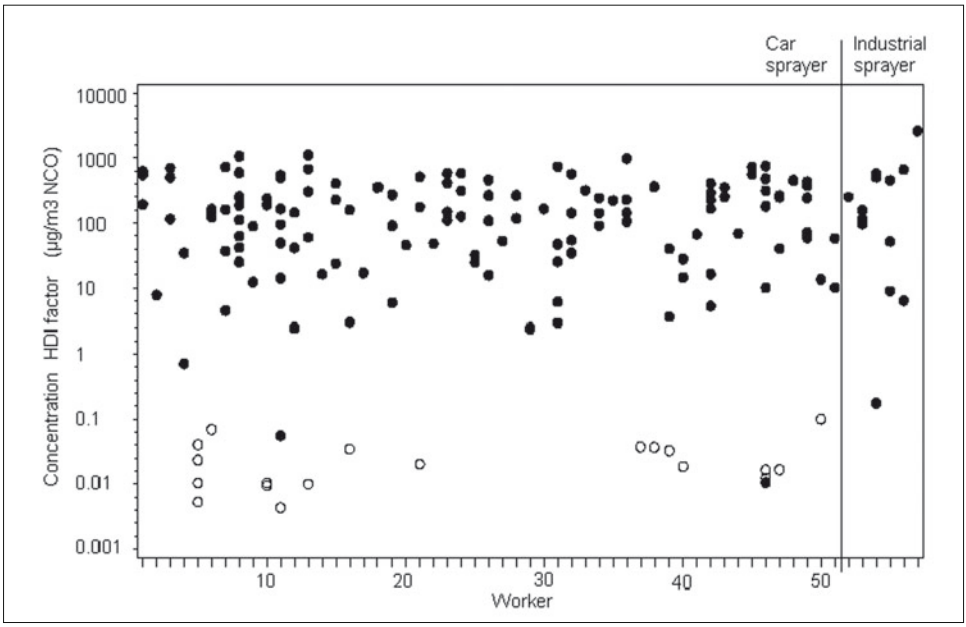

Figure 2.
Variability in isocyanate exposure for each sampled worker and by type of industry (car spray painting and industrial spray painting). Total isocyanate exposure (monomer and different oligomers) is expressed in µg/m^3 NCO. Closed circles refer to detectable levels, open circles refer to measurements below the detection limit, which are plotted as 2/3 of the detection limit (Pronk et al. [75], with permission of the publisher).

are exposed to lower concentrations than industrial spray painters, but that the variability within a worker (over time) is large, up to several orders of magnitude. Reliable estimation of exposures for epidemiological study requires a sufficient number of samples and attention to design options for the exposure component.

Sampling for allergen exposure

Good quantitative epidemiological studies with state of the art exposure assessment are as yet lacking for most allergens. An important explanation is the absence of methods to measure the exposure accurately for most sensitizers. Exposure-response modeling for sensitizing agents is also complex. Asthma is a variable condition, and a sensitized individual reacts to levels to which he or she did not react before becoming sensitized. This complicates identification of levels that induce sensitization or that induce asthmatic reactions. However, these issues do not make exposure-response analyses impossible. For many diseases, differences in susceptibility exist between individuals, but exposure-response relationships have been described.

Low-molecular-weight sensitizers are usually gaseous or partially in solid phase and have to be measured using conventional equipment for gaseous or mixed phase (solid and gaseous) pollutants in combination with chromatography and, in some cases, mass spectrometry. High-molecular-weight sensitizers have to be analyzed with immunochemical techniques to measure the allergen content of the dust.

Currently, dust sampling equipment is based on particle size-selective sampling using health-based definitions of particular size fractions, such as respirable dust, thoracic dust, and inhalable dust [77]. Since sensitization and respiratory symptoms can occur in the upper and lower airways (rhinitis and asthma, respectively) inhalable dust, reflecting the dust particles that can penetrate the respiratory system, is the dust fraction that is measured most commonly in modern epidemiological studies. Information on up-to-date equipment can be found in occupational hygiene textbooks [78, 79].

Other dust sampling approaches have also been used, like the nasal sampler [36, 80–82]. The major application is in situations where conventional sampling equipment cannot be used, for instance, behind face masks [82].

In a very large study among wood workers, passive samplers that hold pieces of sticky tape were used to capture particulates from different angles. These samplers can be calibrated against conventional dust sampling equipment. The advantage is that no expensive sampling pumps have to be used. A disadvantage is that very little dust is sampled, often not enough for immunochemical analysis. The group that used these samplers was able to take several thousands of samples over a relatively short time period and related measured dust exposure with asthma occurrence in wood workers [83].

Measuring sensitizers

Immunochemical methods for measurement of high-molecular-weight agents make use of antibodies, specifically directed against the antigen(s) to be measured. These antibodies form measurable antigen-antibody complexes, which can be detected by different labels – isotopes, enzymes, fluorescents or luminescents – in either inhibition or sandwich assays. Enzyme-linked immunosorbent assays (ELISA) with chromogenic substrates are most commonly used. Validation studies for each immunoassay are necessary, and the outcome depends on sensitivity and particularly specificity of the antibodies used. Specificity of antibodies, as well as the properties and purity of calibration standards or other reference preparations, can be assessed by gel electrophoresis and immunoblotting. Sensitivity of inhibition assays depends mainly on the avidity and concentration of the inhibited antibodies in the assay. With high-avidity antibodies, a sensitivity of 10–20 ng/ml for protein allergen molecules of 10–20 kDa can be reached. Sandwich assays can be much more sensitive, depending on the quality of the reagents; if sufficiently specific, the detection system can be markedly amplified by using various secondary reagents, and in some assays, sensitivities in the pg/ml range are possible.

Considering the large number of aeroallergens pertinent to asthma, few studies are available that evaluate the comparability of immunoassays that have been used to measure them [84–89]. The optimal situation for analyzing allergens immunochemically is reached when the allergen has been identified, and purified allergen and monoclonal or polyclonal antibodies against the allergen are available.

Low-molecular-weight sensitizers such as platinum salts, isocyanates and other chemical agents have to be analyzed using standard chemical techniques such as atomic absorption spectrometry and gas and high-pressure liquid chromatography. Many low-molecular-chemical sensitizers are highly reactive and this complicates sampling and analysis.

Exposure assessment strategies

How should the exposure of a population be characterized? Usually several sources of information have to be combined. Two quantitative exposure assessment strategies exist: measurement for each individual in the population, or measurements for so-called homogeneous exposure categories, for instance, on the basis of job titles. Often, exposure assessment on the individual level is considered the gold standard. However, this strategy is most sensitive to variability of the exposure over time. High variability over time is known to lead to potentially strong underestimation of the exposure-response relationship relative to the variability between individuals in the population. Intuitively, this can easily be understood. When the variability is large and few measurements per individual are taken, the average exposure for a certain

worker will be estimated poorly and can be overestimated or underestimated. This will occur for each worker, and this misclassification of exposure leads in a regression analysis to underestimation of the exposure-response relationship. This underestimation becomes smaller when more repeated measurements per individual have been taken. Categorization of the population in homogeneous exposure groups and use of the measured average exposure per exposure group in an exposure-response relationship is less sensitive to variability over time. In most cases, this strategy is known to lead to unbiased relationships between exposure and response. However, differences within an exposure category are associated with unexplained differences in health risk. This leads to a reduction of power of this strategy in comparison with the individual exposure assessment strategy. Despite the lower power, homogenous exposure groups are the most commonly used strategy. Alternative strategies used in respiratory epidemiology, which have the same properties as the grouping strategy, are estimation on the basis of determinants of exposure [90] and strategies, which consist of combining categorical structures, and individual exposure data [6, 91].

Exposure assessment in epidemiology has developed into a discipline on its own and covers issues such as categorizing the population into exposure categories, allocation of sampling effort over these categories to obtain accurate estimates of the average exposure, use of exposure modeling approaches to estimate the exposure, and evaluation of different exposure assessment strategies as part of an optimization process. These issues have received little attention so far in the field of allergen exposure, since the emphasis has been on instrumental issues, such as developing assays and monitoring techniques. However, more and more examples have been published in which these principles have been applied.

Analysis of exposure-response relationships

In epidemiological studies, exposure-response relationships are usually evaluated with dichotomous outcomes, even when based on a measurement on a continuous scale, for example, IgE titer. The most evaluated exposure-response relationships in allergic respiratory disease are exposure-sensitization and exposure-symptom relationships. However, examples exist in which time to sensitization has been evaluated [15].

Exposure leads to sensitization, and sensitization in conjunction with further exposure may lead to an inflammatory airways response that is accompanied by symptoms, bronchial hyperresponsiveness, airflow variability, etc. For both steps, risk modifying variables have been identified. Atopic workers have higher risk of sensitization against high-molecular-weight sensitizers. Smoking and gender might modify the sensitization risk as well, but the evidence for their roles is weak and depends on the sensitizing agent. In the case of the low-molecular-weight sensitizer, platinum salts, smokers are at higher risk, and atopy is not a risk modifier for most

low-molecular-weight sensitizers. The consequences of these observations are that the exposure-response relationship is potentially modified by these factors, and that the slope of the relationship may differ for different sub-categories of workers. Recent papers have evaluated these variables in epidemiological analysis of quantitative exposure-response relationships for several high-molecular-weight sensitizers, such as wheat allergens, fungal α-amylase, and rat urinary proteins [92–96]. In all cases, the slope of the exposure-response relationship was steeper for atopics compared to non-atopics. In some cases, there was a suggestion that an elevated sensitization risk only occurred in non-atopics among the highly exposed, and the exposure-response relationship was also shifted somewhat to the right.

When one is interested in the relationship between exposure to allergens and work-related symptoms, all modifiers on the causal pathway between exposure and symptoms have to be considered. Workers with work-related sensitization are at higher risk for having or developing symptoms, but atopic workers without work-related sensitization may also have high symptom rates, as may smokers and older workers. Despite the fact that Becklake recognized this problem some time ago [97], most studies have not addressed these modifiers explicitly in their analytical strategies by careful stratification by all potential modifiers.

Power and biological relevance

It is simple to make power calculations in the design phase of a study. Several textbook and internet sites give guidance for specific types of endpoints for a range of designs (see for instance: http://www.cs.uiowa.edu/~rlenth/Power/). Power calculations help optimize the study design and give insight into what can be expected from a study of a given size. Power calculations are extremely useful in situations where major constraints exist in terms of potential number of individuals who can be included. For instance, study of a disease cluster in a company or section of a company is usually limited to a fixed number of workers. If low numbers lead to an extremely low power to detect a biologically relevant increase in disease risk, one might decide not to study a cluster in greater detail at all, or to use completely different approaches such as risk assessment based on literature reviews and exposure assessment studies. Studying clusters in a situation where the power to detect an excess risk is extremely low can be misleading. With large surveys it is possible to detect small differences in prevalence or incidence between populations or in continuous endpoint variables like lung function or inflammatory markers such as eNO (Tab. 2). For instance, a survey including several thousand individuals can detect differences between exposed and non-exposed that are lower than 1%, and such small changes may defy biological interpretation.

These changes are usually not considered relevant on the level of one individual and are smaller than the measurement error found on one occasion and the intra-

Table 2. Required population size for exposed and controls to be able to detect a difference in FEV_1 between these two populations (2xN) with α=0.05 and β=0.20 and a population standard deviation of 0.5 l (after Berry [100]).

Δ (liter/second)	N
0.030	4356
0.060	1089
0.090	484
0.120	272
0.150	174
0.180	121
0.210	89
0.240	68
0.270	54
0.300	44

individual variation in lung function assessed over a period of a few weeks. The major question does not refer to statistical significance, but to biological relevance of small changes in respiratory function. Several considerations exist:

- It is known that FEV_1 reductions of several hundred milliliters can be associated with an approximate doubling of the number of individuals with abnormal lung function when the whole distribution has been shifted [98].
- Similarly small lung function reductions are also associated with an elevated mortality for respiratory diseases and total mortality, underpinning the importance of small changes in lung function on the population level.

What may put this in perspective is to consider the effect of smoking on lung function. In population studies, usually differences between (ex)-smokers and non-smokers are between 100 and 300 mL. While such effects are small in the clinical context, a strong population level determinant like smoking is associated with a relatively small change in function. This epidemiological paragraph has been extensively discussed by Rose et al. [99]. Lung function is one of the parameters for which a considerable amount of evidence exists to place small changes in a broader context. For many recent inflammatory markers, such contextual information is usually not present, making it more difficult to interpret findings from surveys, which are usually smaller than effects seen in clinical cases.

In large longitudinal population studies, the frequency of follow-up measurements and the time between surveys determine the power to a great extent [100].

Very frequent measurement is not useful with long follow-up. Schlesselman [101, 102] has given exact formulas to calculate the population size based on the standard deviations and different combinations of α, β, Δ, measurement frequency, and follow-up duration.

Cross-shift studies are a special case of longitudinal studies with an extremely short follow-up time (8 hours or less). Even when measurements can be performed with very small analytical errors (< 1–3%), the signal-to-noise ratio of these studies is usually not very good, because the expected changes over the work shift on the population level may be relatively small and, therefore, difficult to detect. Peak expiratory flow (PEF) monitoring in OA cases is effective because the changes seen in PEF may be as large as 30–50%.

Research needs

Although much progress is being made on eosinophilic OA, less survey work exists for irritant asthma or asthma with neutrophilic inflammation. The recent recognition that the latter phenotypic group may account for half of asthma cases [103] is particularly pertinent to work-related asthma. Neutrophilic airway inflammation may be triggered by bacterial endotoxin, particulate air pollution, solvents, cleaners, and ozone. Surveys of agricultural workers and office workers in damp indoor spaces are now associating bioaerosol exposures, such as endotoxin and fungal biomass markers, with work-related asthma symptoms [104]. The approaches to exposure assessment in non-industrial damp spaces differ from that for allergen exposure, in that settled dust biomass measurements have a role, whereas air sampling has generally not been associated with work-related respiratory symptoms. In principle, the techniques for surveys examining asthma symptoms in relation to exposures for low level irritant or biomass exposures should be similar to those for eosinophilic asthma, with the exception of examining immune health outcomes or intermediates, such as sensitization. Target industries for such surveys will likely be motivated by population-based studies that indicate asthma excess that has not been clinically recognized.

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