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Développement et validation de modèles pour le diagnostic de l'asthme professionnel

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Développement et validation de modèles pour le diagnostic de l'asthme professionnel

(Development and validation of models for diagnosing occupational asthma)

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Résumé

Le diagnostic de l'asthme professionnel (AP) est toujours un défi. Le test de provocation bronchique spécifique (TPS), comme une méthode de diagnostic de référence, n'est pas aisément accessible. Cette étude diagnostique rétrospective vise à évaluer des outils diagnostiques actuels et à développer des scores cliniques pour AP (définis comme ayant le résultat positif en TPS). Les données concernant les travailleurs soupçonnés d'avoir de l'AP qui, d'une part, ont été exposés aux agents de haut-poids-moléculaire élevé (HPM) (n=139) et à bas-poids-moléculaire (BPM) (n=285), et d'autre part, ont travaillé encore un mois avant de l'évaluation de TPS. Par ailleurs, les modèles de régression logistique sont développés dans chaque groupe d'exposition. Ainsi, concernant des tests objectifs, les valeurs de différents tests distinctifs sont ajoutées aux caractéristiques cliniques, et enfin, le résultat a été évalué. Les modèles ont été testés pour l'exactitude, et pour la validation interne par la procédure bootstrapping. Suite à cela, les modèles finaux sont traduits en scores cliniques et le score total est stratifié en groupes à risque. Chez les travailleurs exposés à des agents BPM, si le test de la méthacholine est fait isolément, le modèle prédictif n'a pas montré de meilleures valeurs diagnostiques que le test de provocation. Cependant, dans le groupe HPM, le modèle final, y compris le sexe, l'âge> 40 ans, la durée des symptômes ≥1 an, la rhinoconjonctivite, l'utilisation de corticostéroïdes inhalés, le test de provocation à la méthacholine, et le test de la piqûre épidermique spécifique, avait un bon calibrage et une validation interne raisonnable. Par ailleurs, la catégorie de sujets avec une probabilité élevée d'avoir AP avait une meilleure spécificité et une meilleure valeur prédite positive par rapport à la combinaison de test de provocation à la méthacholine et de la piqûre épidermique spécifique dans la détection de l'AP, cependant n'avait pas de signification statistique. En conclusion, ce modèle quantifie la probabilité individuelle d'AP. Dans les centres

où l'accès à TPS est difficile ou impossible, notre modèle serait utile dans le diagnostic d'OA, néanmoins, la validation externe du modèle reste nécessaire.

Mots-clés : asthme professionnel, modèle diagnostique, prévention, score clinique.

Abstract

The diagnosis of occupational asthma (OA) is challenging since the use of specific inhalation challenge (SIC) as the reference test is not widely accessible. This retrospective diagnostic study is aimed to evaluate current diagnostic tools and to develop clinical scores for OA (defined as positive SIC). Data from workers with suspected OA who were exposed to high-molecular-weight (HMW) (n=139) and low-molecular-weight (LMW) agents (n=285) and still working one month before the SIC were evaluated. Logistic regression models were developed in each exposure group. The added values of different objective tests to clinical and exposure characteristics were evaluated. The models were tested for accuracy, and, validated internally by the bootstrapping procedure. The final models were translated into clinical score and the sum scores were stratified into risk groups. In workers exposed to LMW agents, the predictive model did not perform better diagnostically than the methacholine challenge test alone. In the HMW group, the final model including sex, age >40 years, symptom duration ≥1 year, rhinoconjunctivitis, inhaled corticosteroid use, the methacholine challenge test, and specific SPT had a good accuracy and reasonable internal validation. The high probability category of the predictive model had a better specificity and positive predicted value compared to the combination of methacholine challenge test and specific SPT in detecting OA but did not reach the statistical significance. Our results suggest that this model could quantify an individual's probability of OA. This model emphasizes the necessity of performing both tests in order to have a more accurate diagnosis in workers exposed to HMW agents. In centers where access to SIC is difficult or impossible, our model might be of benefit in diagnosing OA. Nevertheless, external validation of the model is necessary.

Key words: occupational asthma, diagnostic model, prevention, clinical score.

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List of abbreviations and symbols

ACCP = American College of Chest Physicians

ACGIH = American Conference of Governmental Industrial Hygienists

AOEC = Association of Occupational and Environmental Clinics

AP = Asthme professionnel

ATS = American Thoracic Society

AUC = Area under the receiver operating characteristic curve

BHR = Bronchial hyper-responsiveness

BOHRF = British Occupational Health Research Foundation

BPM = Bas poids moléculaire

CER = Comité d'éthique de la recherche avec les êtres humains

CI = Confidence interval

CNESST = Commission des normes, de l'équité, de la santé et de la sécurité du travail

COPD = Chronic obstructive pulmonary disease

CSST = Comité de la santé et de la sécurité du travail

DLCO = Diffusing capacity of the lungs for carbon monoxide

DPBS = Dulbecco's phosphate buffered saline

ERS = European Respiratory Society

FeNO = Fractional exhaled nitric oxide

FEV1 = Forced expiratory volume in one second

HLA = Human leukocyte antigen

HMW = High molecular weight

HPM = Haut poids moléculaire

HR = Hazard ratio

HSCM = Hôpital Sacré-Cœur de Montréal

IIA = Irritant-induced asthma

IL = Interleukine

IgE/G = Immunoglobuline E/G

KDa = Kilo Dalton

LMW = Low molecular weight

LR = Likelihood ratio

MAR = Missing at random

MCAR = Missing completely at random

MSDS = Material safety data sheet

NO = Nitric oxide

NOS2 = Nitric oxide synthase

NPV = Negative predictive value

NRL = Natural rubber latex

NSBHR = Non-specific bronchial hyper-responsiveness

OA = Occupational asthma

OASQ-11 = Occupational Asthma Screening Questionnaire-11 items

OASYS = Occupational Asthma expert SYStem

OR = Odds ratio

PAR = Population Attributable Risk

PEL = Permissible exposure level

PEF = Peak expiratory flow

PPB = Parts per billion

PPV = Positive predictive value

RADS = Reactive airway distress syndrome

ROC = Receiver operating characteristic

SD = Standard deviation

SE = Standard error

SIC = Specific inhalation challenge

SPT = Skin prick test

STARD = Standards for Reporting Diagnostic accuracy studies

TLV = Threshold limit value

TPS = Test de provocation spécifique

WAA = Work-aggravated asthma

WEA = Work-exacerbated asthma

WRA = Work-related asthma

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Introduction

Work-related asthma (WRA) includes different subtypes such as work-exacerbated asthma (WEA) and occupational asthma (OA). OA can be initiated either by exposure to high-molecular-weight (HMW) or some low-molecular-weight (LMW) agents causing sensitizer-induced asthma, or by exposure to inhalant irritants that may cause different variants of irritant-induced OA (IIA) (1). More than 360 work-related agents have been identified to cause OA and the number of the causal agents is constantly growing (1). A systematic review of six longitudinal general population-based studies found that 16.3% of all adult-onset asthma is caused by occupational exposures (2). Subjects with OA would have a lower quality of life since this disease is associated with job loss and early retirement hence, high financial burden (3). Yacoub et al. (4) assessed the mental status and quality of life in subjects with OA who were removed from exposure to the causal agents for more than 2 years. They found that, 35% of subjects had anxiety disorders, and 23% had dysthymia. They also had moderately impaired disease-specific quality of life. The American College of Chest Physicians (ACCP) recommended an algorithmic approach for approaching the patients who developed lower respiratory symptoms and are suspected to have OA (5). However, diagnosis of OA remains a challenge in clinical practice. Typically, physicians take a stepwise approach initiated with a thorough medical and occupational history and then continue with one or a combination of the available objective tests. The protocol and tests used for diagnosing OA depend on the country and region. Specific inhalation challenge (SIC) as the reference test is not widely available in North America (6, 7). In Québec, the performance of SIC is mandatory in the vast majority of the cases for confirming the diagnosis of the disease in order to claim insurance compensation (8). However, SIC is offered only in specialized centers across Canada (9).

The secondary and tertiary levels of prevention of OA both focus on the early detection of the individuals who develop the disease in order to avoid further health deterioration (10). The prediction models and scores can improve secondary prevention by helping physicians in detecting individuals with work-related disease, choosing appropriate diagnostic tests, and/or making the decision to refer workers for further investigations (11). In the Netherlands, a surveillance program for bakers (12) is based on a risk prediction questionnaire model and scores (13) for detection of work-related sensitization. Application of this model allows a step-wise approach where only bakers with an elevated risk of sensitization to flour would be referred for further medical examination for work-related allergy. Another study was conducted by Jonaid et al. (14) among bakers with a high risk of sensitization who were referred to specialized clinic. A diagnostic questionnaire model for baker's asthma was developed in this population and showed good ability in distinguishing bakers with and without the disease. Diagnosis was confirmed with the presence of asthma symptoms, sensitisation to at least one of the bakery allergens, and a change in non-specific bronchial hype-responsiveness (NSBHR) as SIC is not performed in the Netherlands. This stepwise approach seems to be useful, especially for small enterprises where delivery of adequate examination is difficult, and may contribute to cost reduction.

To our knowledge, there is no risk prediction model that quantifies an individual's probability of having occupational asthma by utilizing clinical and exposure characteristics as well as objective tests other than the SIC (spirometry, bronchial responsiveness testing by methacholine challenge, and skin-prick tests (SPTs)). Development and validation of such a model and converting the results into an easy-to-use score is important because it may facilitate the decision to refer a patient for further investigation (11).

This thesis describes a diagnostic study, performed according to Standards for Reporting of Diagnostic Accuracy (STARD) guidelines, in 424 subjects with lower respiratory symptoms due to exposure to LMW agents (285 subjects) and HMW agents (139 subjects) who were evaluated by SIC for suspicion of OA at the Hôpital du Sacré-Cœur de Montréal between 1983 and 2011. These subjects were still working one month before the SIC.

The study had two objectives: (1) To evaluate the diagnostic parameters of different objective tests in predicting the presence of OA; (2) To develop and validate risk prediction models for estimating the individual's probability of having OA.

Logistic regression analyses were used to develop the models in the multiple-imputed dataset. A clinical and exposure characteristics model was developed from the subjects' medical and occupational histories. We further evaluated the added value of skin-prick tests for common and work-related agents, spirometry, and methacholine challenge tests. The accuracy of models was evaluated by using calibration and discrimination measures. The models were validated internally by using bootstrapping procedures. The coefficients from the final regression models were transformed into easy-to-use numbers (i.e. clinical scores).

The predictive model targets the secondary prevention by quantifying an individual's probability of occupational asthma. Once validated, it would allow physicians to optimize the risk estimation and to detect workers with a higher risk of OA in the absence of the SIC.

1. Chapter one

1.1. Definition and Subtypes of Work-related Asthma

Work-related asthma (WRA) refers to all cases diagnosed with asthma either caused by exposure to a variety of substances in the workplace or an exacerbation of a pre-existing asthma after entering into the workplace. Different phenotypes of WRA have been defined based on past medical history of the patients, work exposures and immunologic response; occupational asthma (OA), and work-exacerbated asthma (WEA) (15). Several clinical entities must be considered as differential diagnosis specially those diseases that have the same presentation as asthma: eosinophilic bronchitis (15), irritable larynx syndrome (16), bronchiolitis, multiple chemical sensitivity syndrome, etc. (17). The prevalence of WRA is constantly increasing as the number of the causative agents related to work are continuously introduced in the literature (18). Figure 1 shows the categorization of WRA adapted from Baur et al. (19).

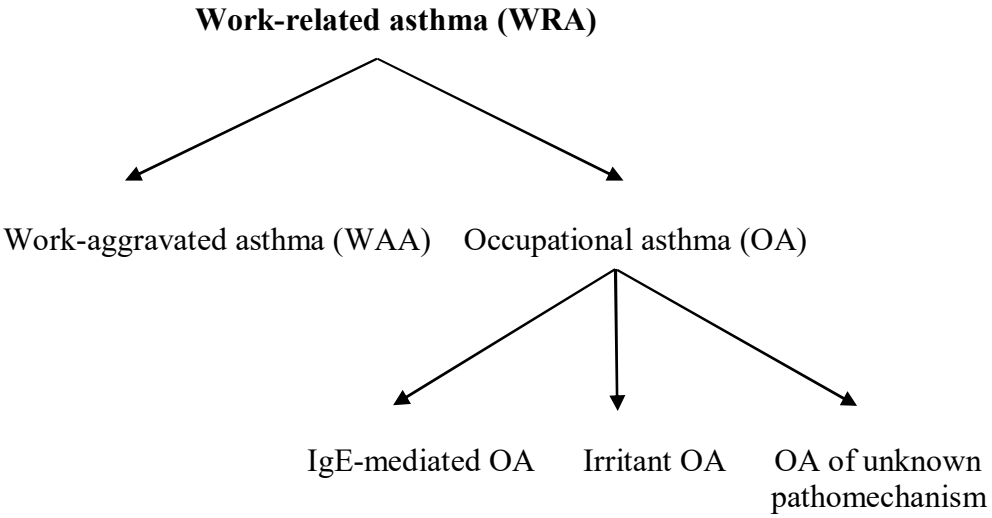

FIGURE 1. SUBTYPES OF WORK-RELATED ASTHMA ADOPTED FROM BAUR ET AL. (19)

1.1.1. Work-exacerbated asthma

Participants with WEA have also been diagnosed with a pre-existing or concurrent asthma, and show worsening of respiratory symptoms upon exposure to substances in the workplace (10). Diagnosis and prevention of WEA are very important as every episode of severe asthma exacerbation is associated with a more rapid decline in lung function (post-bronchodilator FEV1) in children and adults (20). In spite of the importance of early diagnosis and prevention of WEA, a surveillance program in the United States estimated that only 5.2% of reported WEA cases had recorded evidence of pulmonary function tests in their medical records, used to confirm the relationship between asthma exacerbation and work exposure (21). In Ontario, it is also reported that the respiratory function tests were often performed for supporting the relation of asthma with the workplace in subjects with a suspicion of OA (76%) rather than in subjects with WEA (11%) (22). It seems that in a clinical setting, the identification of a WEA case is based on self-reports of a work-related pattern of respiratory symptoms or medication use (23).

Henneberger et al. (23) proposed four criteria for WEA cases definition: 1) pre-existing or concurrent asthma, 2) asthma-work temporal relationship, 3) conditions exist at work that can exacerbate asthma, 4) asthma caused by work (i.e., occupational asthma) is unlikely. They proposed that pathologic pathway to WEA includes inflammatory changes, increased airway responsiveness and reduced flows.

1.1.2. Occupational asthma

Occupational asthma, a variant of WRA, is a common respiratory disease worldwide, defined as asthma caused by exposure in the workplace (24). In other words, it is “a disease characterized by variable airflow limitation and/or hyper-responsiveness and/or inflammation due to causes

and conditions attributable to a particular occupational environment and not to stimuli encountered outside the workplace" (10). OA mostly occurs among bakers and pastry makers, other food processors, spray painters, hairdressers, wood workers, health care workers, cleaners, farmers, laboratory technicians and welders in exposure to different sensitizing and irritative agents (24). A comprehensive list of agents and their related professions responsible for OA can be found at: <http://www.asthme.csst.qc.ca/info_med/index.html>.

There are two main types of OA: immunologic/allergic OA and non-immunologic/non-allergic OA. Immunologic OA, also known as OA with latency or sensitizer-induced OA, is produced by exposure to high-molecular-weight (HMW) or low-molecular-weight (LMW) agents, which provoke an immunological sensitization response. On the other hand, non-immunologic/non-allergic OA, also known as OA without latency or irritant-induced OA, is produced by exposure to inhaled irritant substances at work (25, 26).

1.1.2.1. Sensitizer-induced asthma

HMW agents can induce WRA through an IgE-mediated mechanism. Once a person is sensitized, a very low dose exposure to trigger agents will provoke immune response immediately (within minutes of such an exposure) or may provoke a late or dual response (10).

The pathophysiology of LMW-induced OA is poorly understood. It has been hypothesized that LMW agents cause sensitization; however, a few of them induce asthma through an IgE-mediated pathway, such as complex platinum salts during the manufacture of catalysts (27) or cytotoxic drugs (28), as well as rhodium salts used in electroplating (29). These specific LMW agents act as haptens, and are then combined with a body protein to create complete antigens (30). Diisocyanates (31) as well as plicatic acid (32) can make IgE in blood, however, it can be found only in a small proportion of the workers developing OA. Diisocyanate can produce

specific IgG antibodies, and monocyte chemoattractant protein-1 which is more specific and sensitive for OA (31). Moreover, sensitization to diisocyanates can be seen in workers who are exposed to high concentration of the chemical in a spill. It has been suggested that it may cause an epithelial injury, facilitating the penetration of diisocyanates to the underlying tissues and provoke sensitization (30).

The risk of IgE-mediated sensitization to occupational agents may increase with frequent exposures to particularly higher levels of the agents (1, 10). It has been suggested that the level of exposure at a certain time is a more relevant factor than cumulative doses of exposure or current level of exposure for development of OA. For example, in a cohort of Dutch bakers, a bell-shaped exposure-response relationship between the wheat allergen exposure and sensitization and OA was shown (33). Moreover, a case-referent study on a cohort of animal laboratory technicians demonstrated that there was an increase in the risk of sensitization to animal allergens when the allergic symptoms were initially reported within the first 2 years of initial exposure (34).

Inhalation is not the only mean of sensitization, it can also be caused by skin contact as it can be seen in workers exposed to isocyanate (35-37). Inhalation of some substances might produce different pattern of IgE sensitization in different groups. For example, in an exposure to soybean, citizens became sensitized to the LMW-protein components concentrated in the hull (Gly m 1 and Gly m 2) while bakers mostly became sensitized to HMW allergens in the hull and flour(38).

The latency period between exposure to causative agents and development of respiratory symptoms that are suggestive of OA can range from weeks to years. The latency for LMW sensitizers (e.g., diisocyanates and plicatic acid) and some HMW sensitizers is within 2 years of

exposure (39, 40). A latency period of ≥2 years can be associated with exposure to some HMW agents such as flour and latex (17, 41, 42).

1.2.Epidemiology of Occupational Asthma

1.2.1. Etiological agents causing occupational asthma

A recent study performed by Association of Occupational and Environmental Clinics (AOEC) provided a web-based listing of agents associated with new onset work-related asthma in adults from 2002 to 2015, and estimated that there are more than 327 occupational agents linked to WRA (43) and this list is constantly growing (44). Typically, based on their molecular weights, these agents are classified into two main groups: high-molecular-weight agents (HMW) (>10 KDa) and low-molecular-weight agents (LMW) (<10 KDa) (45).

1.2.1.1. High-molecular-weight agents

HMW agents induce OA through an IgE-mediated mechanism. Glycopeptides and proteins of animals and plants origins are the most frequent HMW agents inducing OA. Table 1, adapted from Tarlo and Lemière (46), and Cartier (47) summarizes the common HMW allergens causing OA.

1.2.1.2. Low-molecular-weight agents

Table 2 summarizes the common LMW agents causing OA (47-65).

The most frequent OA inducing LMW agent is diisocyanate, which may result in an increase in serum level of specific IgE antibodies that is a highly specific but not a very sensitive test for diagnosing OA (66, 67).

TABLE 1. COMMON HMW AGENTS IN SENSITIZER-INDUCED OA

HMW agents	
Agents	**Workers at risk of exposure**
Animal allergens	Farmers, persons who work with laboratory animals, veterinarians
Plants	Greenhouse workers, farmers
Plant products (e.g., natural rubber latex)	Latex-glove makers and users, makers of other latex products
Cereals and grains	Farmers (Cit s 3, an orange tree aeroallergen), grain workers, bakery workers (wheat proteins(natural or purified), thaumatin-like proteins, lipid transfer protein 2G), handling and peeling oranges (orange zest (flavedo))
Other foods (e.g., milk powder and egg powder)	Food-production workers (Microbial transglutaminase), cooks
Fungi	Office workers, laboratory workers, Garbage sorter of packaging material and driver in same plant (exposed to Aspergillus fumigatus)
Enzymes	Laboratory workers, pharmaceutical workers, bakery workers, process operator in a dishwashing tablets factory (exposed to savinase from subtilase family), production and packaging of detergents (Genetically engineered bacterial a-amylase Termanyl)
Insects	Farmers, greenhouse workers
Fish and crustaceans	Workers handling herring or snow crabs (contact to crustaceans, mollusks, fin fish), aquarium fish food production –packing and canning (exposed to Red midge larvae, Gammarus species and Tu. Tubifex, a segmented earthworm), preparing frozen meals containing seafood(Squid (Loligo vulgaris))
Vegetable gums (e.g., guar and acacia)	Printers, including carpet makers
Pests and arthropods	Workers exposed to coffee grounds (Chrysonilia sitophila, asexual state of Neurosporasitophilia), pork butcher worker(Penicillium nalgiovensis in dry sussage molds), driver of van transporting dry-cured ham (Tyrophagus putrescentiae, a dust mite)

TABLE 2. COMMON LMW AGENTS IN SENSITIZER-INDUCED OA

LMW agents	
Agents	**Workers at risk of exposure**
Diisocyanates (e.g., toluene diisocyanate, hexamethylene diisocyanate, and methylene diphenyl diisocyanate)	Makers of rigid or flexible polyurethane foam, installers of polyurethane foam insulation, urethane spray painters, those who work with urethane adhesives or urethane molds in foundries, (Carmine red (E-120) natural red pigment)
Acid anhydrides (e.g., phthalic anhydride, maleic anhydride, and trimellitic anhydride)	Makers of epoxy resins for plastics
Acrylic monomers	Chemical-industry workers, dental workers, aestheticians applying artificial nails
Wood dusts (e.g., from red cedar and exotic woods, Phenol-formaldehyde resin, Spruce wood dust)	Carpenters, sawmill workers, forestry workers, makers of wood products, foundry workers, Sawmill owner (exposed to Spruce wood dust)
Complex platinum salts	Refinery workers, jewelry workers
Biocides(glutaraldehyde and chlorhexidine, 4,4-Methylene bismorpholine, Nitrogen trichloride)	Health care workers, machine tool setter operator (exposed to semisynthetic metalwork fluid), swimming pools
Antiseptics and disinfectants (Chlorhexedine, peracetic acid–hydrogen peroxide mixture and orthophthalaldehyde using in endoscopic units)	Nurses Working on medical wards
Persulfates and henna	Hairdressers
Cyanoacrylate	Eyelash extension glue
Drugs (antibiotics (e.g., vancomycin, colistin, thiamphenicol, 7-aminocephalosporanic acid, cefteram and 7-amino-3-thiomethyl-3-cephalosporanic acid), potassium tetrachloroplatinate (K_2PtCl_4 – halogenated platinum compound-cytotoxic drugs), antineoplastic drugs, anesthetic drugs, diuretics (lasamide), 5-aminosalicylic acid and thiamin. etc.)	Pharmaceutical workers, pharmacists
Aliphatic amines (ethylenediamines and ethanolamines)	Lacquer handlers, soldering workers, spray painters, professional cleaners
Natural dyes	Screen printer (exposed to Carmine red (E-120), a natural red pigment), at spice blenders and butcher shops
Metals and alloys (mostly from the first transition series such as cobalt, nickel and chromium, and stellite)	Welders, plumbers, machinists

1.2.2. Prevalence, Incidence and Population-attributable risk

Occupational asthma is one of the most common work-related respiratory diseases globally (68). The prevalence and incidence of OA vary in different populations due to variation in geographical patterns, type, level and duration of exposure to sensitizing and irritating agents, and preventive methods efficacy (2, 69-74).

Apprentices represent an ideal population for studying the natural course of OA. In an inception cohort of 408 Canadian apprentices exposed to HMW agents (animals, latex, flour, and enzymes), probable OA, defined as the occurrence of new skin sensitization to a program-specific allergen and significant increase in bronchial responsiveness was found in 8.3% of subjects during the apprenticeship, and, 3% post-apprenticeship (75). Furthermore, El-Zein and her collaborators (76) followed 194 apprentice welders for a duration of 15 months in Quebec using assessment of respiratory function with spirometry, methacholine challenge test and questionnaire. The incidence of probable OA was 3%, defined as the presence of at least one lower welding-related respiratory symptoms (cough, wheezing, and/or chest-tightness) and an increase in bronchial responsiveness (a two-fold or ≥3.2-fold decrease in the provocative concentration causing a 20% fall in the forced expiratory volume in one second (PC20) from baseline to the end of the study).

A systematic review of six longitudinal general population-based studies found that OA accounted for about 17.6% of adult asthma (2). Another study estimated that 15% of adult asthma was attributable to the workplace agents, and the incidence of OA was about 22 to 40 cases per million in/among active workers every year (77). Moreover, an international longitudinal study of 13 countries among workers aged between 20-44 years reported that about 10-25% of new asthma diagnoses occurred in an occupational setting. This study used a job-

exposure matrix for evaluating occupational exposure to causative agents. After calculation, the authors also found that the incidence of OA was 250-300 cases/million workers/year (78).

Based on the medico-legal reports, OA is the most common respiratory disease in Quebec (79). A physician based surveillance system of occupational respiratory diseases (PROPULSE) in Québec estimated that the incidence of the OA was 42 cases/million female workers/year and 79 cases/million male workers/year between October 1992 and September 1993 (80). Moreover, it was estimated that between 1988 and 2002, OA was the second common work-related respiratory disease in Quebec. Consequently the greatest proportion of the workers who were entitled to get compensation from the *Commission de la santé et de la sécurité du travail (CSST)* belonged to this group (81). The mean cost of OA was estimated to be about 50,000 CAN$ between 1986 and 1988 (82), and 93,000 for the period 1988–2002 (83).

1.2.3. Host risk factors

Age and sex: Advancement in age has been identified to increase the risk of OA among farmers, but this is the only study about this risk factor (84). A higher prevalence of OA has been reported among female subjects compared to male subjects since female workers were more exposed to occupational sensitizers (21.1% of female workers versus 13.4% of male workers) (85). Moreover, the distribution of occupations by sex might explain different types of exposure between men and women. For example, OA due to cleaning agents could be seen more among women (86, 87) while men are at more risk of developing OA due to exposure to epoxy, diisocyanates and acrylate (88).

Atopy: Atopy is a known independent risk factor for OA in exposures due to HMW agents (89, 90) such as flour (91, 92) and animal allergens (93, 94). In the other words, atopic workers are at increased risk of OA in exposure to HMW agents (95). On the other hand, the atopic workers are

not at risk of OA in relation to LMW agents that do not induce asthma through an IgE-mediated mechanism (96). Gautrin and Malo followed the apprentices exposed to HMW agents in Montréal (exposed to flour, laboratory animal allergens and latex). They found that atopy to work-specific agents was associated with a probability of having OA in subjects exposed to animal-based HMW agents (39, 97). However, pre-exposure sensitization to common allergens which are structurally the same as workplace allergens might be a more important determinant of OA than atopy to work-specific agents (93, 97).

BHR and rhinitis: In a cohort study conducted by Omland et al. (98) on 1964 farming-school students and 407 non-farming subjects aged 16 to 26 years, bronchial hyper-responsiveness (BHR) at baseline (OR: 11.7; 95% CI: 2.4 to 56.4) was associated with OA. BHR and rhinitis before entering to workplaces containing HMW agents are the independent risk factors for the development of IgE sensitization to HMW allergens (99). The presence of work-related nasal symptoms during the exposure to sensitizers often proceeds to the development of OA (100, 101). It has been found that 11.4% of those workers exposed to laboratory animal allergens with rhinitis developed OA during the period of 30 to 42 months after the initial exposure (99).

In subjects exposed to HMW agents, an IgE-mediated sensitization leads to the presence of upper respiratory symptoms that precede asthma symptoms: rhinorrhea, ocular pruritus, or nasal congestion. The presence of rhinoconjunctivitis and wheezing are associated with OA in exposure to HMW agents rather than LMW sensitizers (102-104). However, Riu et al. (105) showed that there was an increased risk of rhinitis among adults exposed to occupational LMW agents (OR: 1.4; 95% CI: 1.0 to 2.1).

Genetics: Certain genotypes relate to the presence of human leukocyte antigen (HLA) class II, as well as the genes related to T-helper 2 cells make some individuals more susceptible to OA upon

exposure to HMW and LMW agents (99). It has been proven that workers with certain genotypes are more susceptible, or in contrast, more immune to OA. The pathophysiology of these gene expressions is not clear. It has been hypothesized that as a protective mechanism, some genes play a role in the regulation of the immune system response to causative agents of OA (106).

Smoking: It has been documented that smoking increases the risk of OA in welders exposed to metal dust, volatile fumes, and in workers exposed to paper dust (107-109). The confounding role of smoking has not been fully investigated in all epidemiologic studies. It seems that smoking increases the risk of sensitisation to HMW agents but not to LMW agents (110, 111). For example, in a study on snow crab processing workers, smoking was significantly associated with OA (OR: 3.1; 95% CI: 1.3 to 7.4) (112). However, a few studies have reported direct evidence that smoking can increase the risk of OA (112-115).

Others: Ingestion of spice dust by food processing workers (116) and ingestion of grain products by bakers (91) have been reported to be strongly associated with OA in these professions. In contrast, no association has been found between OA and the consumption of seafood among seafood processors (71). Obesity has also been suggested to increase the risk of OA through an uncertain mechanism (92, 117). Moreover, low socioeconomic status is related to exposure to work-related agents and possibly, future OA (118). Further research is needed to explore the nature and magnitude of this relationship.

1.3. Diagnosis and Evaluation of Occupational Asthma

Despite the fact that OA is the most common work-related lung disease in industrialised countries, and the second most common disease reported after pneumoconiosis in developing countries (95, 119), physicians may face some difficulties in diagnosing OA, which will cause

the underestimation of this disease among high risk populations. This may be due to the fact that there is a limited access to an occupational health service, or the health care providers have little knowledge of potential hazard of exposure to workplace agents and therefore do not dedicate enough time in the primary health settings prior to referral. All these causes may delay referring patients with work-related symptoms by physicians (120-122). Chest physicians may also fail to perform appropriate objective tests (121, 123).

The American College of Chest Physicians (ACCP) recommended algorithmic approach for diagnosing OA (5). Following this algorithm, there are several tools that can be used in the diagnosis of OA. Recently, Vandenplas et al. (120) generated an updated algorithmic approach based on the availability and feasibility of the tests in clinical practice (see Figure 2). However, the diagnosis of OA is still challenging and sometimes inappropriately investigated. It might be due to financial and professional concern of the workers (22, 124, 125), a delay in referring the patients with work-related respiratory symptoms to specialist by general practitioners (121), or failure in performing appropriate objective tests or taking proper diagnostic steps by specialists (121, 126).

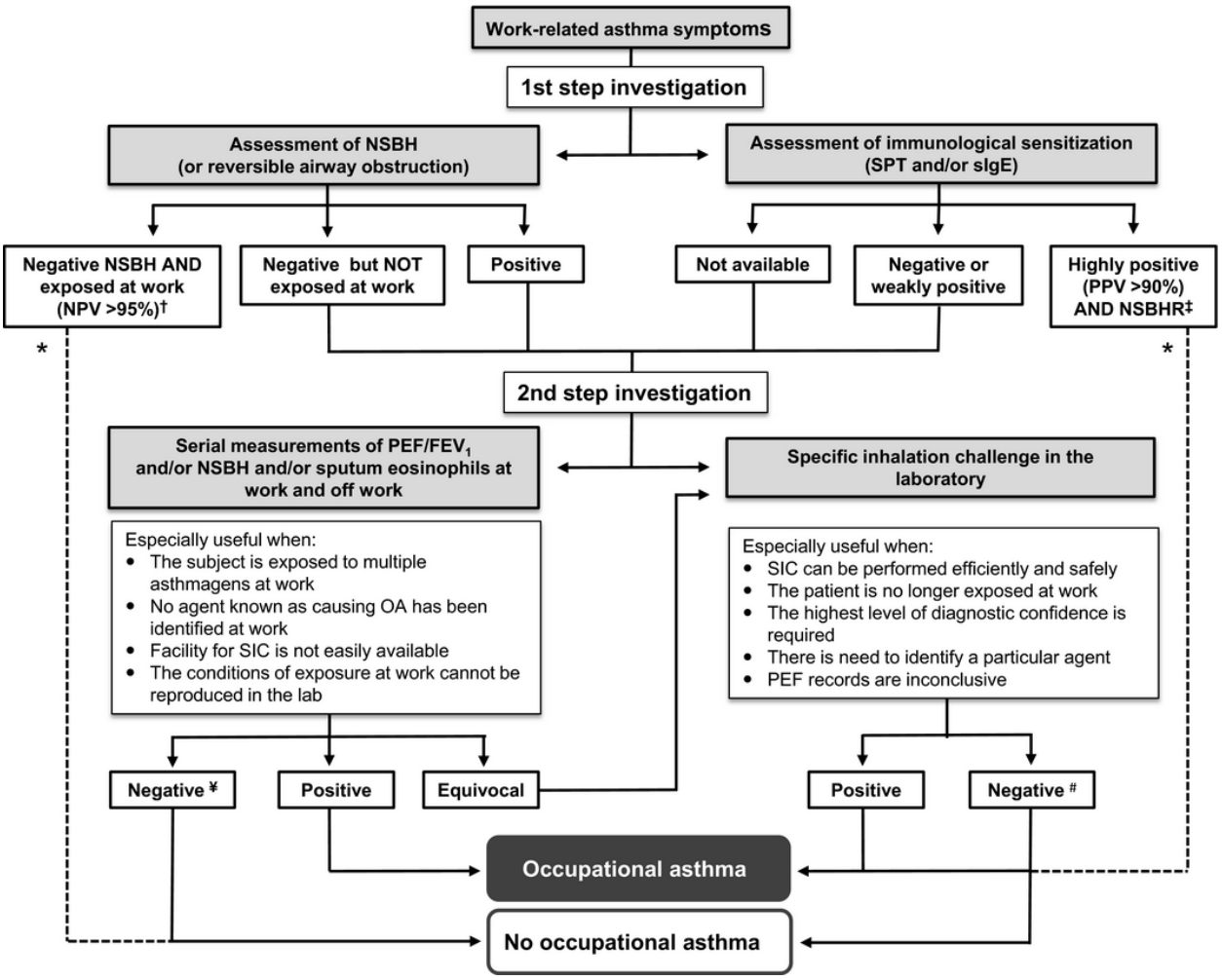

FIGURE 2. APPROACH TO DIAGNOSIS OF OCCUPATIONAL ASTHMA SUGGESTED BY VANDENPLAS ET AL. (120).

Abbreviations: FEV1, forced expiratory flow in one-second; NSBH, non-specific bronchial hyper-responsiveness; NPV, negative predictive value; OA, occupational asthma; PEF, peak expiratory flow; PPV, positive predictive value; sIgE, specific immunoglobulin E; SPT, skin prick test.

Any diagnostic workup for OA in subjects with work-related respiratory symptoms should begin with diagnosing asthma. For the next step, the link between asthma and occupational exposure should be confirmed by a stepwise approach: the first step is taking the occupational and medical history of the worker suspected to have OA in order to estimate the clinical likelihood of the disease, and the second step is to confirm the relationship between occupational exposure and

occurrence of asthma by combination of different objective tests depending on the availability and feasibility of the tests, which may differ between regions and countries (9, 120, 127).

1.3.1. Medical and occupational history

Any assessment of referred workers suspected of having OA should start with taking a thorough and detailed medical and occupational history including information about the workplace in order to recognize possible offending agents, duration of exposure, onset and duration of respiratory symptoms, upper and lower respiratory symptoms, and work impairment due to respiratory symptoms. An incomplete history can delay diagnosis. Notably, patients must be asked about the presentation of the symptoms when away from work to identify the possible late responses to offending agents especially LMW agents (128). Table 3 summarizes the key points of occupational history, extracted from Tan J. et al. (129).

In addition, material safety data sheets (MSDSs) can be used as a complementary tool to identify the physical and chemical characteristics of the sensitizers to which workers are exposed at the workplace (130). They also provide information on generic chemical names, standardized threshold limit values (TLV), and standardized permissible exposure levels (PEL) of causal agents (129, 131). However, they cannot identify all sensitizers particularly if their concentration in the product or in its ingredients is low (i.e., <1%) (17, 132). MSDSs are available at: http://www.msds.com or http://www.ilpi.com/msds.

MDSs must be used with caution. For instance, Heederik et al. (133) estimated an exposure threshold of sensitization to wheat allergens in a population of Dutch bakers. They found that when asthma symptoms were accompanied by sensitization, a steeper exposure–response relationship were observed, which means that the “lowest observed effect levels” were lower

compared to the proposed TLV by American Conference of Governmental Industrial Hygienists (ACGIH).

Typically, the respiratory symptoms might be provoked by starting a work shift and disappear shortly after leaving the workplace or during weekends and holidays (134). Questionnaires for identifying the work-related respiratory symptoms are highly sensitive but have low specificity (6). Vandenplas et al. (102) found that "improvement of symptoms at weekends and on vacations" item used in an "open" questionnaire, which was administered in 212 patients with confirmed OA by specific inhalation challenge (SIC), had a sensitivity of 75% and a specificity of 55% for diagnosing OA. Generally, this "open" questionnaire falsely categorised 8% of the OA subjects as non-OA and 34% of non-OA subjects as having OA. Previously, Malo et al. (135) found that improvement of symptoms at weekends and on vacations had sensitivities of 77% and 88% and specificities of 44% and 24%, respectively. They also found that an "open" questionnaire had a sensitivity of 87%, but a specificity of only 27% for diagnosing OA. They concluded that the low specificity of the questionnaire might be due to the fact that workers without OA might develop respiratory symptoms during the work period similar to the workers with OA, or some individuals without OA might experience an improvement of the symptom when away from work similar to those individuals with a definite diagnosis of OA. Given the low positive predictive value (PPV) and the negative predictive value (NPV) of the open questionnaire (63% and 83%, respectively), it can be concluded that the open questionnaire is not a satisfactory tool for diagnosing OA and other objective means of diagnosis must be used (135). Workers may also falsify questionnaires because of the fear of losing their jobs (124).

Moreover, irritant-induced asthma may have variable clinical presentations. For example, workers exposed to diisocyanates may represent a late airway response with lack of correlation with the workplace or duration of exposures (129).

Pralong et al. (136) evaluated the capacity of the Occupational Asthma Screening Questionnaire–11 items (OASQ-11) to screen patients with suspicion of having OA in the clinical setting. The prevalence of OA was 12% in the population that underwent the analyses. The final proposed models composed of eight symptoms (i.e. wheezing, dyspnea, cough, asthma attack, asthma medication, cough at work, wheezing at work, chest tightness at work), age, and exposure duration could identify 89% of the OA cases. Developing a distinct prediction model in the same population on the basis of the same questionnaire for prediction of WEA showed that the questionnaire items, which showed good sensitivity (e.g. wheezing) in OA prediction model, did not showed enough sensitivity in predicting WEA (137). This shows that it is important to develop different models for prediction of OA and WEA.

TABLE 3. KEY ELEMENTS OF THE OCCUPATIONAL HISTORY IN THE EVALUATION OF OCCUPATIONAL ASTHMA

I.	Demographic information
	A. Identification and address.
	B. Personal data including sex, race and age.
	C. Educational background with quantification of the number of school years completed.
II.	Employment history
	A. Current department and job description including dates begun, interrupted and ended.
	B. List all other work processes and substances used in the employee's work environment. A schematic diagram of the workplace is helpful to identify indirect exposure to substances emanating from adjacent work stations.
	C. List prior jobs at current workplace with description of job, duration and identification of material used.
	D. Work history describing employment preceding current workplace. Job descriptions and exposure history must be included.
III.	Symptoms
	A. Categories:
	1. Chest tightness, wheezing, cough, shortness of breath.
	2. Nasal rhinorrhea, sneezing, lacrimation, ocular itching.
	3. Systemic symptoms such as fever, arthralgia and myalgia.
	B. Duration should be quantified.
	C. Duration of employment at current job prior to onset of symptoms.
	D. Identify temporal pattern of symptoms in relationship to work.
	1. Immediate onset beginning at work with resolution soon after coming home.
	2. Delayed onset beginning 4–12 h after starting work or after coming home.
	3. Immediate onset followed by recovery with symptoms recurring 4–12 h after initial exposure to suspect agent at work.
	E. Improvement away from work.
IV.	Identify potential risk factors.
	A. Obtain a smoking history along with current smoking status and quantitate number of pack years.
	B. Asthmatic symptoms preceding current work exposure.
	C. Atopic status

	1. Identify consistent history of seasonal nasal or ocular symptoms.
	2. Family history of atopic disease.
	3. Confirmation by epicutaneous testing to a panel of common aeroallergens.
	D. History of accidental exposures to substances such as heated fumes or chemical spills.

1.3.2. Diagnostic tests

To make a valid diagnosis of asthma, some complementary tests can be done. In this section, different diagnostic tools used in clinical and laboratory setting are discussed.

1.3.2.1. Immunological tests (skin-prick test (SPT) for specific work-related allergens or blood specific IgE measurement)

Skin-prick test (SPT) is used to detect sensitization to work-related allergens via an IgE-mediated pathway or type I hypersensitivity reaction. A positive test is defined as the presence of cutaneous reactivity, a ≥3 mm diameter wheal, 15-20 minutes after the application of the specific allergen extracts in the absence of any reaction to the diluent (glycerine, 50%) and in the presence of a positive reaction to histamine phosphate (1/200 mg/ml) (138). Test extracts for many of HMW and LMW agents are not commercially available and lack standardization and validity (10, 120).

SPT shows a high sensitivity and a low specificity for detecting OA in workers exposed to HMW agents (128). A positive test confirms the sensitization to a HMW agent but not the presence of OA because the PPV is very low (17). A negative test makes the diagnosis of OA very unlikely (17). For LMW agents, the sensitivity and specificity of SPT in detection of sensitization is lower than HMW agents as the immunologic pathway to OA caused by LMW agents is not well-known. Only a few LMW agents are associated with OA through an IgE-mediated mechanism, such as acid anhydride compounds, chloramine, persulfates, reactive dyes, and platinum salts

(10, 139). However, since the test extracts are not standardized (9) or do not have enough potency and reliability (140), incorrect results are likely to be generated. SPT is not commonly performed in clinics for diagnosing OA induced by LMW agents (140). Thus, in vitro tests such as basophil histamine release or assay of monocyte chemoattractant protein-1 by peripheral blood mononuclear cells might provide a higher sensitivity and specificity; however, they are not sufficient for a definite diagnosis of OA since they have not been validated in clinical settings (17, 141).

Vandenplas et al. (142) investigated the validity of SPT and IgE test in workers exposed to latex. They found that compared to the result of the SIC test, SPT had the sensitivity and specificity of 100% and 21%, respectively. All SIC-positive and 79% of SIC-negative workers had positive SPT results. A systematic review including 77 studies used SIC as the "reference standard" found that the pooled sensitivity of SPT and serum IgE level was >73% for diagnosing OA in workers exposed to HMW agents (127). However, specific IgE level of the serum showed a higher specificity (pooled estimate: 79.0%; 95% CI: 50.5% to 93.3%). For LMW agents, SPT showed a higher sensitivity for detection of OA when combined with methacholine challenge test (pooled estimate: 100%; 95% CI: 74.1% to 100%). However, the specificity of SPT and serum specific IgE were almost equal (pooled estimate: 88.9% and 86.2%, respectively). This review concluded that in the absence of the specialized tests such as SIC, a combination of methacholine challenge test with SPT or a measurement of IgE serum level can be used for diagnosing OA. Moreover, Park et al. (143) found that in 42 workers with confirmed OA who were exposed to reactive dyes, the sensitivity of the SPT and serum IgE was 91% and 86%, respectively. The PPV was 80% and 63% and NPV was 89% and 80%, respectively for SPT and

serum IgE test. These results show that SPT has more reliable results than serum IgE measurement for the screening of OA.

In conclusion, as SPT shows a high sensitivity and a low specificity for diagnosing OA, it must be used in conjugation with other confirmatory tests (6, 127, 138). It has been suggested that a positive SPT to HMW agents combined with a positive methacholine challenge can increase the probability of the diagnosis to >90% in workers with an occupational and clinical history suggestive of OA (17), although a negative combined test results does not provide an adequate negative predictive value for ruling the disease out (127). SPT is recommended to be used as a diagnostic tool for HMW agents (evidence level A)[1], while it is moderately recommended for detection of OA in subjects exposed to LMW agents (evidence level B) (144).

1.3.2.2. Respiratory function tests

1.3.2.2.1. Serial peak expiratory flow rate recording

Peak expiratory flow (PEF), one of the recommended first-line tools for diagnosing OA, is both a sensitive and a specific tool, at the same time, inexpensive and reproducible (10, 45, 145). It can be used to assess reversible bronchial obstruction in individuals who are currently working and are exposed to "routine levels" of the causative agents, when they are at or away from work (146, 147).

PEF can be used to measure airflow limitation in subjects with suspected OA and shows a high specificity and sensitivity if done in a serial pattern (6). The desirable frequency of PEF measurement has been investigated in many studies but it is still controversial (148-150). A more

[1] "Recommendations were graded from (A) to (C) in favor and against the specific diagnostic test or treatment, with (A) level recommendations having the highest quality body of literature."–Jolly et al. (REF#144)

frequent and longer measurements of PEF increase the specificity and sensitivity of the test but decrease the accuracy of the test due to errors in recording. Recently, the recording period of 4 weeks with at least one week away from work has been recommended (128, 151, 152). If the number of the PEF recordings is less than 4 times per day, or PEF recording is done for less than 3 weeks, the diagnostic value of this test will fall (128, 151). The use of respiratory-related medications should be minimal and the working shift patterns should be unchanged. Any changes in work shifts or new exposure to irritants or respiratory infections during the testing period must be recorded as it can influence on the accuracy of interpretations (10). Anees et al. (150) found that the PEF recording of 3 consecutive workdays in any work period and at least four readings per day for a total of 3 weeks, could produce a sensitivity of 78% and specificity of 92% for diagnosing OA. Moore et al. (153) found that a PEF recording of 8 times per day during 8 workdays and 3 rest days could result a specificity of 91% and sensitivity of 68% for diagnosing OA.

PEF rates recording at and away from the workplace are compared by an expert or by the aid of OASYS software (Occupational Asthma System) in order to detect any decline in pulmonary function caused by exposure to work-related agents (148, 154, 155).

The visual interpretation of PEF results is more reliable and is considered as significant if there is ≥20% change in the mean values at work compared to the mean values away from work (120, 156). Anees et al. (157) found that the mean PEF difference of ≥16 L/min between workdays and rest days was a significant index for distinguishing workers with OA from those without OA (sensitivity of diagnosing: 70%). A systematic review of more than 31 studies found that the pooled sensitivity and specificity of serial PEF recording for the diagnosis of OA were 82% (95% CI: 76% to 90%) and 88% (95% CI: 80% to 95%), respectively (158).

The interpretation of PEF results can be difficult due to exposure to unknown causal agents, low compliance of the patients, severe asthma (17), and a decreased level of exposure due to relocation of the workers to an area with lower concentration of the offending agents (10). As a result, it is moderately recommended to use this tool for diagnosing WRA when a patient has already been diagnosed by other diagnostic tools (evidence level B) (144).

1.3.2.2.2. Pre and post-exposure specific inhalation challenge test

Specific inhalation challenge (SIC) is considered as the reference test for diagnosing OA (10). When the results of the available objective tests are equivocal, or a worker is exposed to more than one sensitizer, the SIC can help in identifying the causal agent and in clarifying the final diagnosis of sensitizer-induced asthma (10, 159, 160). The evidence-based guidelines issued by the British Occupational Health Research Foundation (BOHRF) acknowledged that: ‘‘a carefully controlled SIC comes closest to a gold standard test for some agents causing OA’’, but ‘‘a negative test in a worker with otherwise good evidence of OA is not sufficient to exclude the diagnosis’’ (90). The consensus statement issued by the ACCP recommended that: ‘‘in individuals with suspected sensitizer-induced OA, conducting SIC (where available) is suggested when the diagnosis or causative agent remains equivocal’’ (10). In Québec, SIC is the recommended method to confirm the diagnosis of OA (8).

Indications and contraindications of SIC

Based on the consensus statement provided by ERS Task Force, the indication for performing SIC in a patient suspected of OA are as follows: “1) confirmation of the diagnosis of occupational asthma when other objective methods are not feasible, are less efficient or have failed to provide definitive results; 2) identification of the cause of occupational asthma when other objective methods are not feasible, are less efficient or have failed to provide definitive

results; 3) the identification of a new (not formerly described) specific cause of occupational asthma; and 4) research into the mechanisms of work-related asthma" (161, 162).

The contraindications of the SIC are as follow: severe airway obstruction, baseline forced expiratory volume in one second percent predicted (FEV1%) ≤70%, unstable asthma, unstable or recent cardiovascular disorders, uncontrolled epilepsy, recent respiratory tract infection (<4 weeks), pregnancy, IIA, lack of trained staff and specialized facilities and equipment, and patient's inability to understand the procedures (10, 161, 163-165).

SIC procedure

The goal of this procedure is to expose a subject to a suspected causal agent in order to produce a fall in forced expiratory volume in 1 second (FEV1), an increase in bronchial responsiveness, and/or an increase in inflammatory markers of the respiratory tract (166, 167). There is no standardized protocol for the use of SIC. A "realistic" approach was initially developed by Pepys and Hutchcroft (10, 168). In this method, it is aimed to reproduce the exposure to work-related agents in respect to the chemical and physical characteristics of the agents. The concentration of the probable agents is not directly measured; instead, duration of challenge exposure is considered as the surrogated of the dosage of the agent (169). Therefore, this method can lead to misleading results due to lack of tight control on the concentration and dose of the agent (170, 171). Consequently, closed-circuit apparatuses have been developed where the concentration and dose of the inhaled agent can be continuously measured throughout the procedure. The latter method presents less immediate asthmatic reactions and more safety (172). The cessation of inhaled corticosteroid before the SIC is still controversial.

Patients must stop taking theophylline, inhaled bronchodilators, leukotriene receptor antagonists, cromoglycate and antihistamines before the test according to their duration of action (163, 173).

It is also recommended to withhold the oral or inhaled corticosteroids 72 hours before the SIC since they might diminish the bronchial responsiveness to sensitizers (161, 174-176).

SIC consists of three or four days of control and challenge:

The control day is a day without any exposure to work-related sensitizers. The baseline FEV1 values using spirometry and/or PEFs using portable instruments are measured after 30-60 minutes of exposure to a control agent for a period of 6-8 hours (161, 163, 164). The control agent is an irritant and/or an agent with the same physical characteristics of the suspected occupational agent causing OA. The control agents may be selected among the following various agents: "lactose powder for SIC with agents in powder form (flour, drugs and persulfate); pine dust for SIC with wood dusts; vinyl gloves for SIC with latex gloves; and solvents for polyurethane products and other resins" (161, 169). The purposes of the control day are to verify the functional stability which is important for interpretation of FEV1 variability after exposure to the suspected agent, to provide a comparison for any bronchial reaction on the challenge day, and to detect any non-specific irritant reaction to control agent which would suggest an irritant reaction to the suspected agent that cannot meet the definition of a specific bronchial hyperreactivity (161, 177).

On the challenge day, patients are exposed to the suspected occupational agents with the levels lower than occupational exposure limits (OEL) to prevent severe asthmatic reactions and/or irritant responses (161). The suspected agent must be delivered with the same chemical and physical characteristics of the agent to which a worker is exposed in the workplace (168). The duration of exposure to HMW agents can be increased progressively on the first challenge day until an immediate bronchial reaction or until the maximum duration of exposure is reached. For LMW agents, the duration of exposure must be increased gradually in a period of 2 days with a

cumulative exposure limited to <30 min on the first challenge day (161, 163, 178) in order to avoid severe late asthmatic reactions (179).

FEV1 values are measured immediately after exposure to suspected agent, and every 10 minutes during the first hour after the exposure, twice during the second hour after exposure, and then hourly for the following 6 hours, for the total of at least 8 hours in order to record any relapsing bronchospasm (169, 180).

Non-specific bronchial hyper-responsiveness (NSBHR) using methacholine challenge test are measured before (on the control day) and should be reassessed at the end of SIC, particularly if SIC indicate no significant changes in airway calibre (9, 161). The purpose of NSBHR measurement is to decrease the risk of false-negative results especially when there is no significant change in NSBHR post-SIC. If there is a significant change in NSBHR compared to the baseline values (i.e. more than two- to three-fold reduction in post-challenge PC20/provocation dose causing a 20% fall in FEV1 (PD20) values compared to baseline), a repeated challenge must be performed before excluding the diagnosis of OA (9, 161, 181).

Sputum eosinophil count is measured on the control day and on the last day of exposure to the suspected agent. If there is a >3% increase in sputum eosinophilia compared to baseline value, there is a possibility of having an asthmatic reaction after another challenge day (182). Recently, Racine et al. (183) investigated the diagnostic accuracy of this test before and after SIC. They found that a sputum eosinophil count is less effective than a methacholine challenge test for diagnosing OA. Moreover, a post-SIC sputum eosinophilia is more accurate than a positive methacholine challenge for distinguishing workers with and without OA (AUC = 86%; 95% CI: 0.8-0.9, P < .001; AUC = 69%; 95% CI: 0.6-0.8, P = .010, respectively).

Notably, if the FEV1 fluctuation in the control days is ≥10%, the challenge must be stopped as it is considered instable asthma (161). Moreover, it is beneficial to distinguish non-specific irritant reactions in exposure to control agents from specific BHR in exposure to specific work-related sensitizers since it may lead to falsely positive SIC results (161, 177).

Another day of active challenge test may proceed the second challenge day if the changes in FEV1 are equivocal or negative and challenge with a higher dose is considered appropriate, or when there is a significant increase in sputum eosinophils or in fractional exhaled nitric oxide (FeNO) after the challenge, or there is a more than two- to three-fold reduction in post-challenge PC20 values compared to baseline values on the control day (184, 185).

SIC interpretation

A ≥15-20% of sustained fall in FEV1 value (PC20) recorded in two consecutive assessments is considered as positive SIC test if the fluctuation of FEV1 6-8 hours after the exposure during the control day is <10% (161, 169). It has to be consistent with an asthmatic reaction. No SIC should be considered as negative unless the challenge lasted for at least 240 minutes without ≥20% fall in FEV1 (169).

If the results of FEV1 are inconclusive, in the presence of the following conditions, another challenge day must be repeated before excluding the diagnosis of OA:

1) a significant change in NSBHR (two to three-fold reduction in post-challenge PC20 compared to baseline value) (181, 185);
2) an sputum eosinophilia of >3% compared to baseline value (161);
3) a post-challenge increase in FeNO of >30–40% compared to pre-challenge value (161, 186, 187) an increase >20% for baseline values over 50 ppb or >10 ppb for values lower than 50 ppb (188).

SIC limitations

Although a positive test confirms OA, the negative test does not rule out diagnosis of OA (17). The major challenge in SIC is the possibility of the production of false-negative and false-positive results.

False-negative results also can occur in the absence of specific bronchial responsiveness to offending agent when away from exposure, inadequate or false timing and concentration of exposure, and usage of asthma-specific medications before the challenge (10, 161). Lemière et al. found that after cessation of exposure to sensitizers, specific bronchial responsiveness to HMW and LMW agents may decrease but never completely disappears in most of the cases unless the NSBHR may normalize (189), although the required dosage of the occupational agents for the production of a positive SIC will significantly increase (190). As a result, in patients with negative SIC (no significant change in FEV1 values) and/or a decrease in specific bronchial responsiveness, it is recommended to increase the duration of challenge or the concentration of the suspected occupational agent (169). It is also recommended to measure the NSBHR level (185) and sputum eosinophilia (182) before and after the challenge as it might lead to repeating the challenge that ultimately helps accurate diagnosis of OA by reducing the number of false-negative results.

Another challenge in interpretation of the SIC is false-positive results. Mostly, the false-positive results are due to non-specific bronchoconstriction associated with exposure to irritants stimuli, which may mimic the same pattern due to exposure to sensitizers (10, 15, 90, 161). False positive results can be prevented by reducing non-specific reactions. This can be reach by exposing workers to a control agent in order to identify those who may produce non-specific reactions, and also by exposing the subjects to concentrations below the OEL (161).

SIC adverse effects

Although SIC is a precise test for diagnosing OA, its performance needs special equipment and laboratory environment (191). Fever, skin reaction, anaphylactic shock and asthma exacerbation might possibly be induced during the test (181, 191-193). The use of closed-circuit equipment, adequate exhaust ventilation, and protective clothing help in improving the safety of this test (161, 194). To consider the potential adverse effects and the need for special equipment, the level of recommendation for usage of this test has been reduced from strongly recommended to recommended (evidence level C) (144). The ACCP Consensus Statement established that "in individuals with suspected sensitizer-induced OA, conducting a SIC (where available) is suggested when the diagnosis or causative agent remains equivocal. However, this testing should only be performed in specialized facilities, with medical supervision throughout the testing" (10).

1.3.2.2.3. Respiratory challenge tests using non-specific agonists such as methacholine, mannitol, adenosine, or histamine

Non-specific bronchial hyper-responsiveness (NSBHR) will be confirmed by challenge tests using non-specific agonists including direct stimuli (e.g., methacholine, histamine), or indirect stimuli (e.g., exercise, hyperventilation, cold air, nonisotonic aerosols, mannitol, and adenosine) that cause a >20% fall in FEV1 value (PC20) (195-198). The direct stimuli act through direct stimulation of smooth muscle receptors, and indirect stimuli act through the release of mediators from immune cells. Indirect stimuli are hypothesized to be more relevant to be used since it produces similar respiratory symptoms in asthma (198). The methacholine is the preferred agonist to be used to histamine because it may cause fewer side effects if used in high concentration dosage. The normal results cannot rule out the presence of asthma since this test only shows a moderate sensitivity and specificity at or away from the workplace (6).

A provocative concentration of a substance causing a 20% fall in FEV1 (PC20 value) using a methacholine or histamine challenge must be measured after a weeks of exposure to work-related agents, and once again, after being away from work for a period of two weeks in order to detect any changes in the value of PC20 suggesting airway hyper-responsiveness. A ≥3 fold change in the value of PC20 can be suggestive of OA (10). This test is more reliable when all the baseline FEV1 values are similar (10). Patients' medical and occupational history such as pulmonary infection within 6 weeks prior to the test (199), exposure to non-occupational allergens that may lead to an increase in bronchial responsiveness, the use of inhaled corticosteroids or failure to discontinue the bronchodilator therapy before the test (200), or the presence of gastro-esophageal reflux (201) must be considered when interpreting the test because they might influence the level of NSBHR.

Côté et al. (202) found that the methacholine challenge test had a lower sensitivity and specificity (62% and 78%, respectively) for diagnosing OA when compared to SIC in cedar workers suspected to have OA. Beach at al. (127) also measured the sensitivity and specificity of respiratory challenge test in patients exposed to LMW and HMW agents in a systematic review; they demonstrated that negative result of this test, as a single confirmatory test, cannot rule out the presence of OA and must be used in combination with other tests such as specific SPT or specific IgE (HMW agents: sensitivity = 79.3%, specificity = 51.3%; LMW agents: sensitivity = 66.7%, specificity = 63.9%; mixed agents: sensitivity = 83.7%, specificity = 48.4%). Pralong et al. (203) measured the diagnostic utility of methacholine challenge test in 479 patients who were suspected to have OA, and were still working (exposed to causative agents). The workers were categorized based on the SIC results (OA = positive SIC results). The negative predictive value (NPV) of this test could reach 97.7%. The sensitivity of this test also calculated to be 98.1%;

however, the specificity was very low. These studies showed that the methacholine challenge test is a sensitive tool for diagnosing OA in symptomatic patients but it is not very specific. The best utility of this diagnostic tool is for excluding the diagnosis of OA especially if it is done when a worker is still working and is in contact with the offending agent. However, it is not sufficient for a definite diagnosis of OA and must be coupled with another test. This test is moderately recommended to diagnose WRA (evidence level B) (144).

1.3.2.3. Measures of airway inflammation

The >1% increase in eosinophil count of the sputum after being exposed to workplace agents, when FEV1 shows a fall to <20%, and also, the increased fractional exhaled nitric oxide (FeNO) as a surrogate marker for eosinophilic airway inflammation will support the diagnosis of OA, although the absence of the desired result, either for sputum eosinophil count or FeNO, will not rule out the presence of the disease (204). Airway inflammation detection has been proposed as a key feature in the management of asthma (205-208).

1.3.2.3.1. Induced sputum test

Induced sputum test is a useful test since it is an easy-to-perform test before and after SIC. It helps in confirming the diagnosis of asthma in the environment with unknown causative agents, and, can distinguish occupational eosinophilic bronchitis from other variants of WRA (209-211). The sputum eosinophil count can be increased to the level of ≥3% in exposure to HMW and some LMW occupational agents (182, 212). This increase can be seen after a positive SIC test regardless of the type of asthmatic response (212-214), especially after 7-24 hours after exposure (212, 215). Obata et al. (212) found the higher sputum eosinophil count in subjects exposed to red cedar with negative SIC. Some studies showed that the sputum eosinophil count significantly increases when a worker is at work and decreases after cessation of exposure (216, 217). A study

investigated the pattern of respiratory inflammation in subjects with OA and in asthmatic subjects without OA. They were exposed mostly to LMW agents and were working in the same environment. The diagnosis of OA was based on the worsening of the symptoms at work and a positive methacholine challenge test. The sputum specimens were obtained both at work and away from work. The subjects with OA showed a significant increase in the sputum eosinophil count when at work while sputum eosinophilia resolved when they were away from work (218). Another study also investigated the changes in sputum count in subjects exposed to LMW and HMW agents, who were suspected of OA (216). The subjects underwent a SIC for confirming the presence of OA. They found that among subjects with positive SIC, the sputum eosinophil counts significantly increased when at work compared to the period away from work ($p = 0.002$) (15/23 or 65% of subjects with positive SIC). In contrast, in subjects with negative SIC, a higher sputum eosinophil count was detected in only 23% (6/26) of subjects when they were at work compared to the period away from work. Subjects who had a negative SIC had more sputum neutrophilia when at work, probably due to an irritant effect of agents in their workplace.

Sputum neutrophil count can increase in the sputum of exposed workers after negative or positive SIC result. However, it seems that it can be produced by other irritants during the SIC test in the occupational or laboratory setting (212, 214). It has been suggested that neutrophilic airway inflammation can be produced by exposure to irritants (150), inhalation of hypertonic saline (219), inhalation of atmospheric pollutants like ozone (220), exposure to swine confinement (221), grain dust (222), or other occupational agents. Another possible factor in neutrophilia in induced sputum of asthmatic is smoking (223, 224). Sputum neutrophilia is also present in asthmatic subjects without OA (e.g., WEA) (217). The long-term prognosis of subjects with sputum neutrophilia is still unclear.

The procedure of sputum induction is reproducible and non-invasive by the inhalation of a hypertonic saline solution (225). Sputum eosinophilia can be induced by exposure to both HMW and LMW agents (212, 226). A sputum eosinophil count of >2-3% in a patient with negative SIC is indicative of the necessity of increasing the duration of the challenge in order to prevent the false-negative results due to desensitization to agents after being away from the workplace (182, 213, 227).

1.3.2.3.2. Measurement of fractional nitric oxide (NO) (FeNO)

Nitric oxide (NO), one of the biologic mediators involved in the pathogenesis of asthma (228), functions as vaso-bronchodilator, neurotransmitter and inflammatory mediator (229). Asthmatic patients have a high level of NO in their expiration and inducible nitric oxide synthase (NOS2) enzyme expression in the epithelial cells of their airways (230). Measurement of fractional nitric oxide (NO) (FeNO) is a non-invasive and reproducible tool specifically if there is severe asthma that does not allow the other tests to be performed (231). Moreover, this test is strongly recommended for diagnosing eosinophilic variant of asthma (232). Dissimilar results have been found in various research. Allmers et al. (233) found no relationship between bronchial responsiveness, substance-specific IgE and an increase in FeNO in subjects exposed to isocyanate and latex. In contrast, Tossa et al. (234) found that there was a significant relationship between BHR and an increased NO level in 441 atopic or non-atopic apprentices exposed to flour and hairdressing sprays (OR: 2.00; 95% CI: 1.21 to 3.32). They included that FeNO measurement could be used as a reliable screening tool in patients suspected to have OA with recent exposure to agents known for causing OA. Baur and Barbinova (235) investigated the level of exhaled NO in 31 latex-sensitized cases and 14 non sensitized controls and found that the FeNO was increase 24 hours after the positive SIC with a statistically significant relationship

with bronchial obstruction, although the difference between the baseline and post-challenge NO level of was not statistically significant. Piipari et al. (236) also found a significant increase in the NO level 24 hours after the SIC in subjects with a normal or slightly increased level of basal NO and a late bronchoconstriction but not in subjects with high basal NO level and a significant bronchoconstriction.

Moreover, Lemière at al. (167) found that the sputum eosinophilia ≥2.2% could reach a greater sensitivity and positive predictive value (PPV) compared to FeNO in subjects with positive SIC results. This finding indicates that an increased sputum eosinophil count >2% is a better tool to distinguish positive and negative SIC in patients suspected to have OA compared to increased exhaled NO level.

1.4.Which combination of the tests is the best for diagnosing OA?

Classically, all workers showing upper and lower respiratory symptoms at the occupational setting are referred to clinics for further investigations. It is noteworthy that OA must be differentiated from pre-existing asthma or WEA. In addition, other differential diagnoses such as chronic obstructive lung disease, bronchiolitis obliterans, vocal cord dysfunction, endotoxin-induced asthma-like syndromes (e.g., grain fever or byssinosis), and pneumoconiosis should be ruled out. Patients with OA normally have normal chest x-rays, lung volumes with diffusion capacity (DLCO) and an obstructive pattern in PFT (6, 237).

The combination of different tests will increase the probability of a definite diagnosis of OA. It seems that there is a history of respiratory symptoms suggestive of OA in exposure to HMW agents, the combination of a non-specific bronchial challenge test and SPT would result in a post-test probability of >90% of OA, although the negative combined test is not adequate for

excluding the diagnosis of OA (17, 127). A systematic review by Beach et al. (127) found that a positive methacholine challenge test coupled with positive SPT in exposure to HMW agents had the pooled estimate of sensitivity of 60.6% (95% CI: 21.0% to 89.9%) and pooled estimate of specificity of 82.5% (95% CI: 54.0% to 95.0%). This study also reported that when the positive results of the methacholine challenge test, SPT and specific serum IgE were combined, the pooled estimate of sensitivity and specificity were 65.2% (95% CI: 6.7% to 98.0%) and 74.3% (95% CI: 45.0% to 91.0%) for diagnosing OA in workers exposed to HMW agents. The utility of the combination of methacholine challenge and SPT in patients with OA in exposure to LMW agents was reported by the same review (127): the sensitivity and specificity were 100% (95% CI: 74.1% to 100%) and 80.0% (95% CI: 49.0% to 94.3%), respectively. However, further research needs to be done to assess the prediction value of questionnaires and objective tests in reality.

Suarthana and her colleagues (238) developed models for the occurrence of specific IgE-sensitisation to and respiratory symptoms in contact with laboratory animal allergens using standardized questionnaire and objective tests including SPT and bronchial responsiveness test. However, to our knowledge, there is no prediction model that quantifies the diagnostic value of respiratory function tests (i.e. spirometry and bronchial responsiveness testing using methacholine), induced sputum, and SPT for detecting OA using questionnaires and objective tests in the clinical setting.

1.5. Prevention of Work-related Asthma

The prevention of WRA also is done at three levels. The potential primary prevention measures have been outlined by Tarlo and Liss (239) including:

"(i) Identification of highly susceptible workers and locating them to areas without exposure to known sensitizers.

(ii) Limitation of exposure to potential respiratory irritants among those with pre-existing asthma to reduce work-related aggravation of asthma.

(iii) Use of engineering controls, such as elimination of a responsible agent, substitution with a safer substance/chemical, ventilation, process or equipment modification, process enclosure, dust reduction techniques, housekeeping and work practices.

(iv) Administrative controls to reduce number of workers exposed or duration of exposure, e.g. job rotation, rest periods, and shift or location changes where fewer people are working with sensitizers or irritant exposures.

(v) Personal protective equipment (at the worker), which includes respirators, gloves, goggles and coveralls."

Primary prevention showed promising results in controlling OA in workers exposed to NRL gloves. Substitution of powdered NRL gloves with non-powdered NRL gloves and/or low-protein powder-free caused a reduction in incident cases of OA among health care providers (240). Moreover, application of primary prevention measures in industrial hygiene programs in the detergent manufacturing industry to maintain the level of the ambient enzyme at 15 ng protein/m3 (less than the ACGIH TLV of 60 ng protein/m3) caused a decline in the rates of sensitization and incidence of OA (241).

In a situation where primary prevention is not possible, secondary and tertiary level of prevention take place with a focus on the detection of the individuals who developed the disease in order to avoid the deterioration of the condition (10). Secondary prevention consists of early identification of workers suspected of having OA (242), mostly done in a frame of a surveillance

program. Identification of the case often is done by the aid of medical respiratory questionnaires along with immunologic tests such as SPT and spirometry (239). Since OA may occur one year after the sensitization to work-related agents and the onset of respiratory symptoms, surveillance programs should be performed in this interval (30, 100). One example of secondary prevention is conduction of surveillance program in detergent enzyme industry by medical questionnaire and SPT which caused the incidence of OA to remain constant among workers (241).

Tertiary prevention includes environmental control strategies (30) such as removal of the worker with confirmed OA from the workplace in order to prevent exposure to new sensitizers. If a worker has pre-existing OA, efforts should be concentrated on prevention of the progression of the disease to more severe states which may cause irreversible loss of lung function (242). For example, Anees et al. (243) showed that workers with OA who were removed from the workplace showed amelioration in the value of FEV1, 6 months after removal from exposure. If cessation of exposure is not possible, the reduction of exposure is also beneficial in the improvement of respiratory symptoms and NSBHR (6, 244). However, it is not clear if the reduction of exposure is as beneficial as cessation of exposure to sensitizers (245). A systematic review by Rachiotis et al. (246) reported a pooled estimate of 32% of recovery rate (95% CI: 26% to 38%) after on average 31 months of exposure cessation in subjects with OA. Additionally, the pooled prevalence of persisting NSBHR was 73% (95% CI: 66% to 79%).

1.6. The role of prediction modeling in the prevention of work-related asthma

Various modeling tools have been designed to facilitate the complicated clinical decision-making process with implications for both patients and healthcare providers. Two examples of these tools are prognostic and predictive models (247).

Predictive modeling enhance the ability of the healthcare system to assess and manage the financial costs of the healthcare through the identification of at-risk population, who are susceptible to a particular condition. The needed information for development of predictive models are derived from demographic and exposure data, clinical history, and diagnostic tests (247). The predictive models are focused on total population and emphasize long-term behavioral changes (248). There are two main types of predictive models: 1) medical data–based model that has the highest predictive power; 2) prescription drug–based model. The first type of these models has the highest predictive power and consequently can predict consumption of healthcare by a particular population (249).

Diagnostic prediction models are served to evaluate the probability of having an outcome, whereas prognostic prediction models are designed to estimate the probability of developing an outcome (250, 251). In clinical practice, diagnostic models use the data from patients' medical history, clinical examinations and laboratory tests as predictors of an outcome. Based on the estimated probability of having an outcome, physicians are able to do risk stratification for every individual (having a high or low probability of having an outcome). Therefore, they can be guided upon choosing further diagnostic workup in high probability, or refraining from further testing in low probability group (252). An example of a well-known prediction model is the Wells criteria for diagnosing pulmonary embolism in combination with a negative D-dimer test where a negative D-dimer test can accurately rule out pulmonary embolism in about 40% of patients suspected of having pulmonary embolism. Therefore, no further diagnostic testing is indicated for these patients (253).

The prognostic models enable physicians to predict an outcome of a disease and/or a treatment. It is based on the prognostic information related to a patient rather than a disease or treatment.

These models can be used in identifying the eligibility of a patient for a new treatment, selecting appropriate tests and therapies, and supporting decision on continuing or discontinuing a treatment (254). There are two main types of prognostic models: 1) patient population model which is focused on recognizing discrepancies in groups of patients for a specific criterion; 2) individual patient model which is served to provide the best treatment policies in respect to every individual's characteristics (247, 254). For instance, a well-known prognostic model is the assessment of the 10-year risk of coronary heath disease using the Framingham scores that allows physicians to identify the patients with higher probability of the disease whom preventive action should be more precise (11, 255). Another example is the prognostic models that were developed by Suarthana et al. (256) for assessing the value of a questionnaire used alone or in combination with SPT to common allergens and/or NSBHR in predicting the incidence of occupational sensitization and symptoms in apprentices exposed to laboratory animals. They found that a questionnaire model was a useful tool to predict the occurrence of symptoms and sensitization. However, the addition of the objective tests to the questionnaire model did not enhance the ability to predict the symptoms but significantly increased the specificity of the questionnaire model for predicting sensitization to animal allergens.

Risk prediction models and clinical scores have important implications in secondary prevention of OA since they help physicians in early detection of individuals who developed respiratory symptoms at the workplace, and in making decision for further diagnostic testing and referrals (11). They may be used for screening of workers at risk of development of a work-related disease, or, may be applied in the context of a surveillance program for planning lifestyle or therapeutic decisions based on the risk of developing a particular disease (257). In the case of the occupational diseases, surveillance programs commonly use respiratory questionnaires,

spirometry, and skin-prick testing or identification of serum specific IgE to occupational sensitizers in order to identify cases of OA (258). The motive of prediction studies is to risk-stratifying the patients (259) in order to truly assign the therapeutic and diagnostic resources to those patients with a higher risk of a disease based on the model or risk scores.

These risk prediction models always use several predictors including patients' demographic, clinical and work-related characteristics and symptoms, and available diagnostic tools to predict the likelihood of detecting individual with the desired outcome (260). Nevertheless, similar to other diagnostic tools, prediction models are subjected to misclassify a diseased individual as non-diseased, or to under- or over-estimate the risk of having a disease. This may impose a burden of additional cost and disability from the complications of the diseases of the interest. Thus, accurate model development and assuring its diagnostic performance, cost-effectiveness of the model in clinical practice, and choosing a rational cut-off point for risk-stratification of individuals at risk of the disease are important (261).

Surathana et al. (13) developed and validated a risk prediction model including six questionnaire items (naso-conjunctival symptoms in the last 12 months, asthma problems in the last 12 months, shortness of breath and wheeze, work-related upper and lower respiratory symptoms, and type of bakery) for identification of bakers with elevated risk of sensitization to flour. This model was applied in 5,325 Dutch bakers who were later stratified to low, intermediate and high score (probability) groups. They externally validated the model in the first 73 bakers who were referred as having a high risk. Application of this model allows a step-wise approach where only bakers with an elevated risk of sensitization to flour would be referred for further medical examination for work-related allergy. This approach seems to be useful, especially for small enterprises where access to adequate examination is difficult, and may contribute to cost

reduction (12). Jonaid et al. (14) recently developed two distinct models using a self-administered questionnaire, and the medical history of the subjects for predicting bakers' asthma and rhinitis among 436 Dutch bakery workers referred to specialized clinic for occupational respiratory disorder. These bakers were at a high risk for sensitisation to bakery allergens and identified by using the model developed by Suarthana et al. (13). The final models for diagnosing asthma and rhinitis in bakers included work-related upper and lower respiratory symptoms, the presence of allergy and allergic symptoms, use of medication (last year), type of job, type of shift and working years with symptoms (≥10 years). Diagnosis of bakers' asthma was confirmed with the presence of asthma symptoms, sensitisation to at least one of the bakery allergens, and a change in non-specific bronchial hype-responsiveness (NSBHR) as SIC is not performed in the Netherlands. These diagnostic models could differentiate satisfactorily bakers with a higher probability of OA from those bakers with a lower probability of disease. The self-administered questionnaire model demonstrated high sensitivity (98%–100%) and specificity (99%–100%) but very low positive and negative predictive values (32%–41% and 60%–68%, respectively). The medical history model showed similarly a very high sensitivity and specificity and low positive and negative predictive values in differentiating bakers with and without OA especially in those bakers that had the intermediate and high probability of sensitization. The low estimation of the predictive value of the models could be explained by the low prevalence of OA among the study population.

This stepwise concept is adopted in an ongoing medical surveillance program for occupational rhinitis and sensitizer-induced asthma with latency, developed by the "*Agence de la santé et des*

services sociaux de Montréal"[2]. It starts with an information session about the probable upper and lower respiratory symptoms associated with exposure to sensitizers for the at-risk workers exposed to sensitizers in the workplace. A self-administered questionnaire (262) is distributed during the same session to evaluate if the workers have work-related symptoms (Appendix A). An occupational health nurse consequently might interview those workers having symptoms suggestive of occupational-related rhinitis and/or asthma, by administrating detailed medical and occupational questionnaires. Those workers suspected to have occupational rhinitis or OA (≥3 positive answers) may be referred to occupational physicians for further clinical work-up. However, the restricted availability of the objective tests in different geographic regions makes the process of diagnosis complicated. In Québec, the performance of SIC is recommended to confirm the diagnosis of the disease before acceptance of a workers' compensation claim by CNESST (*Commission des normes, de l'équité, de la santé et de la sécurité du travail*) (8). However, SIC is offered only in specialized centers in Canada. The shortage of specialized centers that perform SIC tests might delay the diagnosis of the disease and negatively affect the accuracy of the diagnosis. By developing diagnostic models for OA in workers exposed to high or low-molecular weight agents in the workplace, we can estimate the individual probability of having OA. It enables physicians to optimize the risk estimation and to detect the majority of workers with a higher risk of OA in a timely manner.

[2] Available at:
http://www.dsp.santemontreal.qc.ca/fileadmin/documents/dossiers_thematiques/Sante_au_travail/Comite_d_harmonisation_des_protocoles_medicaux/Asthme_professionnel/Protocole-GuideSurvMed_VTotale_20130825_FINAL.pdf

1.7. Long-term adverse outcome of occupational asthma

Several factors influence on the prognosis of OA. Once the diagnosis is confirmed, the prognosis of OA is often poor. Longer symptomatic exposure to causative agents, lower lung volumes, higher NSBHR, older age and HMW type of agents are related to adverse outcomes of OA. A few studies provided strong evidence on the direct association of smoking and risk of OA (112-115). Atopy is a known independent risk factor for OA in exposure due to HMW agents (89, 90). There is agreement between the ACCP (10) and British Occupational Health Research Foundation (BOHRF) (90) guidelines that longer exposure to work-related agents is associated with worse adverse outcomes of OA. Moreover, persistent exposure to sensitizers is associated with NSBHR and OA (244). Additionally, atopy to allergens, BHR status to methacholine and nasal and respiratory symptoms may contribute to the development of adverse outcomes. Most importantly, the level of bronchial responsiveness at the time of entering to apprenticeship was associated with sensitization to related agents, new respiratory symptoms presentation and BHR post-apprenticeship. The absence of sensitization to related allergens and BHR at the time of entering an apprenticeship was related to remission among those workers with continuous exposure to sensitizers. In contrast, subjects presenting respiratory symptoms during apprenticeship were more at risk of developing persistent sensitization, respiratory symptoms, and BHR with continuous exposure to sensitizers post-apprenticeship (75, 263, 264). Additionally, OA is associated with an excessive decline in lung function (244). For example, in a cohort study, the red cedar wood workers' FEV1 values showed a steeper decline compared to control group (265). Thus, when a diagnosis of OA is confirmed, it is recommended that the worker is removed from further exposure to the causative sensitizer.

2. Chapter two

2.1. Objectives

There are two objectives in this thesis. The first objective of the study is to evaluate the diagnostic value of different diagnostic tools for predicting the presence of OA. The second objective is to develop and validate prediction models for quantifying the individual's probability of having OA using information from clinical and exposure characteristics, SPT, spirometry, and methacholine challenge test. These would enable physicians to optimize the risk estimation and to detect workers with a higher risk of OA in a timely manner.

2.2. Methods

2.2.1. Study design

This retrospective diagnostic study was done according to the Standards for Reporting Diagnostic Accuracy (STARD) guidelines.

2.2.2. Study population and database

The study population constituted subjects with lower respiratory symptoms including dyspnea, cough, wheezing and/or chest tightness, who were exposed to work-related HMW or LMW agents, and were referred to OA clinic at HSCM between 1983 and 2011 to be evaluated for OA (Figure 1 of the manuscript, page 88). Subjects who were exposed to both HMW and LMW agents were excluded from the study (n = 25/1012 total study population). In the HMW group, three subjects with missing specific SPT due to unknown exposing agent were also excluded

from the analyses. We included all subjects who had an SIC to confirm or exclude the diagnosis of OA, and were still working one month prior to the SIC (n = 424/987 total population).

We used an existing clinical database "Banque de tissus" that was developed by the Axe Maladies Chroniques/santé respiratoire and approved by the Comité d'éthique de la recherche avec les êtres humains (CER) of the Hôpital du Sacré-Cœur de Montréal (HSCM) in April 2014. It contains demographic, clinical and exposure characteristics as well as SPT to common and work-specific agents, spirometry, methacholine challenge test, induced sputum, and the SIC tests results. Data on subjects' working status were available at the time of the SIC. The charts of workers with OA and normal bronchial responsiveness while working had been reviewed by Dr. André Cartier for the purpose of another study to determine whether they were exposed to the offending agent at the time of the SIC. However, it was not possible to determine whether subjects were still exposed to the same level of the occupational agents while they were working because they might have been relocated to another department or area with a lower level of exposure. All objective tests were also done prior to the SIC test. The specialists performing SIC tests had access to the results of the previous tests for interpreting the SIC results.

2.2.3. Study variables

1.2.3.1 Reference standard

The reference standard was a positive SIC (i.e. OA), namely a sustained fall in FEV1 $\geq$20% of the baseline value after exposure to the suspected agents in the workplace.

2.2.3.2. Predictors

1. Demographic characteristics: age (>40/≤40 years, the median of age among all the subjects), sex (male/female), smoking habit (current smoker, ever smoked, never smoked).

2. Clinical characteristics: presence of rhinoconjunctivitis (yes/no), lower respiratory symptoms duration (>1/≤1 year), and use of inhaled corticosteroids (yes/no).

3. Exposure characteristics: duration of exposure (>7/≤7 years, the median of the exposure duration among all subjects), and working status at the time of the diagnosis (defined as still working one month before the SIC test).

4. Low FEV1 at the time of diagnosis, defined as FEV1 percentage predicted of ≤80%. The FEV1% predicted calculated by Knudson equation and included in the analyses (266).

5. NSBHR, defined as a provocative concentration to methacholine causing a 20% decrease in FEV1 (PC20) ≤16 mg/ml.

6. Atopy, defined as at least one positive SPT reaction to common aeroallergens.

7. Specific sensitization to a work-related agent(s), defined as at least one positive SPT reaction to work-specific agents.

2.2.4. Measurement tools

2.2.4.1. Clinical characteristics

The demographic, clinical and occupational characteristics were extracted from clinical assessments performed by physicians at the Hôpital du Sacré-Cœur de Montréal. No specific questionnaires were used. Data on occupation status of the subjects was available only at the time of performing SIC. Data on workers' exposure level was not structured in a way that allows a comprehensive assessment of past occupations.

2.2.4.2. Respiratory tests

Respiratory function was measured by using spirometry. A Collins-type spirometer (WE Collins, Braintree, MA, USA)) was used according to the criteria of the American Thoracic Society (267, 268). Predictive value equation by Knudson was used to calculate percent predicted FEV1 (266). BHR was determined through methacholine inhalation tests (163, 269). Methacholine inhalation tests were performed according to the standardized procedure, using a Wright nebulizer (output = 0.14 mL/min-1; Roxon Meditech Ltd, Montreal, PQ, Canada) at tidal-volume breathing for 2 minutes.

SIC tests were performed according to standardized methods (168). Patients were asked to stop taking all medications 8 hours to 3 days before the test according to their duration of action except for inhaled corticosteroids, which were continued at the same dosage but taken in the evening of each test day (161, 163) because it could affect the bronchial response. On the first day of the test, each subject was exposed to a control product. Spirometry test was performed before and serially for a duration of 8 hours after exposure. Methacholine challenge test was also performed prior to the SIC on the control day (baseline). During the following days, subjects were exposed to probable work-related agents causing OA in the laboratory, and if not possible at the laboratory, it was done at the workplace (270).

2.2.4.3. Skin-prick test (SPT)

Sensitization to work-related allergens, and atopy to common allergens were evaluated by SPT. Histamine phosphate (1/200 g/ml) was used as a positive control, and diluent (glycerine, 50%), as a negative control. SPTs were done using 11 common inhalants (mixed tress, mixed grass, and ragweed pollen; Altenaria; Aspergillus; Hormodendrum, feathers, Dermatophagoides farinae; Dermatophagoides pteronissinusa; and cat and dog dander) (271). SPTs were also done with

specific HMW work-related agents to which subjects were exposed. A wheal diameter of 3 mm or more was regarded as a positive response, in the absence of any reaction to the diluent (glycerine, 50%) and in the presence of a positive reaction to histamine phosphate (1/200 mg/mL).

2.2.4.4. Induced sputum test

Sputum eosinophil count ≥2% and sputum neutrophil count ≥60% were set for the purpose of the first objective of this study. The level of the changes in sputum eosinophil, which was associated with positive SIC was reported as ≥2% in the previous study by Lemière et al. (213). Moreover, several studies showed that the mean of sputum neutrophilia after exposure to a causative agent was about 60% (272, 273), which justified the choice of the cut-off point of ≥60% for sputum neutrophilia in this study.

The procedure of induced sputum test was done according to the method suggested by Pin et al. (274). Lung function was monitored before and every 5 minute throughout the procedure. Subjects inhaled the increasing concentrations of hypertonic saline (3%, 4%, and 5%) for up to 30 minutes after salbutamol inhalation (218, 274) and the sputum specimens were obtained 3 times and examined for the level of eosinophil and neutrophil. During the procedure, subjects were asked to clean their nose and mouth before expectoration. Portions of sputum, which were separated from saliva, were treated with 0.1% dithiothreitol (volume equal to four times the weight of selected sputum) for 15 minutes before being diluted with four volumes of Dulbecco's phosphate buffered saline (DPBS).

2.3. Statistical plan

2.3.1. Statistical analysis

Descriptive statistics of the baseline demographic, occupational, and clinical characteristics, as well as objective measurements obtained from spirometry, bronchial challenge test, SPT and SIC were presented in forms of frequencies and percentages for dichotomous predictors, and mean ± standard deviations (SD) for continuous predictors with normal distribution and median (minimum value to maximum value) for continuous predictors with non-normal distribution, as necessary.

2.3.1.1. Objective 1

To answer the first study objective, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of each predictor were calculated and compared between original and imputed data. In addition, positive and negative likelihood ratio (+LR and –LR) as well as the discriminative ability (i.e. through the measurement of the area under the receiver operating characteristic (ROC) curve (AUC)) of each objective test were also calculated.

2.3.1.2. Objective 2

2.3.1.2.1. Model development

To answer the second objective, different models were developed: (1) clinical and exposure characteristics; (2) clinical & exposure characteristics and specific sensitization; (3) clinical & exposure characteristics and atopy; (4) clinical & exposure characteristics and low FEV1; (5) clinical & exposure characteristics and NSBHR; (6) clinical & exposure characteristics and all the tests; (7) clinical & exposure characteristics, specific sensitization, and NSBHR.

Bivariable logistic regression analysis was carried out to examine the relationship between each predictor and the outcome. Subsequently, we used multivariable logistic regression analysis to develop the models (275). Multiple logistic regression with backward stepwise selection using a p-value <0.157 (i.e. Akaike information criterion) was carried out for the clinical & exposure characteristics model (i.e. Model 1) (276, 277). We subsequently added the predictor(s) from the objective test(s).

2.3.1.2.2. Model's accuracy and internal validity

The accuracy of the models was quantified using calibration and discrimination measures. Calibration, the agreement between the predicted probabilities of having OA and the observed frequencies of subjects with confirmed OA was tested with Brier score, which is the squared differences between the actual and binary outcomes (278, 279). It can range from 0 for a perfect model to 0.25 for a non-informative model with a 50% incidence of the outcome (280). A score of 0.1 would be better if the events occurred about half the time than if they occurred almost all the time (or almost never). If the events occur most of the time (or very infrequently), the forecaster would issue a probability close to 1 (or 0) most of the time (281). The discriminative ability of the models was determined by the AUC, which showed the relationship between a false positive rate (1-specificity) and a true positive rate (sensitivity). The AUC can range from 0.5 (no discrimination) to 1.0 (perfect discrimination) that reflects the probability that in all possible pairs of subjects (with and without OA), the higher probability is given to the subject with confirmed OA by SIC (282).

The internal validity of the models was assessed using the bootstrapping procedure. This procedure gives a correction factor for both the models' AUCs and for the regression coefficients

of the predictors in the final models (283). The regression coefficients of the predictors in the final models were multiplied by this correction factor to prevent the models from producing over-optimistic predictions when applied in future clinical practice.

The final models were selected based on the greatest increased in the corrected AUCs compared to Model 1. The differences between the AUC of the models along with 95% of CI and SE were calculated using Hanley and McNeil method (282, 284).

2.3.1.2.4. Clinical scores

To facilitate the application of the models in clinical practice, the final model was transformed to a nomogram in S-Plus, in which each of the corrected regression coefficients was converted to easy-to-use number. Nomogram also provides visualization of the corresponding predicted probabilities of the total scores (285). The sum scores were divided into tertiles in order to stratify the subjects into three risk-groups with a low/intermediate/high probability of having OA. The observed number of the outcome, the mean predicted probabilities, sensitivity, specificity, NPV, PPV, +LR and -LR of having OA of each group were calculated.

To evaluate the applicability in clinical practice, diagnostic properties of the high probability group based on the final model were compared with the combination of specific sensitization and NSBHR. Bayesian approach was implemented using the JAGS program called via R package. We used non-informative prior distributions for comparing the sensitivity and specificity of the two methods (286).

2.3.2. Statistical power

As a rule of thumb in prediction modeling, there should be at least 10 events per candidate predictor variable (i.e. 10:1 event per predictor variable (EPV) ratio) to avoid too high or too low

estimates of the outcome (287). This means we needed to have 10 subjects with OA for each predictor included in the model. We had up to six potential predictors and 61 subjects with a positive outcome in the LMW group and 7 potential predictors and 73 subjects with a positive outcome in the HMW group. Given the number of the predictors in each model, the 10:1 EPV ratio is met.

Atopy was missing in 2.9% of the HMW group and 8.8% of the LMW group. Specific sensitization was missing in 10.1% of the HMW group and 92.3% of the LMW group. Induced sputum was missing in 51.1% of the HMW group and 46.7% of the LMW group.

Deleting subjects with a missing value may lead to a loss of statistical power and biased results, whether the missing value occurs at random (MAR) or completely at random (MCAR). Therefore, multiple imputations with regression method were done as the preferred method to complete case analysis (288, 289) for up to 50% missing data (290). Using R package, twenty imputations were created with linear regression method and no interactions were included. However, we did not impute missing data on induced sputum and did not develop a model with it because the test was not done before 2000. In the LMW group, we also did not impute missing data on specific SPT and did not develop a model with it, because it is rarely used for diagnosing OA in subjects exposed to LMW agents. In extra analysis, complete case analysis with deletion of the subjects with any missing value (14%) was performed. Predictors in the final model remained the same; the corrected regression coefficients were slightly changed, and the AUC was not significantly different. Moreover, the diagnostic parameters of tests were also compared before and after imputation. No substantial difference between the two sets of data was found that allowed us to perform analyses of imputed data.

2.3.3. Software

All analyses were performed using SPSS 24.0 for Windows (Statistical Package for Social Sciences, Chicago, IL), S-Plus 6.0 for Windows (Insightful Corp), and R software.

3. Chapter 3

3.1. Manuscript

Development and Validation of Clinical Scores for Diagnosing Occupational Asthma

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Author Contributions: A.C., C.L, and J.A.P. took part in the acquisition and management of data. J.A.P. initiated the study. M.T. performed the analysis and drafted the manuscript under the supervision of E.S. A.C., C.L, J.A.P., and R.C. critically reviewed the manuscript.

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AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

The diagnosis of occupational asthma remains a challenge in clinical practice. Specific inhalation challenge is the reference test for diagnosis. However, few specialized centers provide facilities for this test. No risk prediction model currently quantifies the diagnostic value of respiratory function, induced sputum, and skin-prick tests for estimating the individual risk of occupational asthma in the clinical setting.

What This Study Adds to the Field

In workers exposed to high-molecular-weight agents, we developed a clinical score that includes clinical and exposure characteristics, coupled with work-specific sensitization and methacholine challenge tests. It could quantify an individual's probability of OA and had better diagnostic parameters than a combination of two objective tests but did not reach statistical significance. Our model confirms the necessity of performing both specific sensitization and methacholine challenge test for diagnosing OA in workers exposed to HMW agents, and might help to diagnose OA in centers where access to SIC is difficult or impossible after external validation.

This article has an online data supplement (Appendix A), which is accessible from this issue's table of contents at www.atsjournals.org

Abstract

Rationale: We aimed to evaluate the diagnostic value of current investigational tools, and to develop clinical scores for occupational asthma (OA), defined as a positive specific inhalation challenge (SIC), as an alternative to this standard reference test.

Methods: This retrospective study analyzed data from workers with suspected OA, who were exposed to high-molecular-weight (n=139) and low-molecular-weight agents (n=285) and still working one month before SIC.

Logistic regression models were developed in each exposure group. The value of adding different objective tests to clinical and exposure characteristics was evaluated. The models were tested for accuracy and validated by bootstrapping procedures. The final models were translated into clinical scores and the sum scores were stratified into risk groups.

Measurements and Main Results: In the high-molecular-weight group, a model including sex, age >40 years, symptom duration ≥1 year, rhinoconjunctivitis, inhaled corticosteroid use, methacholine challenge test, and specific skin-prick test (SPT) had good accuracy and internal validation. The high probability category of the predictive model had better specificity and positive predictive value than the combined methacholine challenge test and specific SPT in detecting OA but did not reach the statistical significance. In contrast, the model in the low-molecular-weight group was insufficient for excluding the diagnosis and methacholine challenge test remained the preferable test.

Conclusions: We have developed the first clinical scores for quantifying an individual's probability of OA with good accuracy and reasonable validity in workers exposed to high-molecular-weight agents. External validation of the model is necessary in order be able to use it in clinical practice.

Abstract word count: 253

Keywords: occupational asthma, logistic regression, modeling, validation, clinical scores

Introduction

Occupational asthma (OA) is one the most common occupational lung diseases worldwide (1). It is a variant of work-related asthma (WRA) in relation to exposure to high-molecular-weight (HMW) and/or low-molecular-weight (LMW) sensitizers or inhaled irritants (1-3). The outcome of OA is best when diagnosis and reduction of or removal from exposure is performed at early stages of the disease (1, 4).

The diagnosis of OA remains a challenge in clinical practice. The protocol and tests used for diagnosing OA depend on the country and region. Specific inhalation challenge (SIC), as a reference test, is not performed in all Canadian provinces (4, 5). In Quebec, SIC is recommended to confirm a diagnosis of the disease (6); however, SIC is offered in only a few specialized centers across Canada.

Prediction models and clinical scores can improve secondary prevention by helping physicians to detect individuals with work-related disease, choose appropriate diagnostic tests, and/or decide to refer workers for further evaluation (7). None of the diagnostic guidelines used prediction models to estimate the individual probability of the presence of OA, or to accurately combine the available objective tools and clinical and work-related characteristics of at risk population in order to facilitate the diagnostic pathway.

In the Netherlands, a surveillance program for bakers (8) has been based on a risk prediction questionnaire model and scores (9) for detection of work-related sensitization. Application of this model allows a step-wise approach, where only bakers with an elevated risk of sensitization to flour are referred for further medical examination for work-related allergy. Further study was done by Jonaid et al. (10) among these referred bakers. Two models for predicting bakers' asthma were developed using a self-administered questionnaire and the medical history. These

diagnostic models could differentiate satisfactorily bakers with a higher probability of OA (confirmed with the presence of asthma symptoms, specific sensitisation, and non-specific bronchial hyper-responsiveness (NSBHR)) from those bakers with a lower probability of disease with a very high sensitivity and specificity but very low predictive values.

To our knowledge, there is no risk prediction model for detecting OA in the clinical setting that evaluates the added value of objective diagnostic test. The aim of this study was to develop prediction models and scores for OA, using information from the subjects' clinical and exposure characteristics, skin-prick test (SPT), spirometry, and methacholine challenge test. Development and validation of such a model and score production could facilitate secondary prevention by quantifying individual's probability of diagnosing OA. It enables physicians to optimise the risk estimation and to detect workers with a higher risk of OA in a timely manner (7).

Methods

We performed a retrospective study, according to Standards for Reporting Diagnostic Accuracy (STARD) guidelines, and analysed a database of workers referred to the Hôpital du Sacré-Cœur de Montréal, Montreal, Quebec, Canada, between 1983 and 2011 for a suspicion of OA. For the purpose of model development, we included all workers exposed to either LMW or HMW agents who were still working one month prior to SIC. Among the study population (n=424), 285 workers were exposed to LMW agents and 139 to HMW agents (Figure 1). They presented with lower respiratory symptoms, including dyspnea, cough, wheezing, and/or chest tightness. The database contains information about symptoms, exposure characteristics, and the results of different tests performed during the diagnostic work-up, including spirometry, peak expiratory flow (PEF) monitoring, SPT, induced sputum, methacholine challenge, and SIC. Pralong et al.

(11) described the process of data extraction of demographic, clinical, and exposure characteristics of subjects as well as the standardized methods used for performing SIC (12-15), spirometry (16, 17), methacholine challenge test (15, 18), induced sputum test (19), and SPT (20). This study was performed with approval from the Hôpital du Sacré-Cœur de Montréal's ethics committee in accordance with Canadian ethical rules.

To identify OA, the reference standard was a positive SIC, namely a sustained fall in forced expiratory volume in one second (FEV1) >20% of the baseline value after exposure to the suspected occupational agent.

We analyzed data on subjects' demographic and clinical characteristics, including age (>40/≤40 years, the median age of subjects), sex (male/female), smoking habit (current smoker, ever smoked, never smoked), the presence of rhinoconjunctivitis (yes/no), duration of lower respiratory symptoms (>1/≤1 year), and inhaled corticosteroid use (yes/no).

We included exposure duration (>7/≤7 years, the median of subjects' exposure duration) and working status prior to SIC (still working one month prior to SIC or off-work).

Atopy and specific sensitization to occupational agents were defined as at least one positive SPT reaction to 11 common aeroallergens and to the specific occupational agent(s), respectively.

Reduced pulmonary function at the time of diagnosis was defined as FEV1 percentage predicted ≤80%. Non-specific bronchial hyper-responsiveness (NSBHR) was defined as the provocative concentration of methacholine causing a 20% decrease in FEV1 (PC20) ≤16 mg/ml. Cut-off points of ≥2% (21) and ≥60% (22, 23) were set for sputum eosinophilia and neutrophilia, respectively.

Statistical Analysis

Descriptive statistics of demographic and clinical characteristics as well as measurements from objective tests and SICs were calculated, according to type of exposure (i.e., LMW/HMW groups). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of each predictor were calculated and compared between original and imputed data. In addition, positive and negative likelihood ratio (+LR/–LR), the discriminative ability (i.e. through the measurement of the area under the receiver operating characteristic (ROC) curve (AUC)) of each objective test were calculated.

Bivariable logistic regression analysis was carried out to examine the relationship between each predictor and the diagnosis of OA. Subsequently, multiple logistic regression with backward stepwise selection, using an inclusion criterion of p value <0.157 (i.e., Akaike information criterion), was carried out when developing model with clinical and exposure characteristics (i.e. Model 1) in order to include all potential predictors (24-26). Different objective tests were added to Model 1 to create new models: specific sensitization in Model 2; atopy in Model 3; low FEV1 in Model 4; NSBHR in Model 5; all objective tests in Model 6; specific sensitization and NSBHR in Model 7. Models were developed separately in each exposure group. Models with specific sensitization were only developed in the HMW group, because this test is rarely done in the LMW group due to its limited availability and potential utility for diagnosis, considering the pathogenic mechanisms (27).

The accuracy of models was quantified using calibration and discrimination measure (Brier score (28) and area under the receiver operating characteristics curve (AUC) (29), respectively). Models were also validated internally using bootstrapping procedures (30). These procedures have been described thoroughly in the literature (9, 31). The final models were selected based on the greatest increased of the corrected AUCs compared to Model 1. The differences between the

AUC of models, along with a 95% confidence interval (CI) and standard error, were calculated using the Hanley and McNeil method (30).

To facilitate the application of models in clinical practice, the final model was transformed to a nomogram in S-Plus, where each of the corrected regression coefficients was converted to easy-to-use number. The sum scores were divided into tertiles in order to stratify the subjects into three risk-groups with a low/intermediate/high probability of OA. Finally, the observed number of the outcome, mean predictive probabilities, and diagnostic properties for each group were calculated.

To evaluate the applicability in clinical practice, diagnostic properties of the high probability group based on the final model were compared with the combination of specific sensitization and NSBHR. Bayesian approach was implemented using the JAGS program called via R package. We used non-informative prior distributions for comparing the sensitivity and specificity of the two methods (32).

Atopy was missing in 2.9% of the HMW group and 8.8% of the LMW group. Specific sensitization was missing in 10.1% of the HMW group. Induced sputum was missing in 51.1% of the HMW group and 46.7% of the LMW group.

Deleting subjects with a missing value may lead to a loss of statistical power and biased results, whether the missing value occurs at random (MAR) or completely at random (MCAR). Therefore, multiple imputations with regression method were done as the preferred method to complete case analysis (33, 34) for up to 50% missing data (35). Using R package, twenty imputations were done with linear regression method and no interactions were included. The diagnostic parameters of tests were compared before and after imputation. No substantial difference between the two sets of data was found that allowed us to perform analyses in imputed

data. However, we did not impute missing data on induced sputum, because the test was not done before 2000. We also did not impute missing data on specific SPT in the LMW group, because it is rarely used for diagnosing OA in subjects exposed to LMW agents.

Analyses were performed using SPSS 24.0 for Windows (Statistical Package for Social Sciences, Chicago, IL), S-Plus 8.0.1 for Windows (Insightful Corp), and R software.

Results

Overall, positive SICs confirming OA were observed in 31.6% of workers (21.4% in the LMW group; 52.5% in the HMW group). In the HMW group, workers with OA presented with more rhinoconjunctivitis symptoms and had a longer duration of lower respiratory symptoms, compared to subjects without OA (Table 1). Additionally, they were more sensitized to common and work-specific allergens. The majority of subjects with a positive SIC had a positive NSBHR (93.2% in the HMW group; 90.2% in the LMW group). Workers with a negative SIC had a statistically significantly lower rate of positive NSBHR ($p < 0.001$), a lower rate of sensitization to work-specific agents ($p < 0.001$), and a lower sputum eosinophilia ($p = 0.001$). They also had a lower duration of exposure to causal agents ($p = 0.233$), and higher sputum neutrophilia ($p = 0.693$) compared to those with positive SICs, but there differences were not statistically significant.

The diagnostic properties of each predictor and its AUCs, along with 95% CI, appear in Table 2. Among the objective tests, the highest sensitivity belongs to NSBHR test (90.2%) in the LMW group, and to specific sensitization (94.5%) in the HMW group. The highest NPV belongs to specific sensitization (90.7%) in the HMW group and to NSBHR test (93.7%) in the LMW group. None of the objective tests presented high PPV values in either exposure group.

HMW Group

Seven models were developed in 139 workers exposed to HMW agents who were still working one month before SIC (Table 3).

Adding NSBHR and specific sensitization to Model 1 (i.e., Model 7) statistically significantly increased the AUC compared to Model 1, Model 2, and Model 5. Figure 1 (see Online Data Supplement, Appendix A) shows the ROC curves of Models 1, 2, 5, and 7.

Model 7 was chosen as the final model for diagnosing OA in the HMW group based on its highest increase in AUC compared to Model 1. This Model also had the best calibration (i.e. lowest Brier score). From bootstrap validation, this model also had a correction factor of 0.82 indicating a reasonable internal validity.

The final model was transformed to clinical scores (Table 4). For example, a male subject who was exposed to HMW agents, 45 years of age, without rhinoconjunctivitis, with lower respiratory symptoms for ≤1 year, who used inhaled corticosteroids, and had specific sensitization and NSBHR (sum score: 56+0+0+0+24+100+79=259) has a probability of OA of 78.1%. In contrast, if this subject did not have BHR nor specific sensitization (sum score: 56+0+0+0+24+0+0=80), the probability of OA would be 0.6%.

The sum scores were divided into tertiles (Table 5). The group with a sum scores ≥230 had the highest number of subjects with OA (n=61/68, 89.7%) and the highest PPV and NPV, compared to other groups. The +LR in this group also showed a moderate increase in the likelihood of OA.

LMW Group

Five models were developed in 285 subjects exposed to LMW agents who were still working one month before SIC (Table 6).

Model 1 consists of sex, age >40 years, symptoms duration >1 year, and exposure duration >7 years. Adding NSBHR to Model 1 (Model 4) statistically significantly enhanced the discriminative ability of the model (delta AUC=0.06, 95% CI: 0.02–0.10). The discriminative abilities of other models were similar to Model 1. The calibration of Model 4 was not good (Brier score <0.147) but it had reasonable internal validity (the correction factor was 0.88 after bootstrap validation). Adding atopy to common allergens and low FEV1 to Model 1 (i.e. Model 2 and Model 3, respectively) did not statistically significantly change the AUC of Model 1.

Model 4 was chosen as the final model for diagnosing OA in the LMW group and converted to clinical scores (Table 7). For example, a male worker of 45 years of age with 5 years of exposure to LMW agents, who had lower respiratory symptoms for only one year and a negative NSBHR test (sum scores: 65+0+0+50+0=115), has a probability of OA of 8.3%. In contrast, if the worker also had NSBHR (sum score 65+0+0+50+100=215), the probability of OA increases to 33.7%.

The sum scores were divided into tertiles (Table 8). The group with the highest sum scores (i.e., 180–268) had the highest number of workers with OA (n=48/146, 32.9%) and the highest diagnostic parameters, compared to groups with a low or intermediate probability of OA.

Sensitivity Analysis

In subjects exposed to HMW agents, an extra model was developed to assess the discriminative ability of combining the methacholine challenge test and work-specific SPT without clinical and exposure characteristics. The AUC was 0.848 (95% CI: 0.71–0.91): it was higher than Model 1, but lower than Model 7 (delta AUC=0.06, 95% CI: 0.01-0.11). Should workers with two positive

tests (n=72, 51.8%) be referred, the diagnostic properties were as follows: sensitivity=82.2%; specificity=81.8%; PPV=83.3%; NPV=80.6%; +LR=4.52 (95% CI: 2.68–7.63); –LR=0.22 (95% CI: 0.13–0.36). Based on the final model, the high risk group has better diagnostic properties than the combined objective tests (PPV: 89.7% vs. 83.3%; specificity: 89.4% vs. 81.8%, respectively). However, these differences were not statistically significant.

DISCUSSION

We developed the clinical models and scores for quantifying an individual's probability of OA by clinical and exposure characteristics, along with objective tests other than SIC, in a population of workers exposed to either LMW or HMW agents in the workplace. To the best of our knowledge, this has not been addressed in the previous studies.

In accordance with the European Respiratory Society (ERS) guideline for the management of work-related asthma (WRA), the specific SPT (single test) in subjects exposed to HMW agents showed a high sensitivity (93.2%) but low specificity (55.8%), when compared to the SIC (4). The sensitivity of the test in our data was higher than the reported pooled estimation of sensitivity (80.6%) in a systematic review by Beach et al. (36), although the specificity did not differ significantly (pooled estimation of specificity=59.6%). NSBHR testing also showed a high sensitivity in both LMW (90.2%) and HMW (93.1%) groups, which was also higher than the pooled estimation of the sensitivity in the same systematic review (36) (79.3% in exposure to HMW agents; 66.7% in exposure to LMW agents). The low specificity of both tests is consistent with the results of other studies (4, 36).

Clinical and exposure characteristics were chosen for use in models based on published literature. Contradictory results have been reported for the association between smoking and OA

(37-40). In our study, smoking was not selected in the final models due to its weak association with OA in both exposure-specific groups.

All finals models showed good discrimination and reasonable internal validity in predicting OA. They were translated into clinical scores and stratified into risk groups. In both exposure groups, the mean predictive probability in the high probability groups was close to the observed OA rate, which indicates good calibration of the scoring rule.

Implications for Clinical Practice

Beach and colleagues concluded that the diagnosis of OA in the absence of SIC should preferably be determined by the combination of a NSBHR and specific sensitization tests, whenever possible (36). In accordance, our final model (Model 7) for workers exposed to HMW agents demonstrates that adding the combination of both tests to clinical and exposure characteristics resulted in the highest discriminative ability in diagnosing OA. It also had statistically significantly higher AUC than the combined two tests. This model emphasizes the necessity of performing both objective tests for diagnosing OA in workers exposed to HMW agents. Based on our final model, the high-risk group also demonstrated better PPV and specificity than the combined objective tests. However, the differences were not statistically significant, probably due to the small size of this subgroup comparison. As a result, the external validation of the model is necessary before using as a diagnostic tool in clinical practice.

In contrast, in workers exposed to LMW agents, the high probability group of the final model had a low PPV, specificity and +LR. In addition, it had a lower NPV for excluding the diagnosis of OA, compared to the methacholine challenge test alone (92.0 vs. 93.7, respectively).

Therefore, for this group of workers, performing a methacholine challenge test remains the preferable method for excluding OA in the absence of SIC.

The higher AUC and better diagnostic properties of the final model in the HMW group compared to the final model in the LMW group suggest that the incorporation of the specific sensitization in the final model might have an important role in improving the likelihood of diagnosing OA. This is in agreement with the systematic review of Beach et al. (36) that also reported a sensitivity of 100% and a specificity of 80.0% for the combined specific sensitization and NSBHR in the LMW group, which was higher than NSBHR alone in the same group (the pooled estimate of sensitivity and specificity were 66.7% and 63.9%, respectively) suggesting the great role of specific SPT in diagnosis of OA.

Our proposed diagnostic model for workers exposed to HMW agents with suspicion of OA targets the secondary and tertiary levels of prevention, and may contribute in cost reduction. The use of the model would be restricted to specialists with the possibility of ordering both skin-prick test and methacholine challenge test. The estimation of the individual's probability of OA can guide physicians in deciding upon further diagnostic testing and referrals; when the probability of the presence of OA is relatively high, further testing (SIC) is indicated; when the probability is low, no further diagnostic testing and/or referral is necessary, and patients should be evaluated for other probable diseases that mimic the asthma symptoms. Those patients with intermediate probability of OA should be re-evaluated after a certain period of time before referring to specialized centers. Providing patients with a more accurate risk assessment combined with an appropriate insight about the prognosis of the disease by physicians would allow workers to make a more educated decision about whether to perform further diagnostic testing and to start an appropriate treatment. Moreover, this model could be used in the assessment of workers with

workers' compensation claims for occupational asthma, in areas without access to SIC evaluations. Application of this model would also aid physicians at the secondary care level (specialists: i.e. respirologists, occupational medicine physicians) in identifying workers who have a high probability of having OA, in order to decide whether they should refer their patients to a tertiary center (41). This model is less beneficial in tertiary centers where SICs are systematically performed.

Study Strengths and Limitations

This study has some limitations. The highly selective population of this study may limit the application of models to clinical settings. Additionally, the model has not been externally validated, which is crucial for confirming the satisfactory performance of the model and generalizability of results to other populations of workers (42, 43). Nevertheless, the correction factor of ≥0.82 after the bootstrapping procedure indicates a reasonable internal validity of all final models (42).

The retrospective design of this study did not allow us to develop models to examine the ability of induced sputum testing to predict OA, since this test had not been administered in workers with suspected OA before 2000. Given the high rate of missing values, the existing database did not provide enough power for developing models with this test.

SIC is subjected to false-negative results due to imprecise techniques of the SIC, exposure to unknown or multiple agents, and absence of specific BHR when workers are off-work for a prolonged period (44-46). An increase in false-negative rate may decrease the power of the test, and the sensitivity of the prediction model. The technical errors causing false-negative results

were less likely to be occurred since the HSCM is a specialised center for the SIC with highly qualified trained staff.

Although SICs were performed according to standard protocols, no measurement of exposure levels was reported before and during the test. De Olim et al. (47) reported that among patients who had negative SICs, there is at least one other sensitizer potentially present in their workplace that was not included in the SIC because it had not been identified by physician assessment (62% of negative SICs). It may affect the degree to which the SIC can be considered as a 'gold standard' for OA diagnosis. In the absence of a gold standard, the case definition (having or not having OA) would be sub-optimal.

The strength of this study lies in using a database with high numbers of workers with confirmed OA in both LMW and HMW groups. As a rule of thumb, in prediction modeling, there should be at least 10 events per predictor variable (i.e., 10:1 event per predictor variable (EPV) ratio) to avoid too high or too low estimates of the outcome (48). Six predictors and 61 subjects had a positive outcome in the LMW group and 7 predictors and 73 subjects had a positive outcome in the HMW group; therefore, the 10:1 EPV ratio in each exposure group was maintained.

We had a total of 14% subjects with missing SPT results. We performed multiple imputation for handling the missing data. Imputation of missing values is superior to complete case analyses since the later method may bias the accuracy of the model in terms of selection of predictor, estimation of the regression coefficients, and the corresponding standard errors (49). In extra analysis, complete case analysis was performed and resulted in the same predictors in the final models and the corrected regression coefficients were slightly changed. The AUC was not significantly different. Therefore, further analyses were performed in imputed data.

We conclude that in workers exposed to LMW agents, the model did not offer a better diagnostic value than the methacholine challenge test alone. In workers exposed to HMW agents with suspicion of OA, our diagnostic model, which incorporated clinical and exposure characteristics with specific SPT and methacholine challenge tests, quantifies an individual's probability of OA with a statistically higher AUC than the combined tests results. The group with high probability of OA showed better diagnostic parameters than combination of the two objective tests, although it did not reach the statistical significance. It confirms the necessity of performing both objective tests for diagnosing asthma in the HMW group. Nevertheless, the model needs to be externally validated in other populations of workers at risk of OA in order to assess its generalizability and potential implications in the clinical practice.

Conflict of Interest Statement:

C. Lemière has received consultancy fees from GlaxoSmithKline Inc. (Canada), AstraZeneca Canada, Teva Canada Innovation, and Merck Canada Inc.; research support from GlaxoSmithKline Inc. (Canada); lecture fees from AstraZeneca Canada and Merck Canada Inc; and royalties from UpToDate. A. Cartier has received research support from Merck Canada Inc. and AstraZeneca Canada; lecture fees from Merck Canada Inc.; and royalties from UpToDate. The rest of the authors declare that they have no relevant conflicts of interest.

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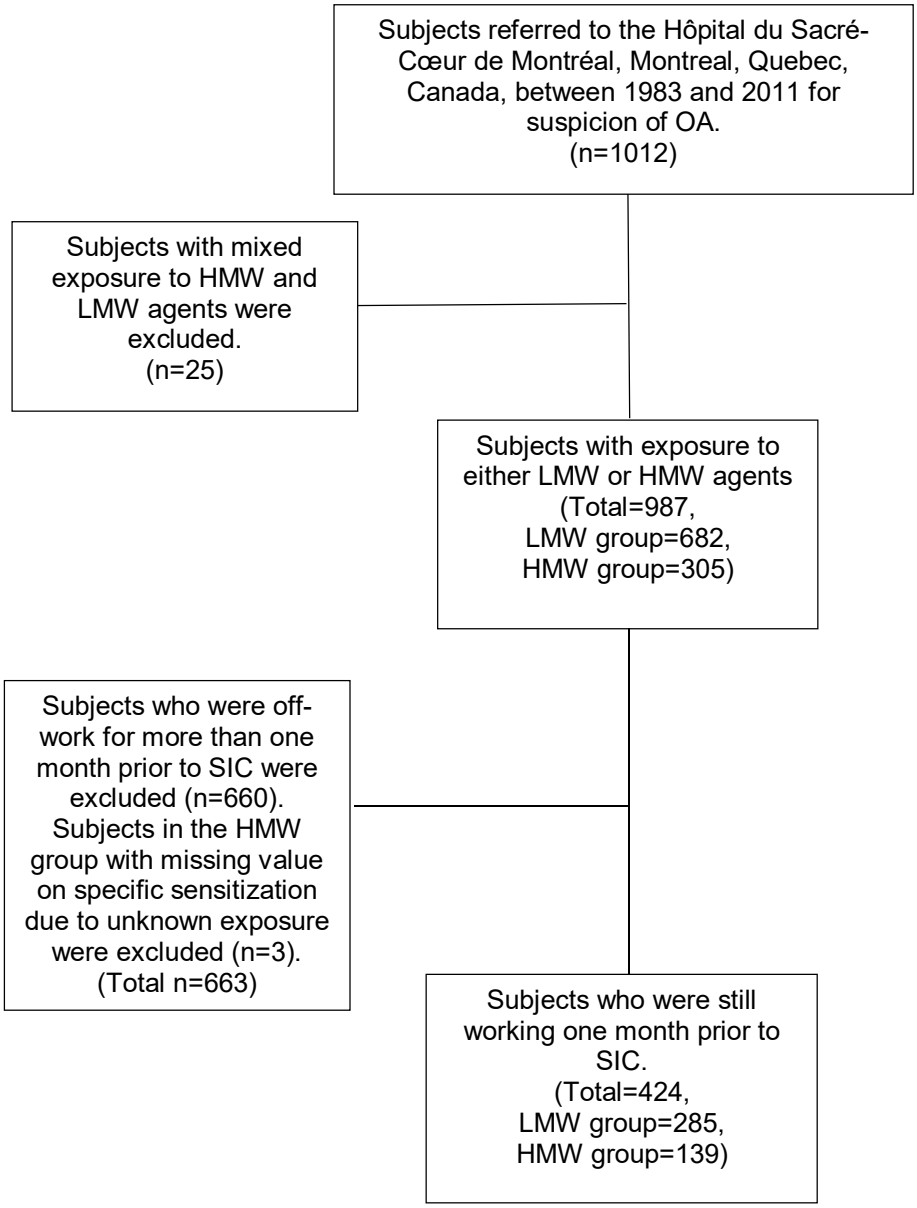

FIGURE 1. THE SELECTION PROCESS FOR STUDY POPULATION

TABLE 1. DISTRIBUTION AND ASSOCIATION BETWEEN THE PREDICTORS AND OA BY TYPE OF AGENTS IN SUBJECTS WORKING ONE MONTH PRIOR TO SIC

	HMW group					LMW group				
	OA	**Non-OA**	**Total**	**OR**	**95% CI**	**OA**	**Non-OA**	**Total**	**OR**	**95% CI**
	N (%)	N (%)	N (%)			N (%)	N (%)	N (%)		
Diagnosed OA	73 (52.5)	66 (47.5)	139			61 (21.4)	224 (78.6)	285		
Clinical characteristics										
Sex (female)	16 (21.9)	39 (59.1)	55 (39.6)	0.19	0.09-0.41	9 (14.8)	69 (30.8)	78 (27.4)	0.39	0.18-0.83
Age	37.6 (1.14)	40.9 (1.43)	39.2 (0.92)	0.97	0.94-1.00	38.1 (1.32)	41.9 (0.70)	41.08 (0.62)	0.96	0.94-0.99
Age >40 years	27 (37.0)	34 (51.5)	61 (43.9)	0.55	0.28-1.08	21 (34.4)	122 (54.5)	143 (50.2)	0.44	0.24-0.79
Smoking habit[a]										
Current smoker	19 (26.0)	15 (22.7)	34 (24.5)	1.31	0.56-3.08	12 (19.7)	57 (26.1)	69 (24.7)	0.69	0.32-1.48
Ever smoked	26 (35.6)	22 (33.3)	48 (34.5)	1.22	0.57-2.64	24 (39.3)	80 (36.7)	104 (37.3)	0.98	0.52-1.85
Never smoked	28 (38.4)	29 (43.9)	57 (41.0)	Ref	Ref	25 (41.0)	81 (37.2)	106 (38.0)	Ref	Ref
Presence of rhinoconjunctivitis	18 (24.7)	9 (13.6)	27 (19.4)	2.07	0.86-5.01	13 (21.3)	32 (14.3)	45 (15.8)	1.62	0.79-3.33
Symptom duration >1 year[b]	54/73 (74.0)	36/64 (56.3)	90/137 (65.7)	2.30	1.12-4.73	35/61 (57.4)	111/219 (50.7)	146/280 (52.1)	1.30	0.73-2.30
Inhaled corticosteroid usage[c]	44 (60.3)	34 (51.5)	78 (56.1)	1.42	0.73-2.80	33/61 (54.1)	102/222 (45.9)	135/283 (47.7)	1.38	0.78-2.44
Exposure characteristics										
Exposure duration >7 years[d]	47/73 (64.4)	35/64 (54.7)	82/137 (59.9)	1.49	0.75-2.97	25/60 (41.7)	128/218 (58.7)	153/278 (55.0)	0.49	0.28-0.87
Objective tests prior to SIC										
SPT-based sensitization										
Atopy to common allergen(s)[e]	67/72 (93.1)	48/63 (76.2)	115/135 (85.2)	4.12	1.40-12.09	39/58 (67.2)	144/202 (71.3)	183/260 (70.4)	0.88	0.48-1.64

Sensitization to work-specific agent[f]	65/69 (94.2)	24/56 (42.9)	89/125 (71.2)	19.36	6.39-58.66	-	-	-	-	-
BHR	68 (93.2)	38 (57.6)	106 (76.3)	10.02	3.57-28.09	55 (90.2)	135 (60.3)	190 (66.7)	6.04	2.49-16.63
Low predicted FEV1 <80%	14 (19.2)	11 (16.7)	25 (18.0)	1.17	0.49-2.83	16 (26.2)	43 (19.2)	59 (20.7)	1.49	0.77-2.89
Induced sputum										
Sputum eosinophil count ≥2%[g]	15/33 (45.5)	6/35 (17.1)	21/68 (30.9)	3.09	1.07-8.91	9/27 (33.3)	32/125 (25.6)	41/152 (27.0)	1.71	0.77-3.78
Sputum neutrophil count ≥60%[h]	10/33 (30.3)	13/35 (37.1)	23/68 (33.8)	0.88	0.33-2.34	7/27 (25.9)	24/125 (19.2)	31/152 (20.4)	1.37	0.56-3.35

[a]missing=6; [b]missing=2 in HMW group, 5 in LMW group; [c]missing=2 in LMW group; [d]missing=2 in HMW group, 7 in LMW group; [e]missing=4 in HMW group, 25 in LMW group; [f]missing=14 in HMW group, 263 in LMW group; [g]missing=71 in HMW and 71 in LMW group; [h]missing=71 in HMW group and 71 in LMW group

TABLE 2. DIAGNOSTIC VALUE OF EACH PREDICTOR OF OA BY TYPE OF AGENTS IN SUBJECTS WORKING ONE MONTH BEFORE THE SIC*

	Sensitivity (%)		Specificity (%)		PPV (%)		NPV (%)		LR				AUC (95% CI)	
									Positive		Negative			
Type of exposure	**HMW**	**LMW**	**HMW**	**LMW**	**HMW**	**LMW**	**HMW**	**LMW**	**HMW**	**LMW**	**HMW**	**LMW**	**HMW**	**LMW**
Clinical & exposure characteristics														
Sex (female)	21.9	14.8	40.9	69.2	29.1	11.5	32.1	74.9						
Age >40 years	37.0	34.4	48.5	45.5	44.3	14.7	41.0	71.8						
Smoking habit														
Current smoker	26.0	19.7	77.3	74.1	55.9	17.1	48.6	77.2						
Ever smoked	35.6	39.3	66.7	62.5	54.2	22.2	48.3	79.1						
Presence of rhinitis	24.7	21.3	86.4	85.7	66.7	28.9	50.9	80.0						
Symptom duration >1 year	73.9	57.4	43.9	49.5	59.3	23.6	60.4	81.0						
Inhaled corticosteroid usage	60.3	54.1	48.5	54.0	56.4	24.3	52.5	81.2						

Exposure duration >7 years	64.4	41.0	47.0	41.0	57.3	15.9	54.4	71.9						
Objective tests														
Atopy to common allergen(s)	93.1	68.8	25.8	29.0	58.1	20.9	77.3	77.4	1.25	0.97	0.27	1.07	0.602 (0.51-0.70)	0.498 (0.42-0.58)
Sensitization to work-specific agents	94.5	-	59.1	-	71.9	-	90.7	-	1.98	-	0.13	-	0.791 (0.71-0.87)	-
BHR	93.1	90.2	42.4	39.7	64.1	28.9	84.8	93.7	1.62	1.50	0.16	0.25	0.678 (0.59-0.77)	0.649 (0.58-0.72)
Low predicted FEV1 <80%	19.2	26.2	83.3	80.8	56.0	27.1	48.2	80.1	1.15	1.37	0.97	0.91	0.487 (0.39-0.58)	0.465 (0.38-0.55)
Induced sputum														
Sputum eosinophil count ≥2%	45.4	33.3	82.9	74.4	71.4	21.9	61.7	83.8	2.65	1.30	0.66	0.90	0.642 (0.51-0.77)	0.591 (0.51-0.67)
Sputum neutrophil count ≥60%	30.3	25.9	62.9	80.8	43.5	22.6	48.9	83.5	0.82	1.35	1.11	0.92	0.466 (0.33-0.60)	0.597 (0.51-0.68)

*The diagnostic properties were only recalculated in the imputed data, except for induced sputum tests that were not imputed.

TABLE 3. THE MULTIVARIABLE MODELS IN WORKERS EXPOSED TO HMW AGENTS

	Clinical & exposure characteristics	**Model 1 + sensitization to work-specific agents**	**Model 1 + atopy to common allergens**	**Model 1 + low FEV1**	**Model 1 + BHR testing**	**Model 1 + all tests**	**Model 1 + sensitization + BHR**
Corrected β	**Model 1**	**Model 2**	**Model 3**	**Model 4**	**Model 5**	**Model 6**	**Model 7**
Intercept	-0.1	-2.62	-1.16	0	-1.75	-4.61	-4.80
Sex	-1.42	-1.42	-1.40	-1.32	-1.68	-1.66	-1.79
Age >40 years	-0.78	-0.45	-0.63	-0.74	-0.79	-0.31	-0.36
Rhinoconjunctivitis symptoms	0.82	0.88	0.90	0.77	0.91	1.02	1.06
Symptom duration >1 year	1.03	1.15	0.93	0.95	1.12	1.21	1.31
Inhaled corticosteroid usage	0.53	0.85	0.49	0.48	0.36	0.69	0.76
Atopy to common allergens			1.24			0.27	
Sensitization for work-specific agents		2.90				2.84	3.19
Low FEV1% (<80%)				-0.09		0.04	
BHR					2.29	2.33	2.52
ROC area (95% CI)	0.770 (0.69-0.85)	0.877 (0.81-0.94)	0.790 (0.71-0.87)	0.770 (0.69-0.85)	0.851 (0.78-0.92)	0.926 (0.89-0.98)	0.927 (0.88-0.98)
Brier score	0.193	0.130	0.183	0.193	0.156	0.10	0.098
Corrected AUC (95% CI)	0.745 (0.67-0.82)	0.857 (0.80-0.92)	0.759 (0.68-0.83)	0.731 (0.65-0.81)	0.830 (0.76-0.90)	0.902 (0.85-0.95)	0.911 (0.86-0.96)

*delta AUC of Model 1 and Model2 = 0.11; 95% CI: 0.07–0.15; delta AUC of Model 1 and Model5 = 0.08; 95% CI: 0.02–0.14; delta AUC of Model 1 and Model 7 = 0.16, 95% CI: 0.10–0.23; delta AUC of Model 2 and Model 7 = 0.05; 95% CI: 0.01–0.09; delta AUC of Model 5 and Model 7 = 0.08, 95% CI: 0.03–0.13.

TABLE 4. CLINICAL SCORES OF THE FINAL MODEL IN WORKERS EXPOSED TO HMW AGENTS

Predictors	Value	Clinical scores
Sex	Male	56
	Female	0
Age	>40 years	0
	≤40 years	11
Rhinoconjunctivitis symptoms	Present	33
	Absent	0
Symptom duration	>1 year	41
	≤1 year	0
Inhaled corticosteroid usage	Positive	24
	Negative	0
Sensitization to work-specific allergens	Positive	100
	Negative	0
BHR	PC20 ≤16 mg/ml	79
	PC20 >16 mg/ml	0

TABLE 5. RISK STRATIFICATION OF SUM SCORES FOR DIAGNOSING OA IN SUBJECTS EXPOSED TO HMW AGENTS

Probability of OA	**Low**	**Intermediate**	**High**
Sum Scores	0-114	115-229	230-344
N	25	46	68
Observed OA (N)	0	12	61
Observed OA (%)	0.0	26.1	89.7
Mean predictive probability (%)	0.1	24.8	85.3
PPV (%)	0.0	26.1	89.7
NPV (%)	36.0	34.4	83.1
Sensitivity (%)	0.0	16.44	83.6
Specificity (%)	62.1	48.5	89.4
+LR	0.0	0.32 (0.18-0.56)	7.88 (3.88-15.99)
-LR	1.61 (1.33-1.94)	1.72 (1.32-2.25)	0.18 (0.11-0.31)
Inhaled corticosteroid usage (%)*	11 (14.1)	27 (34.6)	44 (51.3)

*information available in 78 subjects

TABLE 6. THE MULTIVARIABLE MODELS IN WORKERS EXPOSED TO LMW AGENTS

	Clinical & exposure characteristics	**Model 1 + atopy to common allergens**	**Model 1 + low FEV1**	**Model 1 + BHR testing**	**Model 1 + all tests**
Corrected	**Model 1**	**Model 2**	**Model 3**	**Model 4**	**Model 5**
Intercept	-0.69	-0.46	-0.41	-1.81	-1.36
Sex	-0.97	-0.92	-0.90	-0.97	-0.98
Age >40 years	-0.52	-0.52	-0.52	-0.48	-0.53
Symptoms ≥1 year	0.49	0.49	0.43	0.32	0.35
Exposure duration >7 years	-0.83	-0.82	-0.78	-0.74	-0.77
Atopy to common allergens		-0.35			-0.39
Low FEV1% (<80%)			-0.37		-0.14
BHR				1.49	1.44
ROC area (95% CI)	0.688 (0.61-0.76)	0.701 (0.63-0.77)	0.695 (0.62-0.77)	0.752 (0.69-0.81)	0.765 (0.70-0.83)
Brier score	0.156	0.155	0.154	0.147	0.146
Corrected AUC (95% CI)	0.665 (0.59-0.74)	0.659 (0.58-0.73)	0.664 (0.59-0.74)	0.728 (0.66-0.79)	0.725 (0.66-0.79)

TABLE 7. CLINICAL SCORES OF THE FINAL MODEL IN SUBJECTS EXPOSED TO LMW AGENTS

Predictors	Value	Clinical scores
Sex	Male	65
	Female	0
Age	>40 years	0
	≤40 years	32
Symptom duration	>1 year	21
	≤1 year	0
Exposure duration >7 years	≤7 years	50
	>7 years	0
BHR	PC20 ≤16 mg/ml	100
	PC20 >16 mg/ml	0

TABLE 8. RISK STRATIFICATION OF THE SUM SCORES FOR DIAGNOSING OA IN WORKERS EXPOSED TO LMW AGENTS

Probability of OA	**Low**	**Intermediate**	**High**
Sum Scores	0-89	90-179	180-268
N	54	85	146
Observed OA (n)	1	12	48
Observed OA (%)	1.9	14.1	32.9
Mean predictive probability (%)	3.5	11.8	33.4
PPV (%)	1.9	14.3	31.9
NPV (%)	74.0	75.6	92.0
Sensitivity (%)	1.6	19.7	78.7
Specificity (%)	76.3	67.9	55.8
+LR	0.07 (0.01-0.49)	0.61 (0.36-1.05)	1.78 (1.46-2.17)
-LR	1.29 (1.19-1.40)	1.18 (1.02-1.38)	0.38 (0.23-0.63)
Inhaled corticosteroid usage (%)*	14 (10.4)	38 (27.9)	84 (61.8)

*Information available in 136 subjects

APPENDIX A.

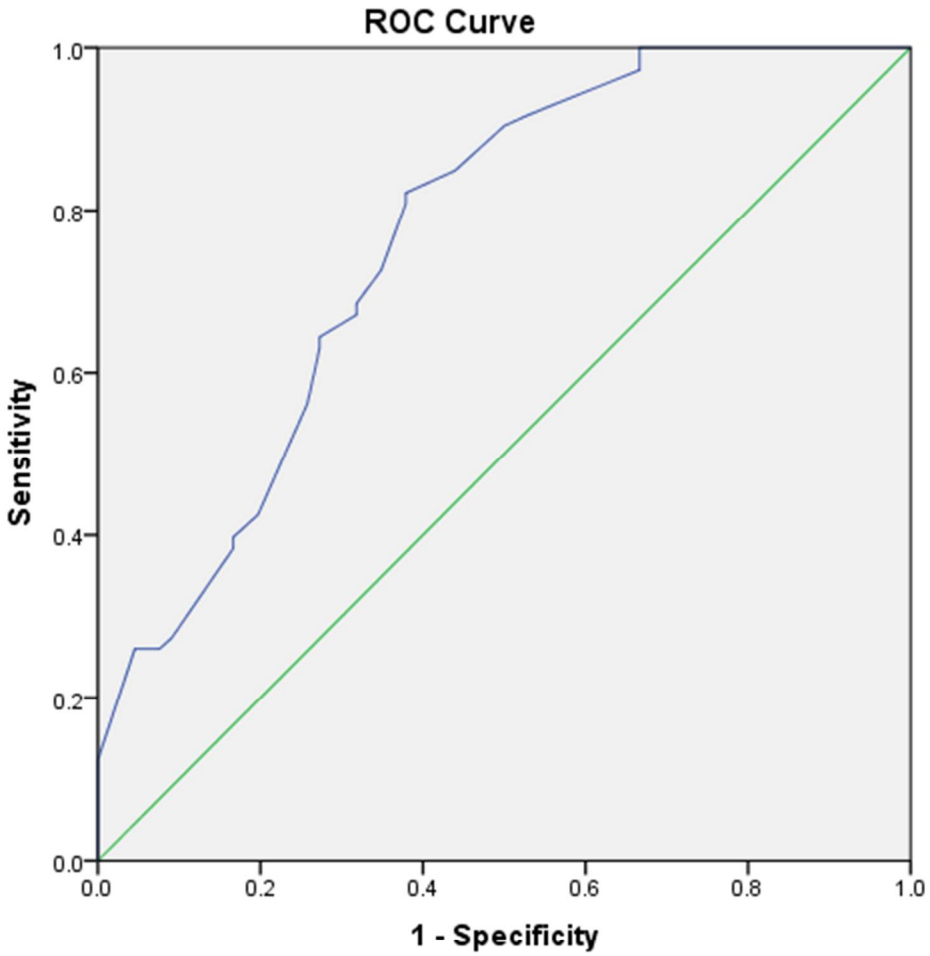

Figure 1A

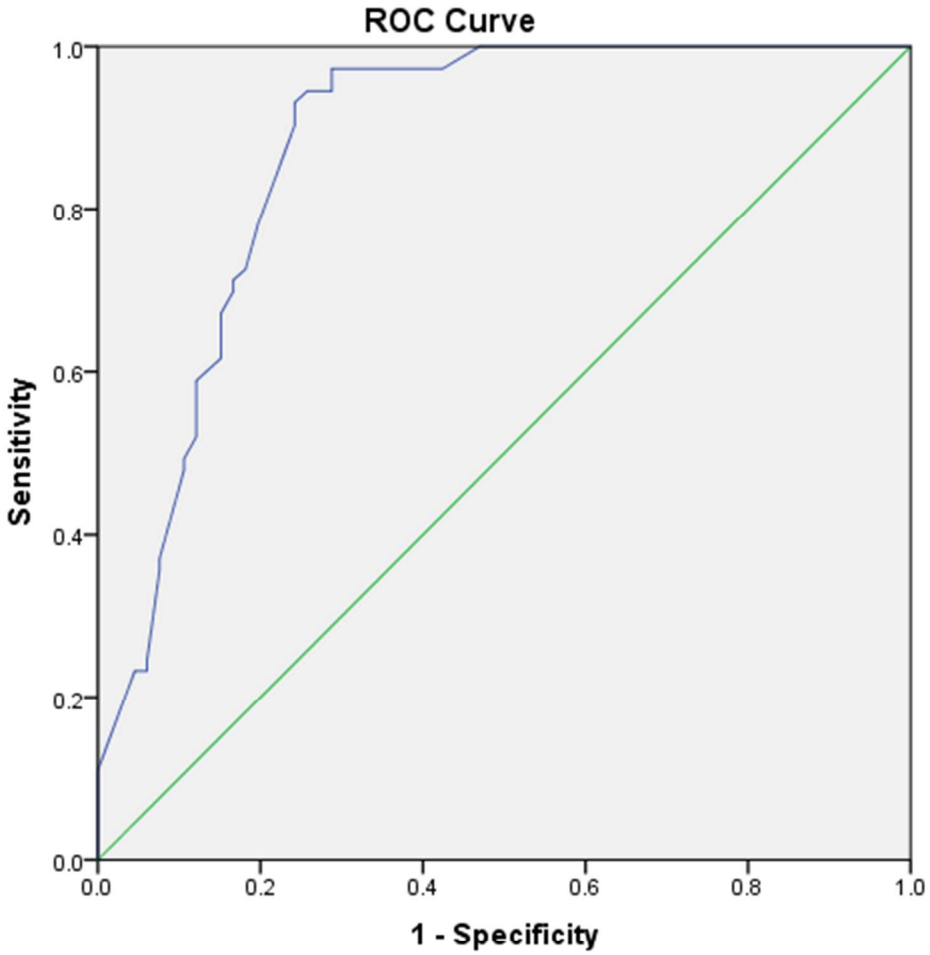

Figure 1B

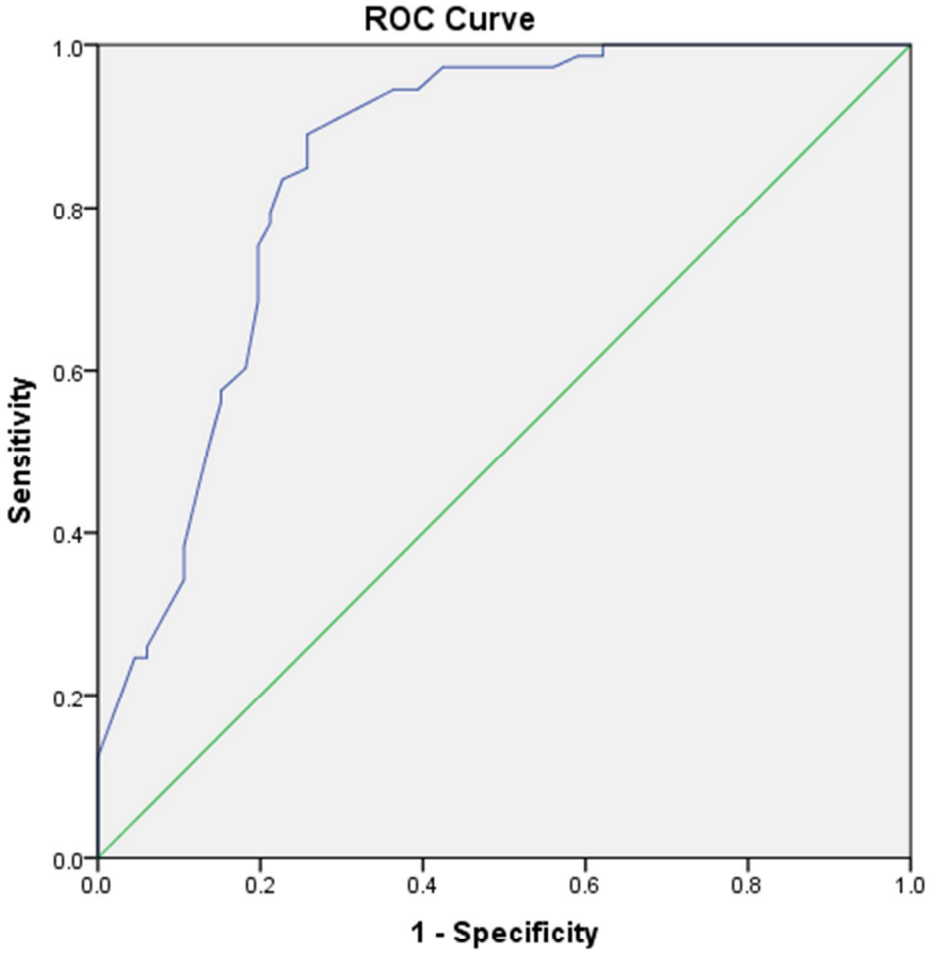

Figure 1C

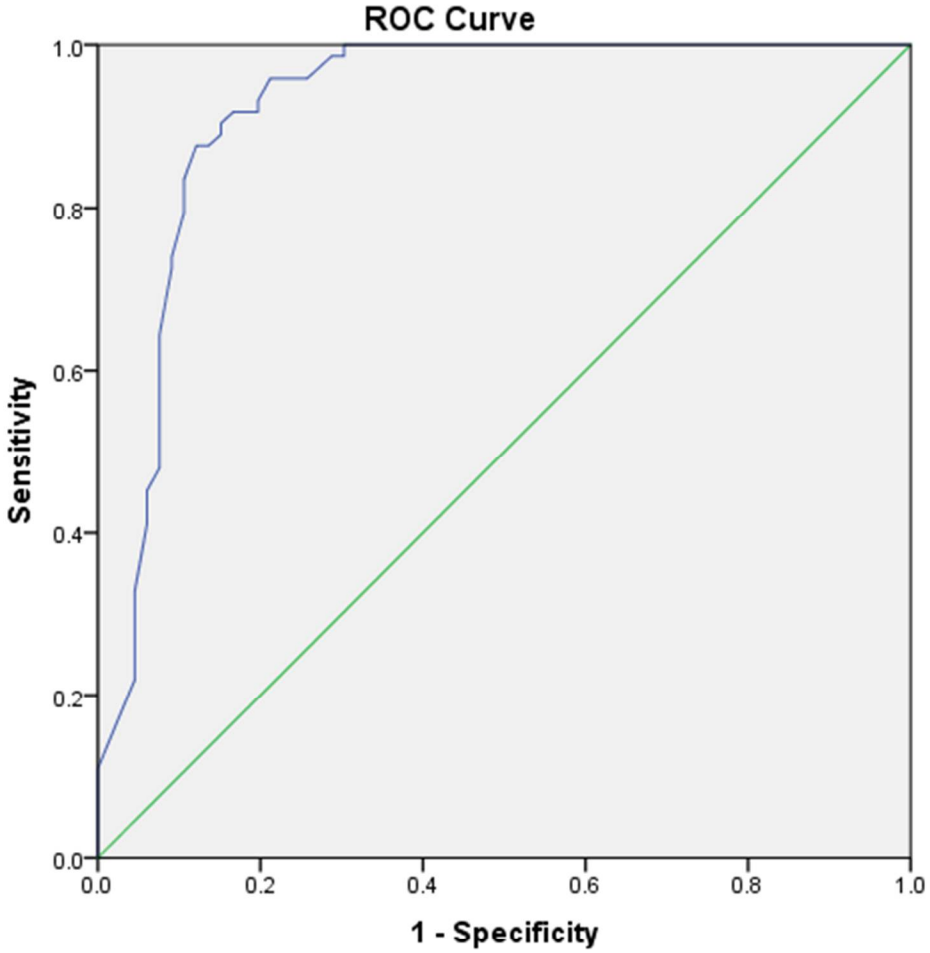

Figure 1D

Figure 1. ROC area of the clinical and exposure characteristics model (A), the clinical and exposure characteristics and SPT-based sensitization to work-specific agents model (B); the clinical and exposure characteristics and BHR testing model (C); the clinical and exposure characteristics and SPT-based sensitization to work-specific agents and BHR testing model (D) in the HMW group. The green diagonal line represents the reference (no discrimination), while the blue curve shows the discriminative ability of Model 7 by plotting the true positive rate (sensitivity) against the false positive rate (1-specificity) at various threshold settings.

4. Chapter 4

4.1. Discussions

To the best of our knowledge, our study is one of the first attempts for the development of the clinical models and scores for quantifying an individual's probability of having OA, using clinical and exposure characteristics along with objective tests other than SIC. The findings of our study show that it is possible to quantify an individual's probability of OA in a population of workers exposed to HMW agents in the workplace. This model could be easily applicable in clinical practice since it is converted to easy-to-use numbers (i.e. clinical scores), and also because the objective tests in the selected model are available in pulmonology departments.

4.1.1. Diagnostic properties of the predictors

Our study demonstrated that the best single test for excluding the diagnosis of OA was the methacholine challenge test (NPV = 93.7%) in the LMW group. In the HMW group, the SPT for sensitization to work-specific agents, and, the methacholine challenge test showed almost equal performances for excluding the diagnosis of OA (NPV = 90.7% and 84.8%, respectively).

The specific SPT had a satisfactory sensitivity in the HMW groups. However, the specificity of this test was moderate. These results are in line with the ERS guideline for the management of WRA which stated that SPT is highly sensitive but less specific for diagnosing OA in workers exposed to HMW agents (6). The systematic review of Beach et al. (127) reported the pooled sensitivity and specificity of specific SPT for diagnosing OA in subjects exposed to HMW agents (sensitivity = 80.6% (95% CI: 69.8% to 88.1%); specificity = 59.6% (95% CI: 41.7% to 75.3%), respectively).

We found that the methacholine challenge test had a high sensitivity and lower specificity in

both HMW and LMW groups. The same study (127) calculated the pooled sensitivity of 79.3% (95% CI: 67.7% to 87.6%) and specificity of 51.3% (95% CI: 35.2% to 67.2%) for diagnosing OA in subjects exposed to HMW agents. In subjects exposed to LMW agents, the pooled estimates of sensitivity and specificity were also 66.7% (95% CI: 58.4% to 74.0%) and 63.9% (95% CI: 56.1% to 71.0%), respectively.

We also calculated the diagnostic properties of combining the methacholine challenge test and specific SPT (sensitivity = 82.2%), which was also higher than the reported pooled estimates of the sensitivity of the combined tests by Beach et al. (127) (the pooled estimate of sensitivity: 60.6%; 95% CI: 21.0% to 89.9%) but almost had equal specificities (the pooled estimate of specificity: 82.5%; 95% CI%: 54.0 to 95.0%). Our measurements of the diagnostic properties of the tests combined together can be regarded as an optimistic measurements since the study population of our study was highly selective due to pre-screening of the workers suspected to have OA through history taking before being referred to HSCM for further diagnostic workup.

4.1.2. Development and validation of the models

Age, sex, rhinoconjunctivitis, and smoking were considered as potential clinical predictors based on published literature (1, 88, 95). However, in our study, smoking was not selected in the final models due to its weak association with OA, either in the total population of the workers or in the exposure-specific groups. The dichotomised predictors were used in the models to facilitate the score calculation and the estimation of OA probability, although it might increase the likelihood of misclassification of individuals into a wrong risk group.

Pralong et al. (203) investigated the predictive value of methacholine challenge test in the same population of workers and found that the performance of the methacholine challenge test could reach an NPV of 97.7% when done while a worker is still working. We also performed a

sensitivity analyses (results not shown) and found that the models, which were developed in the subjects who were still working one month prior to the SIC in each exposure-type group showed better discriminations (AUCs) compared to the models developed in the total population of workers. When stratified into risk groups, the high probability groups also showed better diagnostic properties for diagnosing OA.

As a rule of thumb in prediction modeling, there should be at least 10 events (confirmed OA) per candidate predictor variable to avoid too high or too low estimates of the outcome (287). This means we needed to have 10 subjects with OA for each predictor included in the model (10: 1 EPV ratio). Relative to the number of the subjects and number of the positive outcomes (OA defined as having positive SIC) in the LMW group (6 predictors in each model; number of OA: 61) and in the HMW group (7 predictors in each model; number of OA: 74), we had enough statistical power for developing models in both exposure-specific groups.

The systematic review by Beach and colleagues (127) reported that the diagnosis of OA in the absence of SIC should preferably be determined by the combination of a NSBHR test and specific SPT. In accordance to this statement, our final model demonstrates that adding a combination of specific SPT and the NSBHR test to the clinical and exposure characteristics significantly improved the likelihood of diagnosing OA. It also shows that these two tests are necessary to be performed together in order to have a higher diagnostic accuracy in the HMW group. Application of the clinical scores would not significantly reduce the referral of subjects with a high probability of having OA (referral rate = 48.9%; 68/139 subjects) compared to subjects with a combined positive NSBHR and sensitization to HMW agents (referral rate = 51.8%; 72/139 subjects). However, the PPV and specificity of the high probability group of the prediction model were higher than the combined objective tests but did not reach the statistical

significance. It shows that the scoring rule can potentially be used in identifying the subjects with a higher probability of OA compared to the combined objective tests in areas where access to SIC is difficult or impossible. However, the certain use of the model depends on the results of the external validation.

4.1.3. Diagnostic values of the clinical scores

In both HMW and LMW groups, the mean predictive probability in the high probability group was also close to the observed rate of the subjects with OA. It indicates a good calibration in setting the cut-off points for stratifying the subjects.

In subjects exposed to LMW agents, the low diagnostic properties of the scoring rule indicate that it is not superior to the NSBHR test in isolation since it ultimately did not show a significant increase in the likelihood of identifying the subjects who truly have OA. The scoring rule also did not show a satisfactory NPV for excluding the diagnosis of OA. Thus, the methacholine challenge test still remains the preferable method for excluding OA in the absence of SIC. In contrast, in the HMW group, our model is the first in quantifying the individual's probability of having OA. The higher AUC and better diagnostic properties of the high probability category of the final model in the HMW group suggests that the incorporation of the specific sensitization in the final model might have an important role in improving the likelihood of diagnosing OA compared to the final model in the LMW group where the specific sensitization was not routinely performed and consequently did not included in the final model. Beach et al. (127) also reported that the sensitivity of the combined specific sensitization and NSBHR was 100% (95% CI: 74.1% to 100%) and its specificity was 80.0% (95% CI: 49.0% to 94.3%) that was higher than the pooled estimate of sensitivity (66.7%; 95% CI: 58.4% to 74.0%) and specificity (63.9%; 95% CI: 56.1% to 71.0%) for NSBHR in isolation in the LMW group, suggesting the important

role of the specific SPT in increasing the likelihood of the diagnosis.

The scoring rules in both exposure-specific groups showed a gradual improvement in sensitivity by increasing the sum scores and the number of subjects with OA in each group. However, there is no significant change in the specificity along with increasing the sum scores and observed number of the subjects with OA. Given the higher prevalence of OA in the subjects with a high probability of having OA, our finding is consistent with Whiting et al.'s findings (291), which stated that the sensitivity of a diagnostic test is improved by increasing the disease prevalence and severity while the specificity is not significantly affected.

4.1.4. Application of the diagnostic models in prevention of occupational asthma

The urge to develop a prediction model for diagnosing OA starts with the questions on how to choose the best further diagnostic workup for every patient considering his individual's probability of having OA, or, who is a suitable candidate for performing further diagnostic testing. It is important to quantify the impact of the prediction model in direct patient management in clinical practice in order to recognize to what extent this model can contribute to the management of the disease by physicians, and to the encouragement of the patients in taking an informed decision about further testing and treatment.

Our proposed diagnostic model for workers exposed to HMW agents with suspicion of OA targets the secondary and tertiary levels of prevention, which are focused on detection and early treatment of OA. Moreover, it contributes in controlling the costs of the referral and prescribing inappropriate and/or unnecessary diagnostic tests especially in the current economic crisis in order to allocate resources to only procedures that seem to be effective in enhancing the accuracy of the diagnosis. It is also an easy-to-use tool because it consists of five simple questions on clinical and work-related characteristics of the subjects in addition to positive skin-prick and

methacholine challenge tests that is mostly accessible in specialised centers with active pulmonology departments.

Clinicians' perspectives are mostly focused on deciding on further testing and performing preventive interventions to patients at high risk of having OA. To confirm the diagnosis of OA in workers exposed to HMW agents, our proposed model use the methacholine challenge test and specific SPT as accessible tools in clinics or centers with respiratory tests facilities. Therefore, the use of the model would be restricted to specialists with the possibility of ordering both skin-prick test and methacholine challenge test. It is not useful in areas where access to SIC as the reference standard is possible (for example in Quebec); however, a few centers offer SIC since it need trained staffs and specific test equipment. The estimation of the individual's probability of OA can guide physicians in deciding upon next step of clinical management: when the probability of the presence of OA is relatively high, further testing (SIC) is indicated; when the probability is low, the presence of OA is unlikely and no further diagnostic testing and/or referral of the patient to the specialized center is indicated; they should be evaluated for other probable diseases that mimic the asthma symptoms. Those with intermediate probability of OA can be re-evaluated after a certain period of time by the occupational physicians before referring to specialized centers.

Moving from physicians to patients' perspective, they are mostly concerned with the impact of the disease on their health and employment. Studies suggest that workers who experience asthma symptoms in the workplace are not eager to disclose their symptoms to their manager or medicine due to fear of negative consequences of a diagnosis such as being removed from their jobs and/or submitting a claim to worker's compensation board or failing to file claims. Avoiding from the negative social and economic consequences of the disease makes workers

maintain their job in a hazardous environment where their respiratory symptoms might be worsen, and put worker's health at risk of irreversible adverse effects (292-295). By estimating the risk of having OA for every individual according to his unique characteristics, physicians can take an appropriate risk communication strategy in order to provide an accurate insight about the hazards of not having a definite diagnosis and starting treatment rightfully especially in regard to this point that those patients with significant probability of having OA may develop irreversible deterioration of the respiratory health even after removing from the workplace where they are exposed to offending agents. The translation of the predictive model to clinical score provides workers with a simple and clear tool to calculate the probability of having OA even by themselves (for example by an electronic and online nomogram). Providing patients with a more accurate risk assessment combined with an appropriate insight about the prognosis of the disease by physicians would allow workers to make a more educated decision about whether to perform further diagnostic testing and start an appropriate treatment.

This model may also favor the payers' perspectives. Compensation agencies mostly covers the two major aspects: "(i) diagnosis, compensation, and rehabilitation of the worker at the time of referral; and (ii) long-term compensation for impairment and disability after the diagnosis" (296). In order to have access to basic medical and financial supports and to facilitate return to work in a safe environment through the workers' compensation system, workers need to have a definitive OA diagnosis for consideration of their claim (297). This model may be less relevant in workers' compensation cases where there is access to SIC evaluations and they are required in order to get compensation. For example, in the province of Quebec, workers' compensation claims for OA are examined by a committee consisting of three chest physicians and a SIC evaluation is usually required for a definitive diagnosis of occupational asthma in relation to an

offending agent or agents in the workplace (296). However, our proposed model might aid physicians at the secondary care level (specialists: i.e. respirologists, occupational medicine physicians) in identifying subjects with a high probability of having OA, in order to decide whether they should refer their patients to a tertiary center (296).

In other areas of the world with a compensable insurance systems and legal regulations related to occupational diseases, workers can ask for compensation after doing certain objective tests. However, the heterogeneous compensation system within and between countries urges the need for further research in order to develop medical surveillance for high-risk population of workers that would ensure the timely and rapid referral to diagnostic and medicolegal agencies (296).

Our estimated PPV (89.7%) for OA was relatively optimistic in subjects exposed to HMW agents because the high prevalence of OA in the study population (52.1%). None of the positive or negative predictive values are generalizable to other population of the workers exposed to HMW agents as the probability of including or excluding the diagnosis of OA is dependent on the prevalence of OA in that population (298).

Our model in the LMW group produced low predicted probabilities, although the model showed a good discriminative ability between diseased and non-diseased workers (AUC = 0.728; 95% CI: 0.66-0.79) compared to clinical and exposure characteristics model (Model 1). The specific SPT is not a useful test considering the pathogenic mechanisms of LMW agents in OA (299). Thus, it was not mostly done for the purpose of diagnosis, and developing a model with the SPT alone or in combination with other tests was not possible. The selected cut-off points based on the final model (Model 4) could not significantly reduce the number of subjects who were categorized as having a high probability of OA. A very low PPV of the model did not support its reliability to be applied in the diagnostic setting. Moreover, this model and the methacholine

challenge test alone resulted in an almost equal NPV. It indicates that the model is not beneficial in increasing the likelihood of diagnosing OA, or in decreasing the costs of further tests in the specialized centers.

4.1.5. Strengths and limitations

When evaluating the subjects in the HSCM clinic, the objective tests were done without informing the technicians about the subjects' clinical & exposure characteristics, or the results of the SIC; so, it is less likely that the interpretation of the test results, and consequently, the results of our study were influenced.

This study has some limitations. One of the limitations of our study is that we did not have data to externally validate the models. The external validation of the risk-estimating models is important for determining whether these models are applicable in new populations because the differences between the original population and the population where the external validation is done might influence the performance of the models (300, 301). Although bootstrapping procedure might fit our model in a way that it can predict the outcome with good accuracy, the model might still be over-fitted in a new population of subjects, indicating that external validation is necessary to evaluate the generalizability of the model (302).

Although SIC is considered to be the reference test for diagnosing OA, false-negative or false-positive results may occur. Exposure to unknown or multiple agents, technical errors in SIC performance, and absence of specific bronchial responsiveness, which can occur when a worker is away from exposure for a prolonged period, may lead to false-negative SIC results (17, 162, 191). We excluded all the subjects (n = 25) with multiple exposure to both HMW and LMW agents from the first step of the analyses. All the subjects with exposure to either HMW or LMW agents who were still actively working one month prior to the SIC were included for the purpose

of the final model development. Generally, the characteristics of non-diseased workers (negative SIC) and the performance of the SICs in a center with high quality standards makes the probability of the false-negative results relatively low. Furthermore, although SICs were performed according to standard protocols, no measurement of exposure levels was reported before and during the test. De Olim et al. (303) investigated the results of SICs that were performed in North America. They found that among patients who had negative SICs (not to have OA), there was at least one other sensitizer potentially present in their workplace that was not included in the SIC because it had not been identified by physician assessment (62% of negative SICs). It may affect the accuracy of the interpretation of the results, and consequently, the degree to which the SIC can be considered a 'gold standard' for OA diagnosis. In the absence of a gold standard, the classification of the subjects to cases and non-cases is subjected to be biased.

Notably, we used a dataset which primarily established for a goal other than the development of diagnostic models. This dataset lacks detailed information on induced sputum because the test was not done before 2000. Thus, we could not develop the models for examining the ability of this test in diagnosing OA.

4.2. Conclusion

To conclude, we developed diagnostic models to quantify the individual's probability of having OA in subjects with the presentation of lower respiratory symptoms in relation to occupational exposure. For those workers exposed to LMW agents, our model did not offer a better diagnostic utility for diagnosing OA compared to the methacholine challenge test. Our proposed model in subjects exposed to HMW agents demonstrated that the clinical and exposure characteristics coupled with specific SPT to test sensitization to HMW agents in the workplace and the

methacholine challenge test could quantify the individual's probability of having OA with a better precision compared to combining those objective tests alone based on the higher AUC of the model that was statistically significantly higher than the combination of both tests. We transformed the final models to the easy-to-use number (i.e. clinical scores) that facilitate its application. This model confirms that we need to perform both specific SPT and the methacholine challenge test for diagnosing OA in the HMW group. Application of the clinical scores in centers where access to the SIC is difficult or impossible might be of benefit in diagnosing OA. Yet, the predictive ability of these models needs to be examined in another population of workers before using them with confidence.

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Appendix A

Self-administered questionnaire on asthma*

For each of the following questions, please check the appropriate box. If you are unsure of the answer, check "NO".

1. Have you had **wheezing** or **whistling** in your chest at any time in the last 12 months?

   YES □ NO □

   If "NO", go to question 2.

   If "YES":

   1.1. Have you been at all breathless, when the wheezing noise was present? YES □ NO □

   1.2. Have you had this wheezing or whistling when you did **NOT** have a cold? YES □ NO □

2. Have you woken up with a feeling of **tightness** in your chest or been woken by at attack of **shortness of breath**, at any time in the last 12 months? YES □ NO □

3. Have you been woken by an attack of coughing at any time in the last 12 months? YES □ NO □

4. Have you had an **attack of asthma** in the last 12 months? YES □ NO □

5. Are you currently taking any **medicine** for **asthma** (including inhalers, aerosols or tablets)?

   YES □ NO □

6. When you are at your workplace, do you ever start to feel **short of breath** or get chest **tightness**?

YES □ NO □

7. When you are at your workplace, do you ever start to **cough**? YES □ NO □
8. When you are at your workplace, do you ever start to **wheeze**? YES □ NO □
9. If "YES" to question 6 or 7 or 8:

   Do these problems related to your work lessen or disappear during the weekend or during holidays?

   YES □ NO □

You may suffer from **ASTHMA** if you have checked **"YES"** 3 times or more. In such case, it is important to examine if you work in the cause of your symptoms.

In order to get a complete assessment of this health problem, contact as soon as possible the occupational health nurse.

*adaptation of the questionnaire used for the medical surveillance of workers in the context of the "program provincial isocyanate 2000-2008" implemented by the Réseau de santé publique en santé au travail (Québec).

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