UNIVERSITY OF CAPE TOWN

DETERMINANTS OF FRACTIONAL EXHALED NITRIC OXIDE (FENO) AMONG WORKERS AT RISK OF DEVELOPING OCCUPATIONAL RESPIRATORY ALLERGY AND ASTHMA IN DIFFERENT OCCUPATIONAL SETTINGS

By

Phinias Harris Katolora Mfune

MFNPHI004

A research report submitted to the School of Public Health, Faculty of Health Sciences, University of Cape Town in partial fulfilment of the requirement for the award of the degree of Master of Medicine (MMed) in Occupational Medicine

Supervisor: Prof. Mohamed F. Jeebhay

Co-supervisor: Dr. Roslynn Baatjies

- March 2024 -

DECLARATION

Determinants of fractional exhaled nitric oxide (FeNO) among workers at risk of developing occupational respiratory allergy and asthma in different occupational settings.

I, **Phinias Harris Katolora Mfune,** hereby submit my dissertation for the degree of Master of Medicine (MMed) in Occupational Medicine. I declare that this is my original work (except where acknowledgements indicate otherwise) and that neither the whole work, nor any part of it, has been, is being, or is to be submitted for another degree in this or any other university. This work has not been reported or published prior to registration for the above-mentioned degree.

This dissertation has been submitted to the Turnitin module (or equivalent similarity and originality checking software) and I confirm that my supervisor has seen my report and any concerns revealed by such have been resolved with my supervisor.

Name: Phinias Harris Katolora Mfune

Student number: MFNPHI004

Signature: Signed by candidate

Date: 31st March 2024

ACKNOWLEDGEMENTS

I wish to acknowledge and express my sincere gratitude to the following individuals for their invaluable support and contributions to this dissertation:

- Professor Mohamed Jeebhay, School of Public Health, University of Cape Town, my supervisor, for his dedication and support in guiding, assisting and motivating me throughout this project. His mentorship has been incredibly valuable to me.
- Dr Roslynn Baatjies, Department of Environmental and Occupational Studies, Cape Peninsula University of Technology, my co-supervisor, for her contribution and guidance with the statistical analysis.
- Drs. Roslynn Baatjies, Hussein Mwanga, Dorothy Ngajilo, Anita van Der Walt, Paulino Chamba and Vuyelwa Ndlovu for graciously allowing me to utilize data from their original individual studies for secondary analysis.
- Associate Professor Shahieda Adams, School of Public Health, University of Cape Town, for her valuable input and guidance through my occupational medicine career.
- The Malawi Ministry of Health, specifically the National Tuberculosis Control Programme, for sponsoring my studies at the University of Cape Town through the Southern Africa Tuberculosis and Health Systems Support Project (SATHSSP).
- Ms Lynne Harper, Ms Faranaaz Bennett, Ms Connie Williams, Ms Liza Smith, Ms Bulelwa Manxiwa, Ms Thobeka Mngaza and Ms Carmen De Koker, School of Public Health, University of Cape Town, for assisting with administrative matters throughout my postgraduate studies.

DEDICATION

This dissertation is dedicated:

To God Almighty for His blessings for my strength and good health, which enabled me to complete this dissertation. My guiding verses throughout this journey have been Psalms 116:12-19.

To the memory of my parents, the late Harris Katolora Mfune and the late Tifiness Tembo (Mrs Mfune), for their unwavering love and support in nurturing me to pursue my aspirations. Even in their passing, their spirit continues to inspire and fortify me until we reunite in God's glory.

To my wife, Bridget, for her countless sacrifices, steadfast support, encouragement, understanding, and boundless love. She has been my guardian angel, providing a constant source of strength and a shoulder to lean on. Without her unwavering presence, this endeavour would not have been achievable.

To my children, Ungweru and Uwemi, for their sacrifices and patience throughout my studies. They are my source of motivation and drive to succeed.

To Dr. Grace Chiudzu, former Head of the Obstetrics and Gynecology Department at Kamuzu Central Hospital, Malawi; Dr. Dan Namalika, former Secretary for Health at the Ministry of Health, Malawi; Dr. Frank Sinyiza, former Hospital Director at Mzuzu Central Hospital, Malawi; and Prof. Johnstone Kumwenda from the University of Malawi, College of Medicine, for their unwavering belief in me and their mentorship throughout my medical career.

To my brother, George Katolora Mfune, for stepping in as our parental figure in the absence of our parents.

To all my family members and friends.

PUBLICATIONS

Part of this dissertation has been published:

Mfune P, Jeebhay MF. Determinants of fractional exhaled nitric oxide in workers at risk of developing occupational respiratory allergy and asthma: A literature review. Current Allergy and Clinical Immunology. 2023; 37(1): 31-41. https://hdl.handle.net/10520/ejc-caci-v37-n1-a7

Determinants of fractional exhaled nitric oxide (FeNO) among workers at risk of developing occupational respiratory allergy and asthma in different occupational settings

ABSTRACT

Background: Fractional exhaled nitric oxide (FeNO) offers a potential tool for screening and surveillance of workers at risk of occupational respiratory allergy and asthma.

Objective: This study evaluated determinants of FeNO in workers at risk of occupational respiratory allergy and asthma in diverse industries in southern Africa.

Methodology: Data were analysed from cross-sectional epidemiological studies of bakery, fruit farming, spice milling, poultry farming, wood processing and healthcare industries. All studies used the modified ECRHS questionnaire, assessed atopy using Phadiatop, allergen specific sensitization (sIgE) and NIOX MINO.

Results: Study participants (n=2043) had a median age of 37 years, with 52% female and 25% current smokers. The prevalence of atopy was 44% and sIgE was 18%. FeNo was elevated (≥25 ppb) in 17% and high (≥50 ppb) in 7% of workers. Overall, sIgE explained 20% (adj_R^2 0.1998, $p<0.001$) of FeNO variability, spider mite 35% ($adjR^2$ 0.3529, $p=0.064$), rye 26% ($adjR^2$ 0.2594, $p<0.001$) and wheat 25% ($adjR^2$ 0.2513, $p<0.001$). Host factors significantly associated with FeNO included age, sex, atopy and current smoking status, as was employment duration. Sensitization to wheat (OR_{adj} 5.39), rye (OR_{adj} 4.96), spider mite (OR_{adj} 3.18) and ortho-phthalaldehyde (OR_{adj} 2.51) were significantly associated with FeNO ≥25 ppb, while wheat (OR_{adj} 3.58), rye (OR_{adj} 4.43), garlic (OR_{adj} 9.42), and natural rubber latex (OR_{adj} 7.96) were significantly associated with FeNO ≥50 ppb.

Conclusion: Workers with elevated FeNO are more likely to have elevated sIgE and at increased risk of having occupational respiratory allergy and asthma if symptomatic.

Key words: FeNO, occupational, respiratory allergy, asthma, atopy, sensitization, determinants

ABBREVIATONS AND ACRONYMS

AOA:	adult-onset asthma
BMI:	body mass index
DETs:	diesel engine testers
ECRHS:	European Community Respiratory Health Survey
FeNO:	fractional exhaled nitric oxide
FEV_1:	forced expiratory volume in 1 second
HCWs:	health care workers
HDI:	hexamethylene diisocyanate
HMW:	high molecular weight
HREC:	Human Research Ethics Committee
ICS:	inhaled corticosteroids
IgE:	Immunoglobulin E
IQR:	interquartile range
LMW:	low molecular weight
MDI:	methylene diphenyl diisocyanate
MWCNT:	multi-walled carbon nanotubes
ND:	not done
NO:	nitric oxide
NRL:	natural rubber latex
NSBH:	non-specific bronchial hyperresponsiveness
OA:	occupational asthma

ONS:	ocular-nasal symptoms
OPA:	ortho-phthalaldehyde
OR:	odds ratio
PC20:	Provocative concentration of histamine causing a 20% fall in FEV_1
PICO:	Population, Intervention/(Exposure), Comparison/Comparator and Outcome
ppb:	parts per billion
SIC:	specific inhalation challenge test
SLIT:	sublingual specific immunotherapy treatment
Th2:	type 2 helper T cells
WRONS:	Work-related ocular-nasal symptoms
WRR:	work-related rhinitis

TABLE OF CONTENTS

Contents

TABLE OF FIGURES

SECTION A – PROTOCOL

DETERMINANTS OF FRACTIONAL EXHALED NITRIC OXIDE (FENO) AMONG WORKERS AT RISK OF DEVELOPING OCCUPATIONAL RESPIRATORY ALLERGY AND ASTHMA IN DIFFERENT OCCUPATIONAL SETTINGS.

INTRODUCTION

Fractional exhaled nitric oxide (FeNO) is a non-invasive biomarker of eosinophilic airway inflammation and is able to predict steroid treatment response in asthmatics. This biomarker is primarily used in asthma,[1–7] but is also relevant in various other pulmonary disorders, both allergic and non-allergic, such as chronic allergic rhinitis, acute and chronic eosinophilic pneumonia, chronic obstructive pulmonary disease, bronchiolitis obliterans syndrome, hypersensitivity pneumonitis, viral infection and interstitial lung disease.[1,3,4,8]

Since a number of respiratory diseases can also be caused by work, FeNO represents a safe and easy measure to assess their work-relatedness in an occupational setting.[1,2] However, FeNO may be lower among those who smoke, have bronchoconstriction or use steroid therapy.[8]

Nitric oxide (NO) is a gas produced in the airways and detectable as fractional exhaled NO (FeNO). Its production increases in the airways when type 2 inflammation is present. [5] Induced sputum and FeNO measurements are the most reliable non-invasive procedures to assess the presence of type 2 airway inflammation.[1,5]

It is understood that FeNO levels relate more closely to the risk of disease exacerbation than to disease severity.[5] FeNO levels correlate with asthma exacerbations in severe asthmatic patients independent of blood eosinophils.[5] While FeNO is associated with increased sputum and blood eosinophil levels,[8] it correlates moderately with the former and weakly with the latter.[3]

Aside from its usefulness in the diagnostic work-up of individuals with suspected asthma[9] measurement of FeNO offers a useful tool in the screening and surveillance of working populations at risk of developing occupational asthma (OA) associated with exposure to respiratory sensitisers.[3] Most studies on the determinants of FeNO have generally focused on the general population as opposed to workers in the occupational setting.[1–3,10]

The aim of this study to evaluate the individual/host and occupational-related determinants of elevated FeNO in workers at risk of developing occupational respiratory allergy and asthma. The findings of the study will contribute towards obtaining better insights into FeNO levels in working populations and to assess its applicability in screening and surveillance of high risk occupational groups. This could contribute

towards improved preventive measures for workers at risk of developing occupational respiratory allergy and asthma.

JUSTIFICATION

Fractional exhaled nitric oxide (FeNO) is well described as a marker of eosinophilic airway inflammation related to occupational asthma.(1) Studies have demonstrated the usefulness of FeNO in the identification of Th2 inflammation in asthma.[1] FeNO is also recommended for monitoring asthma interventions including patients on steroid treatment [1–3] and evaluation of preventive interventions.[4]

FeNO levels are increased in workers exposed to high molecular weight agents [3,23] and some low molecular weight agents that induce an IgE-mediated immune response such as isocyanates [1,3,11] and platinum salts.[26] More recent studies, show that FeNO is increased in IgE mediated inflammation, irrespective of the molecular weight of the causative agent.[26]

However, few studies have explored factors that influence high FeNO levels among workers employed in different industries.

PURPOSE AND BENEFITS

Increased FeNO may be an early marker of Th2 inflammation in the airways following exposure to an occupational sensitiser before the worker manifests with allergic respiratory symptoms and asthma. The results of this study will be useful in formulating individual as well as industry-specific medical surveillance programs and preventive measures aimed at improving occupational respiratory allergic disease outcomes and general wellbeing in the workplace.

RESEARCH QUESTIONS

- What are the most important occupational and individual/host-related factors that are associated with FeNO in workers exposed to different sensitisers?
- What individual/host-associated clinical factors predict elevated FeNO in workers at risk of developing occupational allergy and asthma?
- Does working in a specific industry predict elevated FeNO in workers at risk of developing occupational allergy and asthma?

HYPOTHESIS

Occupational and individual/host-related factors predict elevated FeNO in workers at risk of developing occupational respiratory allergy and asthma.

AIM

The aim of this study is to evaluate the determinants of elevated fractional exhaled nitric oxide (FeNO) in workers at risk of developing occupational respiratory allergy and asthma in various industries including baking, spice milling, wood processing, fruit farming, poultry farming and health care.

OBJECTIVES

1. To determine the occupational risk factors (industry type, biological or chemical agent, type of agent, molecular weight of agent) associated with elevated FeNO among workers employed in different industries (baking, wood processing, spice mill, health care, poultry farming, fruit farming).
2. To determine the host-related risk factors (demographic characteristics, atopy, medical history of asthma or rhinitis, treatment) associated with elevated fractional exhaled nitric oxide (FeNO) among workers in these different occupational settings.
3. To determine whether symptom history, occupational symptoms and presence of sensitisation in the past 12 months, is associated with elevated FeNO among workers in these different occupational settings.

METHODOLOGY

Study design, population and sampling strategy

This study involves the analysis of data that was collected in as a part of larger cross-sectional epidemiological studies of bakery, fruit farm, spice mill, poultry farm, wood industry and health workers as previously reported.[13,16,32–34]

The study population comprised 2112 workers involved in bakery (n=424), spice mill (n=150), poultry farming (n=230), fruit farming (n=211), wood processing (443) and healthcare (n=654) work. All workers completed a consent form prior to participating in the study. Ethics approval was obtained from the Human Research Ethics Committee (HREC) of the University of Cape Town for these individual studies (study ethics numbers: Baatjies et al and Al Badri – HREC Ref: 272/2002; van der Walt HREC Ref: 179/2008; Ngajilo – HREC Ref: 601/2015; Ndlovu – HREC Ref: 393/2009; Mwanga – HREC Ref: 212/2013 and HUM00083115), Chamba – HREC Ref: 543/2016; National Bioethics Committee of Ministry of Health of Mozambique (40/CNBS/2015) and University of Michigan Medical School Institutional Review Board (HUM00070862)), as previously reported. (4,16,32–35)

Inclusion criteria

For purposes of this study, the inclusion criteria for analysis will include the following:

1. Participation in the baseline studies.
2. Complete information on sensitization status (IgE levels) to flour dust allergens (wheat, rye, fungal α-amylase) for bakers; spice dust allergens (garlic, chilli pepper and wheat) for spice mill workers; chicken (meat, feathers, serum protein, droppings), storage mite (Lepidoglyphus destructor), sunflower seed, mould mix (containing Penicillium chrysogenum, C. herbarum, A. fumigatus, C. Albicans, A. alternata, S. rostrata) for poultry farm workers; storage mite (Lepidoglyphus destructor) and spider mite (Tetranychus urticae), for fruit farmworkers; and cleaning agents (natural rubber latex; chlorhexidine and ortho-phthalaldehyde (OPA), for health care workers.
3. Complete information on the presence of respiratory outcomes of interest (doctor diagnosed asthma, rhinitis, ocular-nasal and other asthma-related symptoms) at baseline is available.
4. Complete information on occupational respiratory symptoms is available.
5. Fractional exhaled nitric oxide (FeNO) test results are available.

Sample size calculation

According to literature, the prevalence of FeNO ≥25 ppb in asthma patients in Europe is estimated at 14.6% (range 13.0 – 56.9). [36–40] The prevalence of FeNO ≥25 ppb in occupational settings is limited and ranges between 20-25%. Lim et al reported prevalence of FeNO ≥25 ppb in office workers exposed to endotoxin and (1,3)-b-glucan in office dust was 25.5%.[24] Werthmann et al. reported 20% prevalence of FeNO ≥25 ppb in agricultural workers.[7] Using the proportion of FeNO ≥25 ppb among agricultural workers above, the minimum sample size for estimating a single proportion will be 246 subjects based on the following formula:

$$n = \frac{P(1-P)Z^2}{d^2}$$

Where n = sample size; P = proportion of FeNO among agricultural workers (20%); Z → Z statistics = 1.96 for confidence level at 95%; and d = precision (margin of error) of 5%.

However, the original studies collected data from a total of 2112 subjects; therefore this entire sample will be used for analysis so as to have sufficient power to analyse outcome in multivariate regression models.

MEASUREMENTS

Study instruments

The following study instruments were used to assess the presence of the risk factors and fractional exhaled nitric oxide (FeNO) levels. Similar approaches were used in all studies.

Respiratory questionnaire

For all studies, the study questionnaire used was a modified version of the European Community Respiratory Health survey (ECRHS) (41) (Burney et al. 1994) incorporating work-related aspects (exposures and symptoms) adapted for local conditions.[13,16,32–34,42] The interviewer administered questionnaire collected information on important demographic factors, occupational-related respiratory symptoms (ocular nasal, wheeze, tight chest), history of previous illnesses (asthma, rhinitis, and atopy), employment history, degree of exposure to occupational allergens, and smoking status. Information was also collected on medication use, exercise and dietary history (nitrate rich foods).[43] The modified questionnaire was administered in either English or Afrikaans (South African studies), or Swahili (health workers) or Portuguese (wood processing workers). The translated Swahili and Portuguese questionnaires were back-translated to English to ensure validity and repeatability.[13,33]

Fractional exhaled nitric oxide (FeNO) determination

In all the original studies, a hand-held portable nitric oxide electrochemical sampling device (NIOX MINO) at a flow rate of 50 mL/s in accordance with international recommendations [44,45] was used to determine FeNO during the work shift throughout the working week. Subjects followed specific instructions for the measurement, including exhaling residual air, ensuring a ≥6-second exhalation, and inhalation of air free of NO to a total lung capacity before measurement of FeNO. The average of at three technically adequate FeNO measurements was determined. Ambient NO levels were also measured at testing location; no nasal clips were used during FeNO measurement and the testing of workers had no particular variation with regard to time of testing for the different jobs. Workers were instructed to abstain from smoking, eating or drinking at least one hour before the test. This was confirmed prior to testing, and those that did not follow the instructions were tested at a later stage after ensuring their full compliance with these instructions. FeNO was tested prior to spirometry when required. A FeNO level of ≥25 to 50ppb were considered to be elevated and if >50ppb were categorised as high, indicating the presence of allergic airway inflammation. [13,16,32–34,42] BMI was also determined for each participant.

Immunological tests

The method of quantification of serum specific IgE antibodies CAP-FEIA (fluorescence enzyme immuno assay) was performed using the ImmunoCAP 100 System (Phadia, Uppsala, Sweden) according to the manufacturer's instructions using wheat flour (f4), rye flour (f5) and fungal α-amylase (k87) for bakery workers;[34] garlic (f47; *Allium sativum*), chilli pepper (f279; *Capsicum frutesce*) and wheat (f4; *Triticum aestivum*) in spice mill workers;[16] storage mite (*Lepidoglyphus destructor*), sunflower seed (*Helianthus annuus*), mould mix (containing *Penicillium chrysogenum, C. herbarum, A. fumigatus, C. Albicans, A. alternata, S. rostrata*), and in-house chicken (meat, feathers, serum protein, droppings) in poultry workers.[32] In fruit farm workers quantification of specific IgE was performed for specific occupational allergens for spider mite (*Tetranychus urticae*) and storage mite (*Lepidoglyphus destructor*) [42] and natural rubber latex – NRL (*Hevea brasiliensis* – Hev b5 – K218, Hev b6.02 – K220), chlorhexidine (C8) and ortho-phthalaldehyde (OPA) were tested in health workers.[35] Specific IgE were not determined for wood processing workers due to inability to detect IgE using the extract available.

The presence of atopy was defined by a positive Phadiatop test (ImmunoCAP 100 System; Phadia). An ImmunoCAP result of ≥0.35 kU_A/L was regarded as positive.

DATA MANAGEMENT AND ANALYSIS

OPERATIONAL DEFINITIONS AND LIST OF VARIABLES

FeNO levels - FeNO will be treated either as a continuous variable (log-transformed [In] if data is not normally distributed) or categorical variable as follows:

- <25 ppb: will be regarded as normal.
- FeNO 25-50ppb: elevated.
- FeNO > 50ppb: high and considered to indicate the presence of allergic airway inflammation.

Current asthma - respondent answered YES to at least one of the following 2 questions: "Have you had an attack of asthma in the last 12 months?" or "Are you currently taking any medicines including inhalers, aerosols or tablets for asthma?"

Adult-onset asthma (AOA) – doctor-diagnosed asthma and having had the first asthma attack at the age of 16 years or later.

Asthma symptoms – binary variable (Yes/No) generated as a sum of positive responses to four questions on asthma symptoms in the last 12 months (wheeze with breathlessness, woken up with chest tightness, woken by attack of coughing and attack of shortness of breath at rest).

Ocular-nasal symptoms (ONS) - defined as answering YES to "Have you had any nasal allergies including hay fever or itchy and watery eyes/nose in the last 12 months?"

Atopy – serum IgE ≥0.35 kU/L to common inhalants.

Sensitization – serum IgE ≥0.35 kU/L to specific occupational allergens.

Outcome variable

In this study the outcome variable of interest is the FeNO level.

PREDICTOR VARIABLES

INDIVIDUAL/ HOST FACTORS

- Age (continuous variable)
- Sex (binary variable)
- Height (continuous variable)
- Body mass index – BMI (continuous variable)
- Smoking status (categorical variable: current smoker, ex-smoker, non-smoker)
- Medical history (binary variable: current doctor diagnosed asthma, rhinitis/hay fever, recent chest infection) – Yes/No
- Treatment with inhaled corticosteroids – ICS (binary variable: Yes/No)
- Respiratory symptoms (binary variable: current doctor diagnosed asthma, ocular nasal, wheeze, cough, chest tightness) – Yes/No
- Atopy (binary variable: Yes/No)
- Diet (nitrogen containing) (binary variable: Yes/No)

OCCUPATIONAL-RELATED FACTORS

Industry exposures

Industry type will be classified as:

- Biological or chemical agent
- High molecular weight (HMW) or Low molecular weight (LMW) agent

Occupational sensitisation to workplace allergens will be categorised according to sIgE levels to the following allergens and classified either as continuous or categorical based on the industry specific exposure:

- ***HMW agents*:** wheat flour, Rye flour, fungal α-amylase, chilli pepper, garlic, wheat, sunflower seed, mould mix, natural rubber latex (NRL), storage mite, spider mite, chicken droppings, feathers, chicken meat and serum protein.
- ***LMW agents:*** chlorhexidine, ortho-phthalaldehyde (OPA), and wood dust.

Occupational-related exposures

- Exposed or unexposed (binary variable)
- Employment duration in current job (continuous variable)

Occupational-related symptoms

- Chest tightness – (binary variable) - Yes/No
- Wheeze – (binary variable) - Yes/No
- Ocular nasal – (binary variable) - Yes/No

Occupational Sensitization

Presence will be based on the specific IgE level. Sensitization to occupational allergens will be analysed as binary – Yes / No based on whether the worker is sensitised to any one of the sensitisers tested for (see Table below), or categorical (based on degree of response) or as a continuous variable (log-transformed), the latter both based on the level of sIgE to the sensitiser with the highest IgE level. ImmunoCAp results are categorised into the following classes according to levels of specific IgE (kU/L): class 0 = <0.35 – extremely unlikely to have allergy symptoms; class I = 0.35 - <0.70 – low levels of antibody with possible symptoms; class II = 0.70 - <3.50 = Moderate levels of antibody; Class III = 3.50 - <17.50 – high levels of antibody; Class IV = 17.50 - <50.0 – high levels of antibody; class V = 50 - 100.00 - very high levels of antibody; class VI = > 100.00 – extremely high levels of antibody.

Industry	Agent(s)
Baking	Flour (wheat, rye and fungal α-amylase)
Spice milling	Chilli pepper, garlic and wheat
Wood processing	Wood dust - Mutondo wood species
Poultry farming	Sunflower seed, mould mix, storage mite, chicken specific allergens (meat, feathers, serum protein, and droppings)
Fruit farming	Spider mite and storage mite
Health care workers	Natural rubber latex (NRL) – Hev B5 and Hevb6, , chlorhexidine, and ortho-phthalaldehyde (OPA)

Figure 1: Industries and associated exposure agents tested for using specific IgE

Covariates and confounders

Potential confounders to be considered in the analysis elevated FeNO include:

- age (continuous variable).
- sex (binary variable).
- smoking (categorical variable).
- atopy (binary variable based on Phadiatop result - <0.35 kU/L and ≥035 kU/L, also categorical [class I – VI]).
- inhaled corticosteroid treatment (binary variable).
- diet (nitrate rich foods), body mass index (BMI) and physical exercise will be considered in the initial analysis. Atopy will also be assessed as an effect modifier.
- Recent chest infections

DATA ANALYSIS

Data will be analysed using STATA version 12.0. Shapiro-Wilk test will be used to test normality of data distribution. Difference between continuous data variables will be tested using t-test and for categorical data will be tested using the chi-squared test.

Descriptive statistics will be used to summarise the distribution of each measured predictor variable. Frequencies will be summarized as percentages. Associations between FeNO and key determinants (individual/host and occupational related) will be explored using bivariate and multivariate logistic analysis. A p-value of ≤ 0.10 will be used as a criterion to determine the best predictive model for FeNO, using the stepwise forward and backward model selection procedure. Multivariate analysis will include all predictor variables for FeNO that will be significant in the univariate regression analysis. Multivariate logistic regression analyses will be carried out to assess the association between FeNO and individual/host related as well as occupational related factors. Odds ratio (OR) will be used as the measure of effect in multivariate logistic regression analysis. Potential confounders, including age, sex, smoking habits and ICS treatment will be adjusted for in multivariate analysis. Data will be stratified for atopy/sensitization to look for effect modification.

ETHICS AND COMMUNICATION

The original studies received ethical approval from the Research Ethics Committee of the University of Cape Town and other ethics bodies studies (study ethics numbers: Baatjies et al /Al Badri – HREC Ref: 272/2002; van der Walt HREC Ref: 179/2008; Ngajilo – HREC Ref: 601/2015; Ndlovu – HREC Ref: 393/2009; Mwanga – HREC Ref: 212/2013 and HUM00083115), Chamba – HREC Ref: 543/2016; National Bioethics Committee of Ministry of Health of Mozambique (40/CNBS/2015) and University of Michigan Medical School Institutional Review Board (HUM00070862)). This study will not collect any new data since secondary analysis will be conducted on existing datasets from the original studies. Therefore, the participants of the original studies will not be prone to any additional risk. The studies were conducted in accordance with the World Medical Association Declaration of Helsinki.

AUTONOMY

All workers signed informed written consent before participating in the initial studies. Participation was voluntary at no cost to either the worker or the employer.

BENEFICENCE

Study participants were informed of their tests results. Those that had abnormal results were offered referral for further evaluation to the Occupational Medicine Clinic at Groote Schuur Hospital (South African studies) and if necessary, they were referred to the respective doctors for further examination after the initial studies.

Although this study may not add direct individual benefits for the participants, the findings of the analysis will provide more information about the usefulness of using FeNO in screening and medical surveillance programs of high-risk workers in order to identify those at high risk of developing work-related respiratory allergy and asthma in different occupational settings, ultimately improving preventive measures for the workers.

MALEFICENCE

The original studies had negligible risk to participants. There were potentially low risks associated with performing fractional exhaled nitric oxide test and drawing blood, but participants gave informed consent and records of the results indicate that no adverse events were reported. Study participants may have suffered minimal discomfort from the needle prick during the blood sample collection.

Furthermore, there will be no additional risks to the participants since the current study does not require their active physical participation as data have already been collected.

CONFIDENTIALITY

Results of the tests after the initial studies were only shared with the participants. All data was coded with worker and company confidentiality maintained. The hard copy of results were stored in cabinets under lock and key in the School of Public Health at the University of Cape Town. Furthermore, the data was archived digitally and password protected with access limited to researchers only. For public presentations, only summary data will be presented without personal identifiers. Personal information will only be released with the worker's consent should there be a need to do so.

JUSTICE

The increased risk of work-related respiratory allergies and asthma among workers justifies the need for the research with an effort to find preventive ways to reduce the risk of workers developing work-related respiratory allergies and asthma so as to improve the quality of life and productivity of these workers.

DISSEMINATION OF THE RESEARCH RESULTS

The results of the study will be disseminated in the form of:

- MMed dissertation will be submitted to the University of Cape Town.
- Research forum, research day, local/international conference.
- Publications in scientific journal (local and peer review).

FUNDING

There is no additional funding required for this sub-study. The studies were originally funded with support provided through research scholarship grants from the Center for Asthma in the Workplace (Montreal, Canada), Medical Research Council (Republic of South Africa), National Research Foundation FA2006040700028 (Republic of South Africa), Allergy Society of South Africa (ALLSA), and the baking industry, for the bakery study;[34] South African Medical Research Council, Millennium Promise Programme (University of Michigan/Fogarty International Center) – National Institutes of Health (2 D43TW000812-06), Allergy Society of South Africa & National Research Foundation of South Africa for the health worker study;[35] Allergy Society of South Africa-CIPLA Research Award, the National Research Foundation and the Medical Research Council of South Africa supported the poultry farm project;[32] The Women on Farms Project and the University of Cape Town as well as a grant from the National Research Foundation of South Africa [Grant no. IFR 2011041400063] and the Medical Research Council of South Africa, and the Centre for International Health of the Ludwig-Maximilian-University, Munich, Germany (CIHLMU);[42] National Research Foundation and the Medical Research Council of South Africa, Allergy Society of South Africa-Glaxo Smith Kline Research Award supported the spice study;[16] and Millennium Promise Programme (University of Michigan/Fogarty International Center).[33]

REFERENCES

1. Oțelea MR, Fell AKM, Handra CM, Holm M, Filon FL, Mijakovski D, et al. The value of fractional exhaled nitric oxide in occupational diseases – a systematic review. Journal of Occupational Medicine and Toxicology. 2022;17(14):1–12.
2. Nerpin E, Olivieri M, Gislason T, Olin AC, Nielsen R, Johannessen A, et al. Determinants of fractional exhaled nitric oxide in healthy men and women from the European Community Respiratory Health Survey III. Clinical & Experimental Allergy. 2019;49(7):969–79.
3. Coman I, Lemière C. Fractional Exhaled Nitric Oxide (FeNO) in the Screening and Diagnosis Work-Up of Occupational Asthma. Curr Treat Options Allergy. 2017;4(2):145–59.
4. Al Badri FM, Baatjies R, Jeebhay MF. Assessing the health impact of interventions for baker's allergy and asthma in supermarket bakeries: a group randomised trial. Int Arch Occup Environ Health. 2020 Jul 11;93(5):589–99.
5. Pignatti P, Visca D, Loukides S, Märtson AG, Alffenaar JWC, Migliori GB, et al. A snapshot of exhaled nitric oxide and asthma characteristics: experience from high to low income countries. Pulmonology. 2021;28(1):44–58.
6. Olivieri M, Malerba M, Spiteri G, Torroni L, Biscardo CA, Valenza D, et al. Fractional exhaled nitric oxide levels in relation to work-related respiratory burden and sensitization to wheat flour and multigrain in bakers. Clin Transl Allergy. 2021;11(8):1–9.
7. Werthmann D, van Wendel de Joode B, Cuffney MT, Reich BJ, Soto-Martinez ME, Corrales-Vargas A, et al. A cross-sectional analysis of medical conditions and environmental factors associated with fractional exhaled nitric oxide (FeNO) in women and children from the ISA birth cohort, Costa Rica. Environ Res. 2023;233:1–8.
8. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention: 2023 GINA Report [Internet]. 2023. Available from: www.ginasthma.org.
9. Louis R, Satia I, Ojanguren I, Schleich F, Bonini M, Tonia T, et al. European Respiratory Society guidelines for the diagnosis of asthma in adults. European Respiratory Journal. 2022;60.
10. Iavicoli I, Fontana L, Leso V, Macrini MC, Pelclova D. Fractional Exhaled Nitric Oxide and Nanomaterial Exposure in Workplaces. Curr Med Chem. 2020;27(42):7200–12.
11. Engel J, van Kampen V, Lotz A, Abramowski J, Gering V, Hagemeyer O, et al. An increase of fractional exhaled nitric oxide after specific inhalation challenge is highly predictive of occupational asthma. Int Arch Occup Environ Health. 2018;91(7):799–809.
12. Suzuki RA, Irokawa T, Ogawa H, Ohkouchi S, Tabata M, Togashi S, et al. Fractional exhaled nitric oxide (FeNO) and spirometry as indicators of inhalation exposure to chemical agents in pathology workers. J Occup Environ Med. 2017;59(5):467–73.

13. Mwanga HH, Baatjies R, Singh T, Jeebhay MF. Asthma Phenotypes and Host Risk Factors Associated With Various Asthma-Related Outcomes in Health Workers. Frontiers in Allergy. 2021;2:1–12.
14. Wang H, Duan H, Meng T, Yang M, Cui L, Bin P, et al. Local and Systemic Inflammation May Mediate Diesel Engine Exhaust–Induced Lung Function Impairment in a Chinese Occupational Cohort. Toxicological Sciences. 2018;162(2):372–82.
15. Beretta C, Rifflart C, Evrard G, Jamart J, Thimpont J, Vandenplas O. Assessment of eosinophilic airway inflammation as a contribution to the diagnosis of occupational asthma. Allergy: European Journal of Allergy and Clinical Immunology. 2018;73(1):206–13.
16. van Der Walt A, Baatjies R, Singh T, Jeebhay MF. Environmental factors associated with baseline and serial changes in fractional exhaled nitric oxide (FeNO) in spice mill workers. Occup Environ Med. 2016 Sep 1;73(9):614–20.
17. Wild P, Mével H, Penven E, Zmirou-Navier D, Barbaud A, Bohadana A, et al. FeNO levels increase with degree of sensitisation in apprentices at risk of occupational asthma. International Journal of Tuberculosis and Lung Disease. 2017;21(11):1194–200.
18. Ngajilo D, Singh T, Ratshikhopha E, Dayal P, Matuka O, Baatjies R, et al. Risk factors associated with allergic sensitization and asthma phenotypes among poultry farm workers. Am J Ind Med. 2018 Jun;61(6):515–23.
19. Vinnikov D, Tulekov Z, Blanc PD. Fractional exhaled NO in a metalworking occupational cohort. Int Arch Occup Environ Health. 2022;95(3):701–8.
20. Dubini M, Marraccini P, Brass DM, Patrini L, Riboldi L. Occupational asthma and rhinitis due to wheat flour: Sublingual specific immunotherapy treatment. Medicina del Lavoro. 2020;111(3):203–9.
21. Klusáčková P, Dušková Š, Mráz J, Navrátil T, Vlčková Š, Pelclová D. Health effects of exposure to isocyanates in a car factory. Cent Eur J Public Health. 2022;30(1):32–6.
22. Sack C, Ghodsian N, Jansen K, Silvey B, Simpson CD. Allergic and respiratory symptoms in employees of indoor cannabis grow facilities. Ann Work Expo Health. 2020;64(7):754–64.
23. van Kampen V, Engel J, Hagemeyer O, Brüning T, Raulf M, Merget R. Does the measurement of exhaled nitric oxide before and after specific inhalation challenges improve the diagnosis of occupational asthma? Occupational and environmental health. 2019;54:OA3281.
24. Lim FL, Hashim Z, Than LTL, Md Said S, Hashim JH, Norbäck D. Respiratory health among office workers in Malaysia and endotoxin and (1,3)-b-glucan in office dust. International Journal of Tuberculosis and Lung Disease. 2019;23(11):1171–7.
25. Suojalehto H, Suuronen K, Cullinan P, Lindström I, Sastre J, Walusiak-Skorupa J, et al. Phenotyping Occupational Asthma Caused by Acrylates in a Multicenter Cohort Study. J Allergy Clin Immunol Pract. 2020;8(3):971-979.e1.

26. van Kampen V, Migueres N, Doyen V, Deckert A, de Blay F, Vandenplas O, et al. Phenotyping Occupational Asthma Caused by Platinum Salts Compared to Other Low-molecular-weight Agents. J Allergy Clin Immunol Pract. 2023;17(10):1076–93.
27. Vlaanderen J, Pronk A, Rothman N, Hildesheim A, Silverman D, Hosgood HD, et al. A cross-sectional study of changes in markers of immunological effects and lung health due to exposure to multi-walled carbon nanotubes. Nanotoxicology. 2017;11(3):395–404.
28. Pelclova D, Zdimal V, Komarc M, Vlckova S, Fenclova Z, Ondracek J, et al. Deep airway inflammation and respiratory disorders in nanocomposite workers. Nanomaterials. 2018 Sep 16;8(9).
29. Paraskevaidou K, Porpodis K, Kontakiotis T, Kioumis I, Spyratos D, Papakosta D. Asthma and rhinitis in Greek furniture workers. Journal of Asthma. 2021;58(2):1–10.
30. Crivellaro MA, Ottaviano G, Maculan P, Pendolino AL, Vianello L, Mason P, et al. Upper and Lower Respiratory Signs and Symptoms in Workers Occupationally Exposed to Flour Dust. Int J Environ Res Public Health. 2020 Sep 27;17(19):7075.
31. Mason P, Scarpa MC, Guarnieri G, Giordano G, Baraldi E, Maestrelli P. Exhaled nitric oxide dynamics in asthmatic reactions induced by diisocyanates. Clinical and Experimental Allergy. 2016;46(12):1531–9.
32. Ngajilo D, Singh T, Ratshikhopha E, Dayal P, Matuka O, Baatjies R, et al. Risk factors associated with allergic sensitization and asthma phenotypes among poultry farm workers. Am J Ind Med. 2018;61(6):515–23.
33. Chamba P, Baatjies R, Jeebhay MF. Respiratory symptoms, airway inflammation and lung function of workers in the Mozambiquan Wood Processing Industry [PhD Thesis]. [Cape Town]: University of Cape Town; 2023.
34. Baatjies R, Jeebhay MF. Sensitisation to cereal flour allergens is a major determinant of elevated exhaled nitric oxide in bakers. Occup Environ Med. 2013 May;70(5):310–6.
35. Mwanga HH, Baatjies R, Singh T, Jeebhay MF. Work-related allergy and asthma associated with cleaning agents in health workers in Southern African tertiary hospitals. Am J Ind Med. 2022;65(5):382–95.
36. Annangi S, Nutalapati S, Sturgill J, Flenaugh E, Foreman M. Eosinophilia and fractional exhaled nitric oxide levels in chronic obstructive lung disease. Thorax. 2022 Apr 1;77(4):351–6.
37. Lassmann-Klee PG. Fractional exhaled nitric oxide-F ENO-methodology and application in asthma epidemiology in Northern Europe [PhD Thesis]. [Helsinki]: University of Helsinki; 2021.
38. Marcos MC, Cisneros Serrano C. What is the added value of FeNO as T2 biomarker? Frontiers in Allergy. 2022;3:1–6.

39. Wang E, Wechsler ME, Tran TN, Heaney LG, Jones RC, Menzies-Gow AN, et al. Characterization of Severe Asthma Worldwide: Data From the International Severe Asthma Registry. Chest. 2020;157(4):790–804.
40. Dickinson J, Gowers W, Sturridge S, Williams N, Kippelen P, Simpson A, et al. Fractional exhaled nitric oxide in the assessment of exercise-induced bronchoconstriction: A multicenter retrospective analysis of UK-based athletes. Scand J Med Sci Sports. 2023 Jul 1;33(7):1221–30.
41. Burney PGJ, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. European Respiratory Journal. 1994;7(5):954–60.
42. Ndlovu V, Dalvie MA, Jeebhay MF. Asthma associated with pesticide exposure among women in rural Western Cape of South Africa. Am J Ind Med. 2014;57(12):1331–43.
43. Baatjies R, Lopata AL, Sander I, Raulf-Heimsoth M, Batemane ED, Meijster T, et al. Determinants of asthma phenotypes in supermarket bakery workers. European Respiratory Journal. 2009 Oct;34(4):825–33.
44. American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med. 2005;171(8):912–30.
45. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An Official ATS Clinical Practice Guideline: Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Applications. Am J Respir Crit Care Med. 2011 May;184:602–15.

SECTION B – LITERATURE REVIEW

Published in: Mfune P, Jeebhay MF. Determinants of fractional exhaled nitric oxide in workers at risk of developing occupational respiratory allergy and asthma: A literature review. Current Allergy and Clinical Immunology. 2023; 37(1): 31-41 (**Appendix 8**).

DETERMINANTS OF FRACTIONAL EXHALED NITRIC OXIDE IN WORKERS AT RISK OF DEVELOPING OCCUPATIONAL RESPIRATORY ALLERGY AND ASTHMA: A REVIEW

ABSTRACT

Fractional exhaled nitric oxide (FeNO) is a non-invasive biomarker of eosinophilic airway inflammation. FeNO is elevated in allergic asthma and its production increases in the airways in the presence of Type-2 inflammation. Both individual and occupational factors are associated with increased FeNO in workers with occupational asthma. This review focuses on identifying the specific predictors of elevated FeNO in workers at risk of developing occupational respiratory allergy and asthma, with a specific focus on those settings where it is used for medical screening and surveillance. The review found that individual factors such as age and atopy are associated with increased FeNO, whereas smoking and inhaled steroid use are consistently associated with decreased FeNO levels. Occupational factors such as exposure to either high or low molecular weight agents, occupational allergic sensitisation, the nature of work, the duration of employment and the level of exposure to occupational allergen(s) are associated with elevated FeNO. More recent evidence suggests that it is the agent's ability to induce an IgE-mediated mechanism, and not its molecular weight or its nature, that is responsible for elevated FeNO levels in occupationally exposed workers. Stronger epidemiological study designs such as cohort studies are needed that offer better opportunities to investigate these relationships in a more refined manner.

Keywords: FeNO, occupational allergy, asthma, occupational respiratory allergy, sensitisation

INTRODUCTION

Fractional exhaled nitric oxide (FeNO) is a non-invasive biomarker of eosinophilic airway inflammation and is able to predict steroid treatment response in asthmatics. This biomarker is primarily used in asthma, but is also relevant to various other pulmonary disorders, both allergic and non-allergic. These include chronic allergic rhinitis, acute and chronic eosinophilic pneumonia, chronic obstructive pulmonary disease (COPD), bronchiolitis obliterans syndrome, hypersensitivity pneumonitis (HP), viral infection and interstitial lung disease.[1–8]

Since a number of respiratory diseases can also be caused by work, FeNO represents a safe and easy measure with which to assess their work-relatedness in an occupational setting.[1,2] However, FeNO may be lower among those who smoke, have bronchoconstriction or use steroid therapy.[8]

Nitric oxide (NO) is a gas produced in the airways and is detectable as fractional exhaled NO (FeNO). Its production increases in the airways when Type-2 inflammation is present.[4] Induced sputum and FeNO measurements are the most reliable non-invasive procedures with which to assess the presence of Type-2 airway inflammation.[1,4]

It is understood that FeNO levels relate more closely to the risk of disease exacerbation than to disease severity.[4] FeNO levels correlate with asthma exacerbations in severe asthmatic patients independently of blood eosinophils. Whereas FeNO is associated with increased sputum and blood eosinophil levels, it correlates moderately with the former and weakly with the latter.[3,8]

Apart from its usefulness in the diagnostic work-up of individuals with suspected asthma, the measurement of FeNO offers a useful tool in the screening and surveillance of working populations at risk of developing occupational asthma (OA) associated with exposure to respiratory sensitisers.[3,9] Most studies on the determinants of FeNO have generally focused on the general population as opposed to workers in the occupational setting.[1–3,10]

OBJECTIVES OF THE LITERATURE REVIEW

The aim of this review was to evaluate the individual or host and occupation-related determinants of elevated FeNO in workers at risk of developing occupational respiratory allergy and asthma. The findings of the review will contribute to obtaining better insights into FeNO levels in working populations and to assess their applicability in the screening and surveillance of high-risk occupational groups. This could contribute to improved preventive measures for workers at risk of developing occupational respiratory allergy and asthma.

LITERATURE SEARCH STRATEGY

The review focused on the research question: 'What are the determinants of elevated FeNO levels in workers at risk of developing occupational respiratory allergy and asthma in different occupational settings?'

The review included epidemiological and clinical studies of FeNO measurements in workers from different occupations that were published in English from January 2017 to July 2023. The literature search followed the PICO framework to search systematically for relevant articles in PubMed and EBSCOHost (MEDLINE/CINAHL/Africa-Wide Information) databases using specific search terms and identifying relevant articles cited in the references of the articles identified through the search. The following search terms were used:

"[Determinants OR Workers OR occupation OR occupational OR work related OR occupational exposure OR industry OR baking OR wood processing OR spice milling OR spices OR health care OR health care worker OR poultry farming OR fruit farming OR spices OR flour OR dust OR alpha amylase OR α-amylase OR amylase OR latex gloves OR cleaning agents OR high molecular weight agents OR low molecular weight agents OR Asthma OR sensitization OR atopy AND [FeNO OR fractional exhaled nitric oxide]".

The review finally included 26 epidemiological and clinical studies of FeNO measurements in workers from different occupations (see Figure 1).

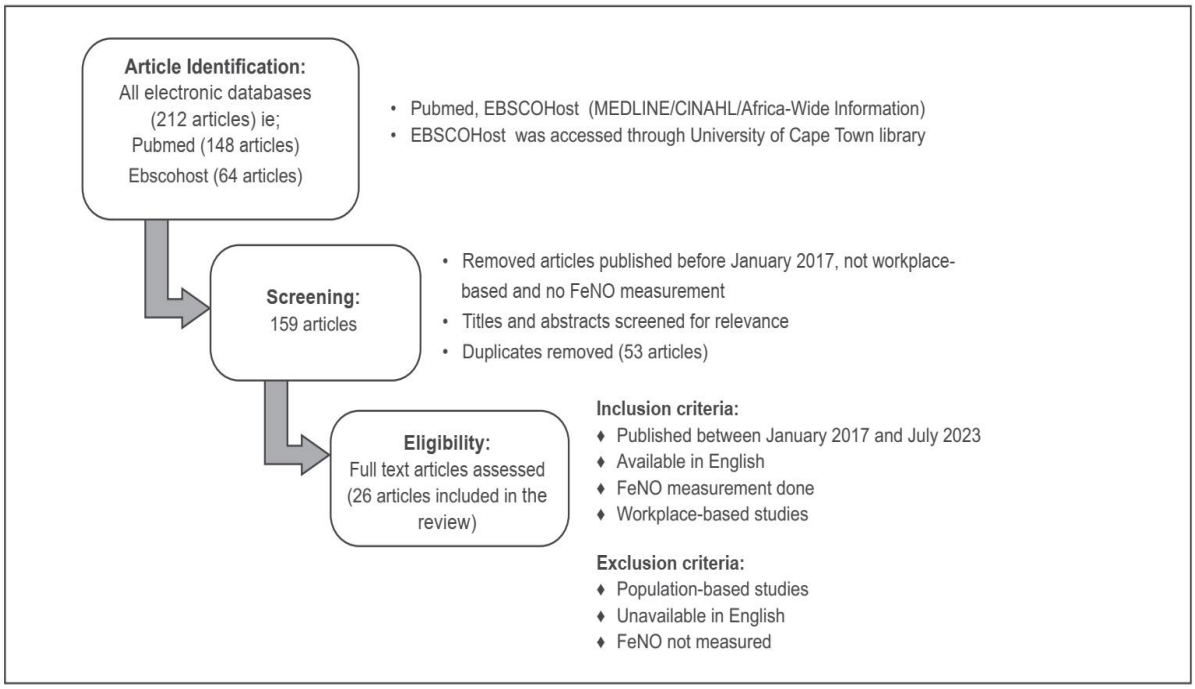

Figure 2: Flow diagram outlining the article search strategy

DETERMINANTS OF FRACTIONAL EXHALED NITRIC OXIDE

Studies of the determinants of FeNO were broadly categorised into either observational epidemiological studies of high-risk working populations exposed to agents of high molecular weight (HMW) or low molecular weight (LMW) and clinical studies of patients with confirmed or suspected occupational asthma (OA). The epidemiological findings are summarised according to workers exposed to agents of HMW (see Table I), LMW (see Table II) or both HMW and LMW (see Table III). The findings observed in patients with either confirmed or suspected OA are presented in Table IV. The review identified various individual (host) and occupational factors associated with elevated FeNO in these various studies.

Table I: Epidemiological studies of risk factors associated with elevated fractional exhaled nitric oxide (FeNO) in workers exposed to HMW agents

Author, year	Study design	Subjects (N)	Exposure or occupational agent	FeNO, ppb mean (±SD); median (IQR)	Determinants (individual/host, occupational)	Predictors of elevated FeNO OR(CI), RR(CI)
Al Badri et al, 2020[7]	Group-randomised intervention (bakery mixer tub lid, dust-control training)	337 bakers	Cereal flours	*FeNO mean difference* Intervention 2.2 (±23) Controls 1.7 (±14.5) *FeNOΔ baseline >25* Intervention 16.9 (±35.2) Control 7.7 (±24.2)	***Individual:*** Current smoker ***Occupational:*** *FeNO change ≥10%* *Intervention group:* Bakers with WRONS Bakers without WRONS *Baseline FeNO > 25:* Bakers with WRONS Bakers without WRONS	 OR 0.92 (0.56–1.52) OR 3.73 (1.22–11.42) OR 1.62 (0.93–2.81) OR 5.03 (1.79–14.16) OR 3.02 (1.35–6.77)
Olivieri et al, 2021[5]	Cross-sectional	174 bakers	Wheat, multigrain	*Sensitisation* Wheat 24.7 (20.1–30.3) Rye 29.3 (21.5–40.0) Alpha-amylase 22.3 (16.2–30.6) *Respiratory symptoms* WRR 25.4 (20.6–31.3) Wheeze 35.5 (25.9–48.6) Chest tightness 27.7 (18.5–41.7)	***Individual:*** Sensitisation Respiratory symptoms	 ND ND
Sack et al, 2020[25]	Cross-sectional	45 cannabis workers (growing facility)	Cannabis dust	FeNO 30 (8–102) *Cross-week change* 3.78 (0.68–6.88) *Cross-shift change* −1.36 (−4.46–1.74)	***Individual:*** Respiratory Ocular Nasal	 ND ND ND
Crivellaro et al, 2020[33]	Cross-sectional	142 bakery workers	Cereal flour dust	FeNO 23.7 (±25.0) *FeNO mean difference* Flour dust concentration >2 mg/m^3 8.60 (p = 0.06)	***Individual:*** Smokers ***Occupational:*** Dust concentration >2 mg/m^3	 ND ND

				Occupational exposure duration (yrs) 0.22 (p = 0.22)	Occupational exposure duration	ND
Ngajilo et al, 2018[21]	Cross-sectional	230 poultry workers (rearing, laying, hatchery, broiler, catching)	Poultry dust and bioaerosols	FeNO >50: 7%	***Individual:*** Current smoker Atopy ***Occupational:*** Casual worker Rearing department	 OR 0.41 (0.13–1.33) OR 3.04 (1.11–8.34) OR 1.76 (0.26–11.94) OR 1.67 (0.31–8.89)
Van der Walt et al, 2016[19]	Cross-sectional	150 spice mill workers	Spice dust (chilli, garlic)	*FeNO baseline >50* Day1 preshift FeNO 67.74 (±1.37) Day1 post shift 60.31 (±1.62) Day2 preshift 64.11 (±1.57) *24-hour FeNOΔ ≥12% increase* Day1 preshift 14.09 (±2.07) - Day2 preshift 20.12 (±2.07)	***Individual:*** **Sensitisation** *FeNO baseline >50 C*hilli pepper Garlic Wheat ***Occupational:*** Work-related respiratory symptoms ***24-hour FeNOΔ ≥12% increase*** Spice dust particulate (>1.18–<3.78 mg/m³)	 OR 17.04 (2.30–126.03) OR 6.80 (1.35–34.20) OR 4.84 (0.88–26.74) OR 5.38 (1.0–28.94) OR 3.77 (1.01–28)
Lim et al, 2019[27]	Cross-sectional	695 office workers	Indoor office dust and biological exposures (endotoxin, (1,3)-β-glucans)	*FeNO >25* Office workers 25.8% Rhinoconjunctivitis 35.2% Doctor-diagnosed asthma 47.7%	***Occupational:*** Sieved dust (1,3)-b-glucan	 OR 2.91 (1.31–6.47) OR 1.10 (1.01–1.20)

CI: confidence interval, FeNO: fractional exhaled nitric oxide, FeNOΔ: change in fractional exhaled nitric oxide, HMW: high molecular weight agents, ppb: parts per billion, IQR: interquartile range, OR: odds ratio, RR: relative risk, ND: not done, WRONS: work-related ocular-nasal symptoms, WRR: work-related rhinitis

Table II: Epidemiological studies of risk factors associated with elevated fractional exhaled nitric oxide (FeNO) in workers exposed to LMW agents

Author, year	Study design	Subjects (N)	Exposure or occupational agent	FeNO, ppb mean (±SD); median (IQR)	Determinants (individual/host, occupational)	Predictors of elevated FeNO OR(CI), RR(CI)
Werthmann et al, 2023[6]	Cross-sectional	277 women living < 5 km from banana plantation	Pesticides, smoke, agricultural chemicals	FeNO >25: 20%	***Individual:***	
					Current smoker	OR 0.28 (0.02–1.42)
					Pregnant	OR 0.89 (0.13–3.60)
					Medical conditions:	
					Asthma	OR 1.60 (0.76–3.22)
					Rhinitis	OR 3.67 (1.81–7.35)
					Wheeze	OR 4.50 (2.25–8.99)
					Occupational/ environmental:	
					Pesticide use	OR 1.46 (0.76–2.93)
					Partners work in agriculture	OR 1.61 (0.77–3.58)
Klusáčková et al, 2022[24]	Cross-sectional	35 workers (26 factory, 9 office work)	Polyurethane and glue containing diisocyanates (MDI, TDI, HDI)	*FeNO day1*	***Individual:***	
				exposed 23 (±10)	Cough	ND
				non-exposed 17 (±11)	Dyspnoea	ND
				FeNO day2		
				exposed 21 (±10)	***Occupational:***	
				non-exposed 16.6 (±8.50)	Isocyanate exposure	ND
Vinnikov et al, 2022[22]	Cross-sectional	80 metal-working workers (33 machine operators, 26 welders/assemblers, 21 administrative)	Welding, machining, assembling, metal coatings application (mild steel, higher nickel-content steel, and stainless steel)	Smoker (0–10/day) 35.8 (±8.9)	***Individual:***	
				Smoker (>10/day) 24.9 (±8.8)	Smoker	ND
				Welders/assemblers 43.5 (±49.3)		
				Machine operators 25 (±15)	***Occupational:***	
				Administrative 22 (±16)	Welders	ND
					Machinists	ND
					Administrators	ND
Paraskevaidou et al, 2021[32]	Cross-sectional	83 furniture workers (35 chemical	Chemicals, wood dust	Chemical exposed 14.42 (±9.13)	***Individual:***	
				Wood-dust exposed 15.24 (±6.93)	Smoker	ND
				Office workers 12.98 (±5.28)	Respiratory symptoms	ND

		exposed, 23 wood dust, 25 office workers)			***Occupational:*** Length of exposure (months)	ND
Pelclova et al, 2018[31]	Cross-sectional	41 office workers (20 exposed, 21 unexposed)	Nanocomposite materials	Pre-shift 24 (6–50) Post-shift 17 (4–40) Unexposed 15 (5–35)	***Individual:*** Age Sex Respiratory symptoms	 ND ND ND
Wang et al, 2018[14]	Cross-sectional	264 diesel exhaust technicians, DET (n=137), 127 unexposed (water utility authority)	Diesel exhaust (high levels $PM_{2.5}$ and elemental carbon)	DETs 16.5 (8.0–43.0) Non-DETs 16.2 (8.0–N34.0) *DETs exposure:* - (0.6–6.3) years, 17.4 (9.0–53.0) - (6.3–9.3) years, 16.4 (8.0–33.0) - (9.3–36.8) years, 15.9 (8.0–30.0)	***Individual:*** Smoker ***Occupational:*** DETs length of exposure (years)	 ND ND
Suzuki et al, 2017[13]	Cross-sectional	63 pathology technicians (33 exposed, 30 unexposed)	Formaldehyde	*Cross-week change in subjects:* *Asthmatics:* Post-work 47.5 (±39.7) *Non-asthmatics:* Post-work 17.5 (±11.0) *Cross-week change in Controls:* *Asthmatics:* Post-work 30.9 (±15.3) *Non-asthmatics:* Post-work 14.0 (±7.2)	***Individual:*** Asthmatics ***Occupational:*** Length of employment (months)	 ND ND
Vlaanderen et al, 2017[30]	Cross-sectional	50 nanotube workers (21 exposed) vs 29 unexposed (age and gender matched)	Carbon nanotubes	*FeNO difference (Lab exposure):* Low 0.25 High 0.08 Intermediate (operator) 0.88 EC exposure trend estimate –0 .020	***Individual:*** Smoker ***Occupational:*** Level of exposure	ND ND

BMI: body mass index, CI: confidence interval, DETs: diesel engine testers, EC: elemental carbon, FeNO: fractional exhaled nitric oxide, HCW: health care worker, HDI: hexamethylene diisocyanate, IQR: interquartile range, LMW: low molecular weight agents, MDI: methylene diphenyl diisocyanate, MWCNT: multi-wall carbon nanotubes, ND: not done, OR: odds ratio, ppb: parts per billion, RR: relative risk, SIC: specific inhalation challenge test, SD: standard deviation

Table III: Epidemiological studies on risk factors associated with elevated fractional exhaled nitric oxide (FeNO) in workers exposed to both HMW and LMW agents

Author, year	Study design	Subjects (N)	Exposure or occupational agent	FeNO, ppb mean (±SD); median (IQR)	Determinants (individual/host, occupational)	Predictors of elevated FeNO OR(CI), RR(CI)
Mwanga et al, 2022[18]	Cross-sectional	699 healthcare workers	Cleaning agents (ortho-phthaladehyde, chlorhexidine, natural rubber latex)	*Sensitisation* OPA: 1.49 (1.15 – 1.93) NRL: 1.82 (1.19 – 2.78) All: 1.57 (1.25 – 1.97)	***Individual:*** *Sensitisation***:** *FeNO ≥ 25* OPA NRL All *FeNO ≥ 50* OPA NRL All	 OR 3.90 (1.74 – 8.75) OR 4.32 (1.15 – 16.29) OR 4.18 (2.03 – 8.59) OR 3.10 (1.01 – 9.50) OR 4.50 (0.91 – 22.43) OR 3.94 (1.52 – 10.21)
Wild et al, 2017[20]	Cohort study	441 apprentices	Baking, pastry-cooking, hairdressing	*Baseline:* Bakers 21.0 ± 23.1 Pastry makers 19.8 ± 17.3 Hairdressers 15.5 ± 15.0	***Individual:*** *Sensitisation to common inhalants:* Weak (1–2 positive SPT) High (≥3 positive SPT)	 OR 1.30 (1.14 – 1.49) OR 1.82 (1.58 – 2.10)
Shiryaeva et al, 2015[11]	Cross-sectional	266 (trawler fishermen, n=127; salmon processors, n=139)	Seafood processing, (slaughtering and fileting of fish)	Trawler fishermen = 28.1 smoker = 9.5 non-smoker = 17.2 asthmatics = 28.1 non-asthmatics = 12.4 Salmon processors = 12.6 smoker (men) = 11.1 non-smoker (men) = 11.5 asthmatics = 12.6 non-asthmatics = 10.9	***Individual:*** Smoker Asthma	 ND ND

CI: confidence interval, FeNO: fractional exhaled nitric oxide, FEV_1: forced expiratory volume in 1 second, HMW: high molecular weight agents, IQR: interquartile range, LMW: low molecular weight agents, ND: not done, NRL: natural rubber latex, OPA: ortho-phthalaldehyde, OR: odds ratio, ppb: parts per billion, RR: relative risk, SPT: skin-prick test

INDIVIDUAL (HOST) FACTORS

AGE

Workplace-based studies that analysed the effect of age on FeNO measurement are very limited, hence reference values for adults are still not available. Age is generally positively associated with FeNO in healthy individuals. In a populationbased study on the determinants of FeNO in healthy men and women from the European Community Respiratory Health Survey (ECRHS III), Nerpin et al reported that men in the upper age quartile (60.3–67.6 years) had 19.2 ppb (95% CI 18.3–20.2) higher FeNO than those in the lowest quartile (39.7–48.3 years).[2] Similarly, women in the two highest age quartiles (54.6–60.2 and 60.3–67.6 years) had 15.4 ppb (14.7–16.2) and 16.4 ppb (15.6–17.1), respectively higher FeNO, compared to the lowest quartile. However, Shiryaeva et al, 2015 found that age was not significantly correlated with FeNO levels.[11]

SEX

Most studies did not report on the association between sex and FeNO. However, various population studies have reported differences in FeNO levels between men and women. In the ECRHS III study, FeNO levels were 21.3% higher in men compared to women ($p < 0.001$).[2] However, an earlier study by Engel et al reported that male patients with OA were less likely (OR 0.69, CI 0.26–1.87) to have a ≥13 ppb FeNO increase after a specific inhalation challenge test.[12] In a study of bakers, no significant difference was reported between median FeNO in men of 18.1 ppb (95% CI 15.3, 21.4) compared to women 15.3 ppb (95% CI 11.7–20.3).[5] Similarly, no significant sex differences were found in pathology workers exposed to chemical agents.[13]

ANTHROPOMETRIC CHARACTERISTICS

In their study of bakers, Olivieri et al reported no significant correlation between FeNO and either height, weight or BMI.[5] Height contributed an 8% significantly higher FeNO in men and 5% in women.[2] Other studies have reported that BMI was not significantly associated with elevated FeNO levels.[2,14,15] Whereas the recent study of women working on banana plantations reported a positive association between BMI and elevated FeNO (OR 1.61, 0.89–2.95), the association was not significant.[6]

ATOPY AND SPECIFIC ALLERGIC SENSITISATION

It is well known that FeNO is higher in atopic individuals subjected to either occupational or environmental exposures.[1–3,10,15] In a study of patients with suspected OA due to HMW and LMW agents, Beretta et al reported that in subjects who undertook a positive specific inhalation challenge test (SIC), a FeNO >25 ppb was more likely to be observed in atopics (OR 2.9, 1.4–6.0).[16] A review of the determinants of FeNO in asthmatics also reported that levels are elevated in asthmatic patients, particularly in those who are atopic.[4]

Engel et al also reported that male patients with a ≥13 ppb FeNO increase after specific inhalation challenge test showed a trend (OR 1.86, 0.75–4.64) to be atopic.[12] In a clinical case series of workers with asthmatic reactions induced by isocyanates, the subjects demonstrated a delayed increase in FeNO 24 hours following exposure in the active (6.6 nL/s) SIC group, of 111.8 ppb, CI (68.5–236.0) compared to sham (3.3 nL/s) SIC of 58.6 ppb, CI (24.1–98.8).[17]

In health workers who frequently use cleaning agents, a strong positive association was observed between those with elevated FeNO ≥25 ppb and specific allergic sensitisation to either ortho-phthalaldehyde (OPA) (OR 3.10, 1.01–9.50) or natural rubber latex (NRL) (OR 4.32, 1.15–16.29).[18] Similarly, in a cross-sectional study of spice mill workers, atopy was strongly associated with sensitisation and FeNO >50 ppb.[19] Workers with FeNO >50 ppb were also more likely to be specifically sensitised to chilli pepper (OR 17.04, 2.30–126.03) or garlic (OR 6.80, 1.35–34.20).[19] Other studies have also shown that laboratory animal workers with a high specific IgE to laboratory animal allergens showed an increase in their FeNO levels when compared to those without specific IgE.[3] Similar findings were reported by Wild et al in apprentices.[20] Highly sensitised apprentices exhibited increased log odds of elevated FeNO compared to non-sensitised (log OR 1.42, 1.10–1.83).[20]

SMOKING STATUS

It is well known that smoking status is inversely related to FeNO levels in adults, including asthmatic individuals, such that it masks the elevated FeNO in asthmatics.[1,3,10] Studies also report significantly lower FeNO values among both acute and chronic smokers compared to non-smokers in the occupational context.[3,4]

In a group randomised trial assessing the impact of interventions for baker's allergy and asthma in supermarket bakeries, Al Badri et al showed that current smokers had a trend (OR 0.92, 0.56–1.52) of having reduced FeNO levels.[7] Similar observations were reported in poultry farmers, bakery workers and banana plantation workers.[5,6,21]

Some studies have been able to demonstrate an inverse dose– response relationship between smoking status and FeNO. Wang et al in their study on diesel exhaust engine testers demonstrated that cigarette smokers with a low internal dose had higher FeNO levels (18.7 ppb) than those with a medium (16.5 ppb) or high (14.4 ppb) internal dose of cigarette smoke.[14] Similar observations were also made in metal-working workers, in that subjects who smoked <10 cigarettes per day had a higher mean FeNO of 35.8 (±8.9 ppb) compared to those who smoked >10 cigarettes per day, having a mean FeNO of 24.9 (±8.8 ppb).[22]

In clinical studies of patients with suspected OA due to HMW and LMW agents, subjects with FeNO ≥25 ppb post-specific inhalation challenge were more likely (OR 2.5, 1.2–5.0) to have never smoked.[16] Similar studies

among patients exposed to HMW, LMW and isocyanates, demonstrated that smokers showed a trend (OR 1.25, 0.45–3.45) of having a ≥13 ppb increase in FeNO after SIC.[12]

INHALED CORTICOSTEROID USE

It is well known that inhaled corticosteroids can cause lower FeNO in certain individuals.[4] Furthermore, FeNO levels decline after inhaled or systemic steroid treatment in steroid-sensitive asthmatic patients.[3,4]

In studies of suspected OA due to HMW and LMW agents, the use of inhaled corticosteroids showed a trend of having reduced odds (OR 0.53, 0.22–1.30) of having a ≥13 ppb increase in FeNO postSIC.[12] Other studies have reported that an increase in FeNO after occupational exposure, especially to HMW agents, may be blunted in patients receiving inhaled or oral steroid treatment.[3] Similarly, FeNO reduction was also reported after a sublingual specific immunotherapy treatment (SLIT) administered to bakers with OA and rhinitis caused by wheat flour.[23]

RESPIRATORY SYMPTOMS

Respiratory symptoms also correlate with increased FeNO in various occupational contexts. Cough, dyspnoea, rhinitis and ocular–nasal symptoms were associated with FeNO >50 ppb in isocyanate exposed car-factory workers.[24] In their study of banana plantation workers, Werthmann et al reported that women with elevated FeNO levels were more likely to report wheeze (OR 4.50, 2.25–8.99), rhinitis (OR 3.67, 1.81–7.35) and asthma (OR 1.60, 0.76–3.22) showed the trend to report a wheeze.[6] In bakery workers, FeNO was higher in flour- and multigrain-exposed subjects having wheeze, chest tightness and shortness of breath. Workers having two asthma-related symptoms had significantly ($p < 0.001$) higher median (IQR) FeNO 35.9 ppb (25.6–50.2) than those with only one, 17.5 ppb (12.7–24.0), or no symptoms, 13.3 ppb (11.4– 15.6).[5] In a cross-sectional study investigating the relationship of workplace exposures and health symptoms at an indoor growing facility, cannabis workers had a trend of having 1.6 higher odds of having work-related nasal symptoms (OR 1.6, 95% CI 0.27–11) and 2.5-fold higher odds (OR 2.5, 95% CI 0.19–37) in individuals with medium and high cannabis-dust exposure. The study reported a mean FeNO of 30 ppb (8–102) among employees with work-related symptoms and a significant increase in cross-week (Monday to Friday) mean FeNO of 3.78 ppb (95% CI 0.68–6.88 ppb; $p = 0.02$).[25] Shiryaeva et al in their study of Russian trawler fishermen and Norwegian salmon industry workers found that asthmatic exhibited notably higher FeNO (28.1 ppb) compared to non-asthmatic trawler workers (12.4 ppb), while there was no significant difference in FeNO between asthmatic (12.6 ppb) and non-asthmatic salmon workers (10.9 ppb).[11]

PREGNANCY

The study of women working in banana plantations in Costa Rica showed an inverse trend between pregnancy and elevated FeNO (OR 0.89, 0.13–3.60), but the association was not significant.[6]

Table IV: Clinical studies of patients with OA (or suspected) and risk factors associated with elevated fractional exhaled nitric oxide (FeNO)

Author, year	Study design	Subjects (N)	Exposure or occupational agent	FeNO, ppb mean (±SD); median (IQR)	Determinants (individual/host, occupational)	Predictors of elevated FeNO OR(CI), RR(CI)
Van Kampen et al., 2023[29]	Retrospective observational	455 with OA due to Pt salts (*n* = 14), HMW (*n* = 225), LMW (*n* = 216)	Platinum (Pt) salts, HMW, LMW agents	Pt vs LMW agents *Baseline:* Pt 30 (13–64) LMW 19 (11–31) HMW 25 (13–41) Isocyanates 17 (12–23) Persulfate 17 (11–26) *Post-challenge:* Pt 77 (44–142) LMW 23 (14–40) HMW 44 (24–84) Isocyanates 23 (15–43) Persulfate 21 (14–34)	***Individual:*** Sex (male) Inhaled SABA use ***Occupational:*** Length of exposure Isocyanates	 ND ND ND ND
Suojalehto et al, 2020[28]	Retrospective observational	598 with OA due to acrylates (n=55), Isocyanates (n=125), other LMW agents (n=418)	Acrylates	FeNO *Baseline:* Acrylate OA 15 (10.0–29.0) Isocyanates OA 20 (12.0–35.0) Other LMW agents OA 19 (10.0–32.8) *Post-SIC FeNO*Δ*:* Acrylate OA 26.0 (8.2–38.0) Isocyanates OA 5.0 (2.0–16.0) Other LMW agents OA 3.0 (–1.0–10.0)	***Occupational:*** Acrylates Isocyanates Other LMW agents	 ND ND ND
Dubini et al, 2020[23]	Case series	5 bakers with OA	Wheat flour dust	*FeNO reduction:* After SLIT 12.20 (9.76–14.64)	***Individual:*** Sex Atopy ICS use***Occupational:*** HMW agents	 ND ND ND ND

Engel et al, 2019[34]	Retrospective observational	122 with suspected OA (pulmonary responders vs non- responders)	HMW and LMW agents	FeNO increase ≥ 13 ppb post- SIC *Baseline: FeNO:* SIC(+) pulmonary response 24 (3–151) SIC(–) 14 (5–119) *Post-SIC FeNO:* SIC positive pulmonary response 54 (6–156) SIC(–) 16 (6–126)	***Individual:*** Sex (male) Smoker Atopy ICS use ***Occupational:*** HMW Isocyanates	 ND ND ND ND ND ND
Van Kampen et al, 2019[26]	Retrospective observational	41 with suspected OA	HMW and LMW agents	*FeNO increase:* At work 20.5 Off-work 16.0	***Individual:*** Smoker Atopy ***Occupational:*** HMW LMW	 ND ND ND ND
Engel et al, 2018[12]	Retrospective observational	148 with suspected OA (pulmonary responders, non-responders, doubtful)	HMW, LMW and isocyanates	*Total:* Baseline 15 (3–158) Post-SIC 21 (5–193) *SIC*(+): Baseline 23 (3–151) Post-SIC 41 (5–156) *SIC*(–): Baseline 14 (3–158) Post-SIC 17 (6–193)	***Individual:*** *FeNO ≥13 ppb after SIC* Sex (male) Smoker Atopy ICS use ***Occupational:*** HMW Isocyanates	 OR 0.69 (0.26–1.87) OR 1.25 (0.45–3.45) OR 1.86 (0.75–4.64) OR 0.53 (0.22–1.30) OR 0.11 (0.01–1.08) OR 1.63 (0.58–4.63)
Beretta et al, 2018[16]	Prospective observational	240 with suspected OA (133 SIC(+) and 107 SIC(–))	HMW and LMW agents	*Positive SIC* PC_{20} ≤16 mg/mL 25.2 (10.9–46.0) PC_{20} >16 mg/mL 18 (17.0–35.3) *Negative SIC* PC_{20} ≤16 mg/mL 14 (8.7–33.0) PC_{20} >16 mg/mL 14.4 (7.8–22.2)	***Individual:*** *In SIC+ FeNO (≥25 ppb)* Never smoker Atopy ***Occupational:*** HMW	 OR 2.5 (1.2–5.0) OR 2.9 (1.4–6.0) OR: 7.2 (1.4–37.7)

Mason et al, 2016[17]	Case series	26 with suspected OA (17 SIC (+) vs 9 SIC(–))	Isocyanates (polyurethane)	*Positive SIC* *Baseline:* Active (6.6 nL/s) 58.6 (24.1–98.8) Sham (3.3 nL/s) 51.2 (25.2–120.3) *SIC+ 24 hrs* Active 111.8 (68.5–236.0) Sham 58.6 (24.1–98.8) *Negative SIC* *Baseline:* Active 17.6 (11–26.8) Sham 13.5 (10.9–19.6) *SIC(–) 24 hrs* Active 19.5 (12–26.8) Sham 17.6 (11–26.8) Atopic SIC+ 122.9 (89.4–156.4) Non-atopic SIC+ 54.1 (41.6–66.6)	***Individual:*** Atopy ***Occupational:*** SIC(+) SIC(–)	 ND ND ND

CI: confidence interval, FeNO: fractional exhaled nitric oxide, HMW: high molecular weight agents, *FeNO*Δ: change in fractional exhaled nitric oxide, ICS: inhaled corticosteroids, IQR: interquartile range, LMW: low molecular weight agents, ND: not done, NSBH: non-specific bronchial hyperresponsiveness, OA: occupational asthma, OR: odds ratio, PC_{20}: provocative concentration of histamine causing a 20% fall in FEV_1, Pt: platinum salts, RR: relative risk, SD: standard deviation, SIC (+): positive specific inhalation challenge test, SIC (–): negative specific inhalation challenge test, SLIT: sublingual immunotherapy treatment

OCCUPATIONAL FACTORS

NATURE OF AGENTS

High molecular weight agents

Exposure to HMW agents has been reported previously to be associated with elevated FeNO.[3,26] In a cross-sectional study of bakers in a wheat and multigrain bakery, workers exposed to wheat, rye, oats, barley, yeast and alpha amylase had elevated baseline FeNO levels.[5] In the spice-milling industry, workers with work-related asthma symptoms were also more likely (OR 5.38, CI 1.01–28.95) to have baseline FeNO >50 ppb.[19]

In clinical studies, exposure to HMW agents at work have also been associated with elevated FeNO levels. Specific inhalation challenge tests with HMW agents inducing an IgE-mediated sensitisation were associated with a ≥12% increase in FeNO.[3] Similarly, subjects exposed to HMW agents on SIC were more likely (OR 7.2, 1.4–37.7) to have FeNO ≥25 ppb.[16] A study of 41 workers that measured FeNO longitudinally at home and at work demonstrated higher FeNO (20.5 ppb) at work compared to periods off work (16.0 ppb), especially among those exposed to flour dust.[26] In a study of 460 office workers by Lim et al, office workers exposed to sieved dust had increased odds of FeNO ≥25 (OR 2.91, 95% CI 1.31–6.47).[27]

Low molecular weight agents

In clinical studies of OA patients with exposure to LMW, agents were generally not associated with elevated FeNO.[26] LMW agents do not typically demonstrate significant longitudinal differences in FeNO (increase ≥20% or >6 ppb) 24 hours after SIC.[1] However, exposure to specific LMW agents may be associated with varying FeNO levels. In a multicentre retrospective observational study of 598 workers, Suojalehto et al observed that workers exposed to acrylate (15.0 ppb) and other LMW agents (19.0 ppb) had lower baseline FeNO compared to isocyanates (20.0 ppb).[28] Furthermore, SIC with isocyanates was associated with significantly increased FeNO levels and an increased odds (OR 1.63, 0.58–4.63) of having FeNO ≥13.0 ppb after SIC.[1,3,12]

Recent studies show that FeNO is increased in IgE-mediated inflammation, irrespective of the molecular weight of the causative agent.[29] Similarly to isocyanates, workers with platinum salt-induced OA were shown to have a significantly greater increase in FeNO post-challenge compared to HMW and other LMW agents.[29] The study suggested that it was the mechanism and not the molecular weight of the agent that was important for inducing FeNO since it is generally understood that OA caused by LMW platinum salts is related to an IgE-mediated mechanism.[29]

Epidemiological studies have also yielded some interesting results. In a cross-sectional study by Suzuki et al, pathology technicians with bronchial asthma and exposed to formaldehyde showed a significant increase in FeNO (26 ppb) compared to control subjects.[13] However, a study of workers exposed to multi-walled carbon nanotubes showed a depression of FeNO with increasing levels of exposure. The FeNO difference was smaller in the high-exposure group (0.0841 ppb) compared to the low-exposure group (0.2478 ppb).[30] Similar findings were reported by Iavicoli et al in their review of seven studies of FeNO and nanomaterial exposure in workplaces.[10] No significant differences were observed between mean FeNO in the exposed (1.20 ppb (±0.32)) and the control groups (1.28 ppb (±0.32)).[10] However, in a study of 41 workers exposed to nanocomposite materials, Pelclova et al reported post-shift FeNO reduction in the exposed group (pre-shift 24 ppb (6–50); post-shift 17 ppb (4–40)).[31]

Few studies have been reported in the wood and furniture manufacturing industry. A study of Greek furniture workers reported no association between baseline and longitudinal FeNO levels associated with wood-dust exposure, despite asthma and rhinitis being common among the chemical and wood workers.[32] The study showed no significant differences between wood- or chemical-exposed workers and office workers, and only 6% of the subjects had FeNO >25 ppb.[32] A limitation of that study was the lack of power and heterogeneity of work practices, ventilation systems, and proper personal protective equipment (PPE) use among the furniture workers. Coman et al previously also reported no difference in FeNO levels in workers who had ceased exposure to red cedar compared to those with continued exposure.[3]

NATURE OF WORK (OR TASK)

Changes in FeNO levels could also be attributed to the nature of the work. In the same workplace, FeNO levels may differ in individual workers depending on the work they perform. In their study of metal-working workers, for instance, Vinnikov D et al reported significantly higher mean FeNO levels in welders or assemblers of 44.3 ppb (±49.3) compared to machine operators with 25 ppb (±15) and office workers with 22 ppb (±16), despite their all working in the same area.[22]

Similar observations were made in poultry farm workers, where working in the rearing department was more likely (OR 1.67, 0.31–8.89) than other areas to be associated with FeNO >50 ppb.[21] The study also found that casual workers were more likely (OR 1.76, 0.26–11.94) to have FeNO >50 ppb compared to permanent workers.[21] This was attributed to various reasons, including workers using poor-quality PPE, being pressurised to take on manual and more hazardous work with limited or no training, being subjected to constant changes in their tasks, and casual workers lacking confidence in raising safety concerns with their supervisors or employers for fear of losing their jobs.[21]

LEVEL OF EXPOSURE

In a study of 150 spice-mill workers, those with higher spicedust particulate exposures (>1.18–<3.78 mg/m³) were almost fourfold more likely (OR 3.77, CI (1.01–28)) to have a 24-hour FeNO delayed increase of ≥12%.19 Among banana-plantation workers with passive pesticide exposure (occupational and para-occupational exposure to agricultural chemicals), a trend of increased FeNO levels were found among those whose partners worked in agriculture (OR 1.61 (0.77–3.58)) and/or in banana plantations (OR 1.80, 0.93–3.48). Generally, women that used pesticides showed a trend (OR 1.46, 0.76–2.93) of having elevated FeNO.[6]

In their study of assessing the impact of an intervention for baker's allergy and asthma in supermarket bakeries, Al Badri et al demonstrated that bakers with ocular–nasal symptoms were more likely (OR 3.73, CI (1.22–11.42)) to demonstrate a significant decline in FeNO of ≥10% if they belonged to the intervention group (bakery lids and training with lower flour-dust levels) compared to the control group (higher flour-dust levels).[7] However, Crivallaro et al did not find a significant FeNO change (mean difference -8.60 ppb p-value 0.06) with an increase in dust concentrations among workers occupationally exposed to flour dust.[33]

SPECIFIC INHALATION CHALLENGE TEST – ALLERGEN DOSE–RESPONSE RELATIONSHIP

In clinical studies, a case series of workers with asthmatic reactions induced by isocyanates, demonstrated a delayed increase in FeNO 24 hours following exposure in the active (6.6 nL/s) SIC test group of 111.8 ppb, CI (68.5–236.0) compared to sham (3.3 nL/s) SIC of 58.6 ppb, CI (24.1–98.8).[17] A SIC test with HMW agents inducing an IgE-mediated sensitisation were also associated with a ≥12% increase in FeNO.[3] Beretta et al also showed that patients exposed to HMW agents during SIC were sevenfold more likely (OR 7.2, CI (1.4–37.7)) to have FeNO ≥25 ppb.[16] Furthermore, subjects with a positive SIC test had a higher FeNO baseline (23.0 ppb) and post-specific inhalation challenge test (41.0 ppb) than the subjects with a negative SIC (14.0 ppb and 17.0 ppb) respectively.[12]

Similar findings were reported in a study assessing the diagnostic accuracy of non-invasive tests before and after SIC for the diagnosis OA; post-SIC FeNO was higher in SIC-positive 54 ppb (6–156) pulmonary responders compared to SICnegative 16 ppb (6–126) pulmonary responders.[34]

CONCLUSION

Occupational respiratory allergy and asthma are associated with elevated FeNO levels. Certain individual (host)-related factors such as age and atopy are more consistently associated with higher FeNO ≥50 ppb or ≥12% increase after SIC. However, some individual-related factors such as smoking and ICS use are associated with lower (<25 ppb) FeNO levels. Whereas an elevated dose of exposure to either HMW or LMW inducing an IgE-mediated inflammatory response is associated with increased FeNO levels, workplace interventions, aimed at reducing exposure to sensitisers, are associated with a significant (≥10%) decrease in FeNO levels in exposed workers. Furthermore, there has been an increasing focus in recent years on the relationship between FeNO and LMW agents, given the inconsistent relationships observed in previous studies. The evidence also suggests that it is the agent's ability to induce an IgE-mediated mechanism, and not its molecular weight or its nature, that is responsible for elevated FeNO levels in occupationally exposed workers.

Future epidemiological studies need to use stronger study designs such as cohort studies in order to investigate the various factors that determine FeNO levels in different occupational settings, which can ultimately influence the development of occupational respiratory allergy and asthma. In this way, temporal responses to host-related and occupational determinants and their relationship with FeNO can be better assessed. Gaining deeper insights into understanding and identifying the risk factors for increased FeNO is important in the surveillance programmes of workers aimed at developing appropriate preventive measures to mitigate risk.

REFERENCES

1. Oțelea MR, Fell AKM, Handra CM, et al. The value of fractional exhaled nitric oxide in occupational diseases: A systematic review. J Occup Med Toxicol. 2022;17(14):1–12. https://doi.org/10.1186/s12995-022-00355-1.
2. Nerpin E, Olivieri M, Gislason T, et al. Determinants of fractional exhaled nitric oxide in healthy men and women from the European Community Respiratory Health Survey III. Clin Exp Allergy. 2019;49(7):969–979. https://doi.org/10.1111/cea.13394.
3. Coman I, Lemière C. Fractional exhaled nitric oxide (FeNO) in the screening and diagnosis work-up of occupational asthma. Curr Treat Options Allergy. 2017;4(2):145–159. https://doi.org/10.1007/s40521-017-0122-8.
4. Pignatti P, Visca D, Loukides S, et al. A snapshot of exhaled nitric oxide and asthma characteristics: Experience from high to low income countries. Pulmonology. 2021;28(1):44–58. https://doi.org/10.1016/j.pulmoe.2020.10.016.
5. Olivieri M, Malerba M, Spiteri G, et al. Fractional exhaled nitric oxide levels in relation to work-related respiratory burden and sensitization to wheat flour and multigrain in bakers. Clin Transl Allergy. 2021;11(8):1–9. https://doi.org/10.1002/clt2.12018.
6. Werthmann D, van Wendel de Joode B, Cuffney MT, et al. A cross-sectional analysis of medical conditions and environmental factors associated with fractional exhaled nitric oxide (FeNO) in women and children from the ISA birth cohort, Costa Rica. Environ Res. 2023;233:1–8. https://doi.org/10.1016/j.envres.2023.116449.
7. Al Badri FM, Baatjies R, Jeebhay MF. Assessing the health impact of interventions for baker's allergy and asthma in supermarket bakeries: A group randomised trial. Int Arch Occup Environ Health. 2020;93(5):589–599. https://doi.org/10.1007/s00420-019-01511-7.
8. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention: 2023 GINA Report [Internet]. 2023. Available from: www.ginasthma.org.
9. Louis R, Satia I, Ojanguren I, et al. European Respiratory Society guidelines for the diagnosis of asthma in adults. Eur Respir J. 2022;60. https://doi.org/10.1183/13993003.01585-2021.
10. Iavicoli I, Fontana L, Leso V, Macrini MC, Pelclova D. Fractional exhaled nitric oxide and nanomaterial exposure in workplaces. Curr Med Chem. 2020;27(42):7200–7212. https://doi.org/10.2174/0929867327666200320154545.
11. Shiryaeva O, Aasmoe L, Straume B, Bang BE. Respiratory symptoms, lung functions, and exhaled nitric oxide (FeNO) in two types of fish processing workers: Russian trawler fishermen and Norwegian salmon industry workers. Int J Occup Environ Health. 2015;21(1):53–60. https://doi:10.1179/2049396714Y.0000000089.
12. Engel J, van Kampen V, Lotz A, et al. An increase of fractional exhaled nitric oxide after specific inhalation challenge is highly predictive of occupational asthma. Int Arch Occup Environ Health. 2018;91(7):799–809. https://doi.org/10.1007/s00420-018-1325-4.
13. Suzuki RA, Irokawa T, Ogawa H, et al. Fractional exhaled nitric oxide (FeNO) and spirometry as indicators of inhalation exposure to chemical agents in pathology workers. J Occup Environ Med. 2017;59(5):467–473. https://doi.org/10.1097/JOM.0000000000000976.
14. Wang H, Duan H, Meng T, et al. Local and systemic inflammation may mediate diesel engine exhaust-induced lung function impairment in a Chinese occupational cohort. Toxicol Sci. 2018;162(2):372–382. https://doi.org/10.1093/toxsci/kfx259.
15. Mwanga HH, Baatjies R, Singh T, Jeebhay MF. Asthma phenotypes and host risk factors associated with various asthma-related outcomes in health workers. Front Allergy. 2021;2:1–12. https://doi.org/10.3389/falgy.2021.747566.

16. Beretta C, Rifflart C, Evrard G, et al. Assessment of eosinophilic airway inflammation as a contribution to the diagnosis of occupational asthma. Allergy: Eur J Allergy Clin Immunol. 2018;73(1):206–213. https://doi.org/10.1111/all.13265.
17. Mason P, Scarpa MC, Guarnieri G, et al. Exhaled nitric oxide dynamics in asthmatic reactions induced by diisocyanates. Clin Exp Allergy. 2016;46(12):1531–1539. https://doi.org/10.1111/cea.12798.
18. Mwanga HH, Baatjies R, Singh T, Jeebhay MF. Work-related allergy and asthma associated with cleaning agents in health workers in southern African tertiary hospitals. Am J Ind Med. 2022;65(5):382–395. https://doi.org/10.1002/ajim.23344.
19. Van Der Walt A, Baatjies R, Singh T, Jeebhay MF. Environmental factors associated with baseline and serial changes in fractional exhaled nitric oxide (FeNO) in spice mill workers. Occup Environ Med. 2016;73(9):614–620. https://doi.org/10.1136/oemed-2015-103005.
20. Wild P, Mével H, Penven E, et al. FeNO levels increase with degree of sensitisation in apprentices at risk of occupational asthma. Int J Tuberc Lung Dis. 2017;21(11):1194–1200. https://doi.org/10.5588/ijtld.16.0758.
21. Ngajilo D, Singh T, Ratshikhopha E, et al. Risk factors associated with allergic sensitization and asthma phenotypes among poultry farm workers. Am J Ind Med. 2018;61(6):515–523. https://doi.org/10.1002/ajim.22841.
22. Vinnikov D, Tulekov Z, Blanc PD. Fractional exhaled NO in a metalworking occupational cohort. Int Arch Occup Environ Health. 2022;95(3):701–708. https://doi.org/10.1007/s00420-021-01801-z.
23. Dubini M, Marraccini P, Brass DM, Patrini L, Riboldi L. Occupational asthma and rhinitis due to wheat flour: Sublingual specific immunotherapy treatment. Medicina del Lavoro. 2020;111(3):203–209.
24. Klusáčková P, Dušková Š, Mráz J, et al. Health effects of exposure to isocyanates in a car factory. Cent Eur J Public Health. 2022;30(1):32–36. https://doi.org/10.21101/cejph.a6805.
25. Sack C, Ghodsian N, Jansen K, Silvey B, Simpson CD. Allergic and respiratory symptoms in employees of indoor cannabis grow facilities. Ann Work Expo Health. 2020;64(7):754–764. https://doi.org/10.1093/annweh/wxaa050.
26. Van Kampen V, Brüning T, Merget R. Serial fractional exhaled nitric oxide measurements off and at work in the diagnosis of occupational asthma. Am J Ind Med. 2019;62(8):663–671. https://doi.org/10.1002/ajim.22996.
27. Lim FL, Hashim Z, Than LTL, et al. Respiratory health among office workers in Malaysia and endotoxin and (1,3)-b-glucan in office dust. Int J Tuberc Lung Dis. 2019;23(11):1171–1177. https://doi.org/10.5588/ijtld.18.0668.
28. Suojalehto H, Suuronen K, Cullinan P, et al. Phenotyping occupational asthma caused by acrylates in a multicenter cohort study. J Allergy Clin Immunol Pract. 2020;8(3):971–979.e1. https://doi.org/10.1016/j.jaip.2019.10.017.
29. Van Kampen V, Migueres N, Doyen V, et al. Phenotyping occupational asthma caused by platinum salts compared to other low-molecular-weight agents. J Allergy Clin Immunol Pract. 2023;17(10):1076–1093. https://doi.org/10.1016/j.jaip.2023.06.014.
30. Vlaanderen J, Pronk A, Rothman N, et al. A cross-sectional study of changes in markers of immunological effects and lung health due to exposure to multiwalled carbon nanotubes. Nanotoxicology. 2017;11(3):395–404. https://doi.org/10.1080/17435390.2017.1308031.
31. Pelclova D, Zdimal V, Komarc M, et al. Deep airway inflammation and respiratory disorders in nanocomposite workers. Nanomaterials. 2018;8(731):1–13. https://doi.org/10.3390/nano8090731.
32. Paraskevaidou K, Porpodis K, Kontakiotis T, et al. Asthma and rhinitis in Greek furniture workers. J Asthma. 2021;58(2):1–10. https://doi.org/10.1080/02770903.2019.1674328.

33. Crivellaro MA, Ottaviano G, Maculan P, et al. Upper and lower respiratory signs and symptoms in workers occupationally exposed to flour dust. Int J Environ Res Public Health. 2020;17(19):7075. https://doi.org/10.3390/ijerph17197075.
34. Engel J, van Kampen V, Gering V, et al. Non-invasive tools beyond lung function before and after specific inhalation challenges for diagnosing occupational asthma. Int Arch Occup Environ Health.

SECTION C: JOURNAL ARTICLE MANUSCRIPT

This manuscript has been prepared to be submitted for publication in the journal, Occupational and Environmental Medicine. The format of the article follows the journal's guidelines for authors (Appendix 7)

Determinants of fractional exhaled nitric oxide (FeNO) among workers at risk of developing occupational respiratory allergy and asthma in different occupational settings

Dr Phinias Mfune

Occupational Medicine Division, School of Public Health, University of Cape Town, South Africa

Ethical statement

Ethical approval for this study was provided by the Human Research Ethics Committee (HREC) of the University of Cape Town (**HREC Ref: 878/2023**).

Conflict of interest

There is no conflict of interest to declare.

Data sharing

Data available on request due to privacy and ethical restrictions.

Funding

No funding was provided for this study.

ABSTRACT

Background: Fractional exhaled nitric oxide (FeNO) offers a potential tool for screening and surveillance of workers at risk of occupational respiratory allergy and asthma.

Objective: This study evaluated determinants of FeNO in workers at risk of occupational respiratory allergy and asthma in diverse industries in southern Africa.

Methodology: Data were analysed from cross-sectional epidemiological studies of bakery, fruit farming, spice milling, poultry farming, wood processing and healthcare industries. All studies used the modified ECRHS questionnaire, assessed atopy using Phadiatop, allergen specific sensitization (sIgE), and NIOX MINO.

Results: Study participants (n=2043) had a median age of 37 years, with 52% female and 25% current smokers. The prevalence of atopy was 44% and sIgE was 18%. FeNo was elevated (≥25 ppb) in 17% and high (≥50 ppb) in 7% of workers. Overall, sIgE explained 20% (adj_R^2 0.1998, p<0.001) of FeNO variability, spider mite 35% ($adjR^2$ 0.3529, p=0.064), rye 26% ($adjR^2$ 0.2594, p<0.001) and wheat 25% ($adjR^2$ 0.2513, p<0.001). Host factors significantly associated with FeNO included age, sex, atopy and current smoking status, as was employment duration. Sensitization to wheat (OR_{adj} 5.39), rye (OR_{adj} 4.96), spider mite (OR_{adj} 3.18) and ortho-phthalaldehyde (OR_{adj} 2.51) were significantly associated with FeNO ≥25 ppb, while wheat (OR_{adj} 3.58), rye (OR_{adj} 4.43), garlic (OR_{adj} 9.42), and natural rubber latex (OR_{adj} 7.96) were significantly associated with FeNO ≥50 ppb.

Conclusion: Workers with elevated FeNO are more likely to have elevated sIgE and at increased risk of having occupational respiratory allergy and asthma if symptomatic.

Key words: FeNO, occupational, respiratory allergy, asthma, atopy, sensitization, determinants

What is already known on this topic

- Various factors have been consistently associated with changes in fractional exhaled nitric oxide (FeNO). There is a consistent positive association between atopy and allergen specific IgE sensitization (sIgE) and FeNO, while smoking and inhaled corticosteroid (ICS) treatment are negatively correlated.
- Occupational asthma to high molecular weight (HMW) but not low molecular weight (LMW) agents has been commonly associated with increased FeNO.

What this study adds

- sIgE is a major determinant of elevated FeNO in a dose-dependent manner.
- Categorization of worker's exposure according to HMW and LMW is not a useful approach in identifying workers with increased likelihood of having elevated FeNO.

How this study might affect research, practice or policy

- Improved insights into determinants of FeNO in working populations will increase its applicability and use in the screening and surveillance of groups at risk of developing occupational respiratory allergy and asthma.

INTRODUCTION

Fractional exhaled nitric oxide (FeNO) has been increasingly used in medical screening and the diagnosis of occupational asthma (OA). Studies on the determinants of FeNO have generally focused on the general population as opposed to workers in the occupational setting.[1–4] In clinical settings, FeNO has been used to detect Th2-driven inflammation, predict inhaled corticosteroid response, evaluate treatment compliance, select patients with severe asthma for biological treatment and monitor patients diagnosed with asthma.[1,5–9] However, its use in occupational settings including surveillance is not as widespread due to limitations in its interpretation as a result of other potential confounders.[1,3,5] Recently, the GLI network, a European Respiratory Initiative (ERS) initiative aimed at developing a single all-age reference equation for FeNO was also unable to do so.[5]

Aside from its usefulness in the diagnostic work-up of individuals with suspected occupational asthma[10] measurement of FeNO offers a potentially useful tool in the screening and surveillance of working populations at risk of developing occupational asthma (OA) associated with exposure to respiratory sensitisers.[3] It is well known that FeNO is elevated in individuals subjected to either occupational or environmental exposures.[1–4,9,11]

Previous epidemiological studies have reported that exposure to high molecular weight (HMW) agents was more consistently associated with elevated FeNO.[3,9,12,13] Generally, the baseline FeNO levels in workers was higher among those exposed to HMW compared to LMW agents.[1] Furthermore, sensitization to specific occupational allergens was significantly associated with elevated FeNO.[14] On the other hand, current smoking status was associated with lower FeNO when compared to non-smokers.[1,3,15]

In clinical studies, the evidence appears to be more consistent based on specific inhalation challenge tests (SIC) with HMW agents (flour, cereals, latex, enzymes) inducing an IgE-mediated sensitization, which were associated with a ≥12% increase in FeNO.[3,16] Patients exposed to HMW agents during SIC were 7-fold more likely to have FeNO ≥25 ppb.[16]Furthermore, subjects with a positive SIC test also had higher baseline FeNO and post SIC test than those with a negative SIC.[7] In other studies, exposure to specific LMW agents (acrylates and isocyanates) may be associated with varying FeNO levels,[17] such that workers with asthmatic reactions induced by isocyanates, demonstrated a delayed increase in FeNO 24 hours after SIC.[18] A more recent clinical study concluded that FeNO increases in IgE-mediated inflammation, irrespective of the molecular weight of the causative agent.[19]

Other occupational factors, such as the nature of work could play a role in influencing changes in FeNO levels in that FeNO levels have differed in individual workers depending on the work they perform.[20]

Furthermore, FeNO levels may also differ according to level of allergen exposure as has been shown and demonstrated in the intervention study of supermarket bakery workers.[21]

The aim of this study was to evaluate the individual and occupational-related determinants of elevated FeNO in workers at risk of developing occupational respiratory allergy and asthma in diverse industries such as baking, spice milling, wood processing, fruit farming, poultry farming and health care in southern Africa.

METHODS

STUDY DESIGN, POPULATION AND SAMPLING

This study involved analysis of data collected as a part of larger cross-sectional epidemiological studies of workers in the bakery,[22] fruit farming,[23] spice milling,[24] poultry farming,[25] wood processing,[26] and healthcare industries[27] in southern Africa, as previously reported.[22–27]

The study population comprised 2112 workers, that were currently employed in supermarket bakeries (n=361), spice milling (n=150), poultry farming (n=230), fruit farming (n=207), wood processing (443) and healthcare (n=652) work. All workers completed a consent form prior to participating in the study. The original studies collected complete data from a total of 2112 subjects, however due to missing information of certain key fields, a full dataset of 2043 subjects was used in the analysis. This dataset had completed information on the respiratory questionnaire, FeNO and serum allergen-specific IgE levels collected during work time.

HEALTH OUTCOME ASSESSMENTS

Respiratory Health Questionnaire

A modified version of the European Community Respiratory Health survey (ECRHS) [28] incorporating work-related aspects (exposures and symptoms) adapted for local conditions was used in all individual studies.[22–26,29] The interviewer administered questionnaire collected information on important demographic factors, occupational-related respiratory symptoms (ocular nasal, wheeze, tight chest), history of previous illnesses (asthma, rhinitis, atopy and recent chest infection), employment history, degree of exposure to occupational allergens, and smoking status. Information was also collected on medication use, exercise and dietary history (nitrate rich foods).[30] The modified questionnaire was administered in either English or Afrikaans (South African studies), or Swahili (Tanzanian healthcare workers) or Portuguese (Mozambiquan

wood processing workers). The translated Swahili and Portuguese questionnaires were back-translated to English to ensure validity and repeatability.[26,29]

Fractional exhaled nitric oxide (FeNO) determination

A similar hand-held portable nitric oxide electrochemical sampling device (NIOX MINO) was used and tests performed in accordance with ATS/ERS recommendations. [31–33] Under the guidance of clinical personnel, workers inhaled NO-free air close to total lung capacity and exhaled for 10 s at a flow rate of 50 ml/s according to the manufacturer's instructions. Two technically adequate measurements were performed. If the measurements differed by 10ppb or more, a third measurement was performed, and the mean of all three measurements was used. The FeNO levels were measured during the work shift throughout the working week. Workers were instructed to abstain from smoking, eating or drinking at least one hour before the test. FeNO was tested prior to spirometry. [22–26,29]

Immunological tests

The presence of atopy was determined using the Phadiatop test (ImmunoCAP 100 System; Phadia Uppsala, Sweden). The method of quantification of serum allergen-specific IgE CAP-FEIA (fluorescence enzyme immunoassay) was also performed using the ImmunoCAP 100 System (Phadia, Uppsala, Sweden) according to the manufacturer's instructions. Specific allergens tested for included wheat flour (f4), rye flour (f5) and fungal α-amylase (k87) for bakery workers;[22] garlic (f47; *Allium sativum*), chilli pepper (f279; *Capsicum frutesce*) and wheat (f4; *Triticum aestivum*) for spice mill workers;[24] storage mite (*Lepidoglyphus destructor*), sunflower seed (*Helianthus annuus*), mould mix (containing *Penicillium chrysogenum, C. herbarum, A. fumigatus, C. Albicans, A. alternata, S. rostrata*) and in-house chicken (meat, feathers, serum protein, droppings) for poultry workers. [25] For fruit farm workers quantification of specific IgE was performed for spider mite (*Tetranychus urticae*) and storage mite (*Lepidoglyphus destructor*) [23] and for health care workers allergens included natural rubber latex – NRL (*Hevea brasiliensis* – Hev b5 – K218, Hev b6.02 – K220), chlorhexidine (C8) and ortho-phthalaldehyde (OPA).[27] Specific IgE was not determined for wood processing workers due to the unavailability of specific wood extracts.

OPERATIONAL DEFINITIONS

FeNO levels - FeNO was defined either as a continuous variable (log-transformed) or as a categorical variable as follows: low < 25 ppb, elevated for values 25–50 ppb, and high for values > 50 ppb.

Doctor-diagnosed asthma - respondent answered YES to at least one of the following 2 questions: "Have you had an attack of asthma in the last 12 months?" or "Are you currently taking any medicines including inhalers, aerosols or tablets for asthma?"*Atopy* – serum IgE ≥0.35 kU/L to common inhalants.

Allergen specific sensitization – serum IgE ≥0.35 kU/L to individual occupational allergens.

HMW agents: wheat flour, rye flour, fungal α-amylase, chilli pepper, garlic, sunflower seed, mould mix, natural rubber latex (NRL), storage mite, spider mite, chicken droppings, feathers, chicken meat and chicken serum protein.

LMW agents: chlorhexidine, ortho-phthalaldehyde (OPA) and wood dust.

STATISTICAL ANALYSIS

Data was analysed using STATA version 18.0. The natural logarithm (ln) of the measured FeNO average was used as the dependent variable since the data followed a log-normal distribution. Linear regression models were developed to describe the individual determinants of variability in FeNO, using geometric mean ratios and 95% confidence intervals (CI). Associations between FeNO and key determinants (host and occupational) were also explored using logistic regression analysis. A p-value <0.05 was regarded as statistically significant. For logistic regression models, results were reported as odds ratio (OR) with 95% CIs. Multivariate saturated linear and logistic regression models to assess the association between FeNO and occupational factors were adjusted for age, gender, smoking, atopy and recent chest infections since these variables appeared to be the most consistent significant confounders identified in the simple regression models.

RESULTS

Study population

Demographic characteristics

The median age was 37 years, 52% were female and a quarter were current smokers (**Table 1**). Almost half (44%) were atopic, with 8% having rhinitis and 9% doctor-diagnosed asthma. There were 18% with work-related chest symptoms, with 14% reporting exposure to high dust levels causing work-related symptoms. The median FeNO level among the participants was 15 ppb (IQR: 10 – 24) among whom 17% had elevated FeNO (≥25 ppb) and 7% with a high FeNO (>50 ppb).

Occupational characteristics

The median employment duration of workers in their current job was 4 years and in the factory/or industry was 8 years. There were 18% of workers that were sensitized to any one of the occupational allergens tested (**Table S1**). More than a third of workers (36%) were exposed only to any one of the high molecular weight agents (wheat, rye, garlic, sunflower seed, spider mite and storage mite) and 22% to low molecular weight agents (chlorhexidine, ortho-phthalaldehyde, wood dust).

Table 1: Demographic and occupational characteristics of study population by industry type

Subject characteristics	**Industry**						
	Total	**Baking**	**Health care**	**Poultry farming**	**Spice milling**	**Wood processing**	**Fruit farming**
N (% of total sample)	**(n= 2043)**	**n = 361 (18%)**	**n = 652 (32%)**	**n = 230 (11%)**	**n = 150 (7%)**	**n = 443 (22%)**	**n = 207 (10%)**
Demographic factors							
Age in years	37 (30 – 47)	37 (32 – 43)	43 (32 – 51)	35 (28 – 42)	32 (28 – 38)	36 (27 – 47)	37 (28 – 45)
Sex (%M:F)	48:52	43:57	23:77	68:32	71:29	94:6	0:100
Height (m)	1.63 (1.56 – 1.69)	1.64 (1.58 – 1.70)	1.60 (1.55 – 1.65)	1.66 (1.60 – 1.71)	1.69 (1.64 – 1.75)	1.67 (1.62 – 1.72)	1.55 (1.51 – 1.60)
Weight (kg)	70 (60 – 83)	79 (66 – 94)	76 (67 – 88)	65 (58 – 73)	72 (62 – 83)	62 (57 – 69)	65 (54 – 75)
BMI (kg/m^2)	26.1 (22.4 – 31.3)	29 (24.8 – 34.3)	29.4 (25.7 – 34.1)	23.2 (20.8 – 27)	25.2 (22.2 – 29.4)	22.4 (20.9 – 24.5)	26.4 (22.5 – 32.9)
Smoking status, n (%)							
- Current smoker	504 (25)	127 (35)	40 (6)	98 (43)	69 (46)	67 (15)	103 (50)
- Ever smoker	666 (33)	186 (52)	85 (13)	101 (44)	84 (56)	106 (24)	104 (50)
- Passive smoker	1066 (52)	195 (54)	219 (34)	153 (67)	99 (66)	224 (51)	176 (85)
Nitrogen containing diet, n (%)							
- Green salad, n (%), n=1812	1668 (92)	333 (92)	611 (94)	-	140 (93)	400 (90)	184 (89)
- Green vegetables, n (%), n=1808	1692 (94)	285 (79)	636 (98)	-	140 (93)	441 (100)	190 (94)
Recent physical exercise, n (%), n=1794	547 (31)	62 (17)	246 (39)	-	49 (33)	159 (36)	31 (15)
Employment history in years							
Employment duration in current job, n=1959	4 (1.5 – 10)	7 (5 – 11)	3 (1 – 7)	3 (1 – 5)	2 (0.83 – 4.5)	5 (1 – 11)	8 (2 – 14)
Employment duration in factory	8 (3 – 16)	10 (6 – 15)	15 (6 – 28)	3.67 (1.75 – 6)	5 (3 - 10.42)	7 (2 – 12)	0 (0 – 8)
Dust particulate concentration in current job (mg/m^3), n=994	4.7 (1 – 5.8)	1 (0.4 – 1)	ND	36.3 (5.5 – 60.9)	3.5 (1.1 – 4.3)	4.7 (4.7 – 4.7)	ND
General dust particulate concentration (n=994)							
- ≥0.5 mg/m^3, n (%)	867 (87)	194 (63)	ND	230 (100)	138 (92)	305 (100)	ND
- ≥1 mg/m^3 , n (%)	630 (63)	0	ND	230 (100)	115 (77)	285 (93)	ND
- ≥5 mg/m^3, n (%)	278 (28)	0	ND	189 (82)	31 (21)	0	ND
- ≥10 mg/m^3, n (%)	143 (14)	0	ND	121 (53)	0	22 (7)	ND

Table 1: Demographic and occupational characteristics of study population by industry type (continued)

Subject characteristics	Industry						
	Total	**Baking**	**Health care**	**Poultry farming**	**Spice milling**	**Wood processing**	**Fruit farming**
N (% of total sample)	**(n= 2043)**	**n = 361 (18%)**	**n = 652 (32%)**	**n = 230 (11%)**	**n = 150 (7%)**	**n = 443 (22%)**	**n = 207 (10%)**
Dominant exposure agent group							
- HMW only, n (%)	741 (36)	361 (100)	0	230 (100)	150 (100)	0	0
- LMW only, n (%)	443 (22)	0	0	0	0	443 (100)	0
- HMW and LMW (both)	859 (42)	0	652 (100)	0	0	0	207 (100)
Past medical history							
- Atopy (positive Phadiatop), n (%), n=1970	861 (44)	150 (42)	284 (44)	78 (34)	67 (45)	193 (50)	89 (44)
- Rhinitis / hay fever, n (%), n=1836	172 (9)	69 (19)	88 (14)	3 (1)	8 (5)	4 (1)	-
- Doctor-diagnosed asthma, n (%), n=1886	157 (8)	46 (13)	46 (7)	2 (1)	13 (9)	27 (6)	23 (11)
- Inhaled corticosteroid use, n (%)	111 (5)	40 (11)	37 (6)	6 (3)	10 (7)	6 (1)	12 (6)
Respiratory symptoms in the past 12 months							
General							
- Recent chest infection n (%), n=1793	385 (22)	158 (44)	64 (10)	–	5 (3)	104 (24)	54 (26)
- Ocular-nasal symptoms, n (%)	573 (28)	118 (33)	233 (36)	42 (18)	25 (17)	106 (24)	49 (24)
- Wheeze, n (%)	352 (17)	99 (27)	108 (17)	35 (15)	17 (11)	29 (7)	64 (31)
Work-related							
- Ocular-nasal symptoms, n (%)	686 (34)	136 (38)	153 (24)	105 (46)	71 (47)	178 (40)	43 (21)
- Chest symptoms, n (%)	357 (18)	99 (27)	75 (12)	54 (24)	26 (17)	64 (15)	39 (19)
- High dust levels causing symptoms, n (%), n=1184	166 (14)	28 (8)	-	110 (48)	25 (17)	3 (1)	-
Fractional exhaled nitric oxide (FeNO) levels							
FeNO (ppb)	15 (10 – 24)	16 (10 – 26)	17 (11 – 24)	13 (9 – 18)	14 (9 – 24)	19 (12 – 27)	10.3 (5 – 17)
- <25 ppb, n (%)	1,552 (76)	263 (73)	498 (76)	193 (84)	115 (77)	314 (71)	169 (82)
- ≥25 - <50 ppb, n (%)	354 (17)	65 (18)	115 (18)	20 (9)	24 (16)	107 (24)	23 (11)
- ≥50 ppb, n (%)	137 (7)	33 (9)	39 (6)	17 (7)	11 (7)	22 (5)	15 (7)

ND: Not done; Numerical variables presented as median and interquartile range (unless otherwise stated); Dominant exposure group - HMW group: baking, poultry and spice milling industry; LMW group: wood industry; HMW and LMW group: healthcare and fruit farming

Individual risk factors associated with elevated FeNO

In the simple linear and logistic regression analysis (**Table 2**), males were more likely to have increased FeNO ($\beta = 0.1619$, $p<0.001$) and was significantly associated with FeNO ≥25 ppb (OR 1.44, 95% CI: 1.17 – 1.76). Current smoking status ($\beta = -0.3003$, p-value <0.001) was negatively associated with FeNO. Whilst, atopy was significantly associated with both elevated FeNO, ≥25 ppb, (OR 3.42, 95% CI: 2.76 – 4.25) and high FeNO, ≥50 ppb, (OR 4.62, 95% CI: 3.08 – 6.95).

With regard to medical history, rhinitis (OR 3.08, 95% CI: 1.94 – 4.87) and doctor diagnosed asthma (OR 3.00, 95% CI: 1.88 – 4.79) was positively associated with FeNO, as was the presence of upper or lower respiratory symptoms including a recent chest infection (OR 2.48, 95% CI: 1.68 – 3.66).

Immunological profiles associated with FeNO

In the simple linear and logistic regression analysis (**Table 3**) sensitisation to any occupational allergen was consistently associated with elevated FeNO levels. Furthermore, those individually sensitised to high molecular weight agents such as wheat (OR 7.40, 95% CI: 3.84 – 14.27), rye (OR 8.60, 95% CI: 3.83 – 19.29), chilli (OR 25.91, 95% CI: 4.84 – 138.81), garlic (OR 6.05, 95% CI: 1.70 – 21.52), natural rubber latex (OR 10.30, 95% CI: 2.37 – 44.87), spider mite (OR 3.00, 95% CI: 1.32 – 6.80) and storage mite (OR 3.90, 95% CI: 1.83 – 8.31) also had higher levels. As for low molecular weight agents, workers exposed to OPA were more likely to have elevated FeNO ≥25 ppb (OR 4.13, 95% CI: 1.75 – 9.76).

Table 2: Univariate analysis of demographic and occupational factors associated with levels of fractional exhaled nitric oxide among workers employed in different industries

Subject characteristics		FeNO (natural log)				FeNO ≥25 ppb (n=491)		FeNO ≥50 ppb (n=137)	
	Prevalence (%)	β	GM	R^2	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Demographic factors (n=2043)									
Age in years, median (IQR)	37 (30 – 47)	0.0009	1.001	0.0002	0.514	1 (0.99 – 1.01)	0.878	1 (0.97 – 1.00)	0.076*
Sex (%M:F)	48:52	0.1619	1.176	0.0130	**<0.001**	1.44 (1.17 – 1.76)	**0.001**	1.10 (0.78 – 1.56)	0.586
Height (m), median (IQR)	1.63 (1.56 – 1.69)	0.9235	2.518	0.0130	**<0.001**	6.02 (1.88 – 19.26)	**0.003**	3.78 (0.53 – 27.16)	0.187
Weight (kg), median (IQR)	70 (60 – 83)	0.0030	1.003	0.0051	**0.001**	1 (1 – 1.01)	0.262	1 (1 – 1.01)	0.394
BMI (kg/m^2), median (IQR)	26.1 (22.4 – 31.3)	0.0022	1.002	0.0004	0.351	1 (0.98 – 1.01)	0.856	1.01 (0.98 – 1.03)	0.712
Smoking status									
- Current smoker	504 (25)	-0.3003	0.741	0.0393	**<0.001**	0.55 (0.42 – 0.71)	**<0.001**	0.41 (0.25 – 0.69)	**0.001**
- Ever smoker	666 (33)	-0.4209	0.657	0.0653	**<0.001**	0.67 (0.53 – 0.83)	**<0.001**	0.56 (0.37 – 0.85)	**0.006**
- Passive smoker	1066 (52)	-0.1621	0.850	0.0130	**<0.001**	0.82 (0.67 – 1.01)	0.060*	0.79 (0.56 – 1.12)	0.186
Nitrogen containing diet									
- Green salad, n (%), n= 1,812	1668 (92)	0.0685	1.071	0.0007	0.265	0.96 (0.65 – 1.42)	0.854	0.76 (0.41 – 1.42)	0.391
- Green vegetables, n (%), n= 1,808	1692 (94)	0.0849	1.089	0.0009	0.212	1.23 (0.78 – 1.95)	0.369	0.66 (0.34 – 1.26)	0.206
- Recent physical exercise, n (%), n= 1794	547 (31)	-0.0117	0.988	0.0001	0.748	0.87 (0.68 – 1.10)	0.246	0.68 (0.44 – 1.05)	0.080*
Employment history in years (n=2043)									
Employment duration in current job, n=1959	4 (1.5 – 10)	0.0015	1.002	0.0002	0.500	1.01 (1 – 1.03)	0.1713	1 (1 – 1.03)	0.928
Employment duration in factory/workplace	8 (3 – 16)	0.0043	1.004	0.0037	**0.006**	1 (1 – 1.01)	0.7309	1 (1 – 1.02)	0.914
General dust particulate concentration (n=994)									
Dust particulate concentration in current job (mg/m^3)	4.7 (1 – 5.8)	-0.0037	0.996	0.0076	**0.006**	0.99 (0.98 – 1)	0.079*	1.01 (1 – 1.02)	0.257
- ≥0.5 mg/m^3, n (%)	867 (87)	-0.1235	0.884	0.0031	0.077*	0.55 (0.37 – 0.82)	**0.003**	0.65 (0.35 – 1.23)	0.184
- ≥1 mg/m3, n (%)	630 (63)	-0.0075	0.993	<0.0001	0.878	0.91 (0.68 – 1.22)	0.511	0.68 (0.42 – 1.10	0.115
- ≥5 mg/m^3, n (%)	278 (28)	-0.1735	0.841	0.0112	**0.001**	0.59 (0.42 – 0.83)	**0.003**	1.04 (0.62 – 1.77)	0.874
- ≥10 mg/m^3, n (%)	143 (14)	-0.1697	0.844	0.0066	**0.011**	0.55 (0.34 – 0.87)	**0.011**	1.46 (0.79 – 2.69)	0.228
Dominant exposure agent group (n=2043)									
- HMW and LMW	859 (42)	0	1			1		1	
- HMW only, n (%)	741 (36)	-0.0236	0.790	0.0116	0.505	1.03 (0.82 – 1.31)	0.778	1.34 (0.91 – 1.96)	0.134
- LMW only, n (%)	443 (22)	0.1728	1.189	0.0116	**<0.001**	1.43 (1.10 – 1.85)	**0.007**	0.78 (0.47 – 1.30)	0.337

*Borderline significant

Dominant exposure group - HMW group: baking, poultry and spice milling industry; LMW group: wood industry; HMW and LMW group: health care and fruit farming industry

Each OR: represents a separate regression model

Table 2: Univariate analysis of demographic and occupational factors associated with levels of fractional exhaled nitric oxide among workers employed in different industries (continued)

Subject characteristics		FeNO (natural log)				FeNO ≥25 ppb (n=491)		FeNO ≥50 ppb (n=137)	
	Prevalence (%)	β	GM	R^2	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Past medical history									
- Atopy (positive Phadiatop), n (%), n=1970	861 (44)	0.3499	1.419	0.0587	**<0.001**	3.42 (2.76 – 4.25)	**<0.001**	4.62 (3.08 – 6.95)	**<0.001**
- Rhinitis / hay fever, n (%), n=1836	172 (9)	0.3471	1.415	0.0215	**<0.001**	2.78 (2.01 – 3.83)	**<0.001**	3.08 (1.94 – 4.87)	**<0.001**
- Chronic bronchitis, n (%), n= 2,015	105 (5)	0.1018	1.107	0.0010	0.153	1.16 (0.74 – 1.81)	0.515	1.15 (0.55 – 2.43)	0.699
- Doctor-diagnosed asthma, n (%), n=1886	157 (8)	0.3168	1.373	0.0141	**<0.001**	2.57 (1.84 - 3.59)	**<0.001**	3.00 (1.88 – 4.79)	**<0.001**
- Inhaled corticosteroid use, n (%)	111 (5)	0.3924	1.481	0.0157	**<0.001**	3.00 (2.03 – 4.42)	**<0.001**	3.41 (2.03 – 5.73)	**<0.001**
Respiratory symptoms in the past 12 months									
General									
- Recent chest infection n (%), n=1793	385 (22)	0.2328	1.262	0.0182	**<0.001**	1.87 (1.46 – 2.38)	**<0.001**	2.48 (1.68 – 3.66)	**<0.001**
- Ocular-nasal symptoms, n (%)	573 (28)	0.2046	1.227	0.0168	**<0.001**	1.97 (1.59 – 2.45)	**<0.001**	2.33 (1.64 – 3.31)	**<0.001**
- Wheeze, n (%)	352 (17)	0.0523	1.054	0.0008	0.209	1.59 (1.24 – 2.05)	**<0.001**	2.03 (1.37 – 2.99)	**<0.001**
- Chest tightness, n (%)	318 (16)	0.0592	1.061	0.0009	0.172	1.28 (0.98 – 1.67)	0.073*	1.50 (0.98 – 2.31)	0.063*
Work-related									
- Ocular-nasal symptoms, n (%)	686 (34)	-0.0134	0.987	0.0001	0.688	1.20 (0.97 – 1.48)	0.097	1.04 (0.71 - 1.49)	0.852
- Chest symptoms, n (%)	357 (18)	-0.0126	0.988	0.0000	0.760	1.16 (0.89 - 1.51)	0.264	1.35 (0.89 – 2.07)	0.159
- Exposure to high dust levels causing symptoms, n (%), n=1184	166 (14)	-0.1775	0.837	0.0074	**0.003**	0.82 (0.56 – 1.22)	0.344	0.93 (0.48 – 1.80)	0.835

*Borderline significant

Dominant exposure group - HMW group: baking, poultry and spice milling industry; LMW group: wood industry; HMW and LMW group: health care and fruit farming industry

Each OR: represents a separate regression model

Table 3: Univariate analysis of immunological profiles associated with levels of fractional exhaled nitric oxide among workers employed in different industries

Subject characteristics		FeNO (natural log)				FeNO ≥25 ppb (n=491)		FeNO ≥50 ppb (n=137)	
	Prevalence (%)	β	GM	R^2	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Immunological profile									
Occupational allergen-specific IgE									
- Occupational allergen-specific serum IgE (sIgE ≥0.35 kU/L) to any allergen, n (%), n=1582	285 (18)	1.4166	1.417	0.0335	**<0.001**	3.64 (2.77 – 4.78)	**<0.001**	4.45 (3.0 – 6.5939)	**<0.001**
High molecular weight agents (HMW)									
- Wheat, n (%), n= 504	116 (23)	0.7244	2.064	0.1528	**<0.001**	9.13 (5.72 – 14.55)	**<0.001**	7.3977 (3.84 – 14.27)	**<0.001**
- Rye, n (%), n=354	100 (28)	0.7139	2.042	0.1663	**<0.001**	7.85 (4.64 – 13.28)	**<0.001**	8.60 (3.8313 – 19.29)	**<0.001**
- Fungal α-amylase, n (%), n=361	10 (3)	0.3606	1.434	0.0057	0.155	2.78 (0.79 – 9.83)	0.112	4.49 (1.10 – 18.25)	**0.036**
- Chilli pepper, n (%), n=150	7 (5)	0.6357	1.888	0.0317	**0.029**	4.82 (1.02 – 22.67)	**0.047**	25.91 (4.84 – 138.81)	**<0.001**
- Garlic, n (%), n=150	29 (19)	0.3051	1.357	0.0256	**0.052**	3.66 (1.54 – 8.69)	**0.003**	6.05 (1.70 – 21.52)	**0.005**
- Sunflower seed, n (%), n=230	29 (13)	0.1249	1.133	0.0034	0.377	1.10 (0.39 – 3.10)	0.856	0.92 (0.20 – 4.24)	0.913
- Chicken meat, serum, feathers, n (%), n=230	8 (4)	0.2500	1.284	0.0037	0.360	2.15 (0.40 – 11.52)	0.372	5.55 (0.99 – 31.02)	**0.051**
- Chicken droppings, n (%), n=230	14 (6)	0.2948	1.343	0.0099	0.132	2.22 (0.66 – 7.49)	0.200	0.96 (0.12 – 7.83)	0.971
- Mould mix, n (%), n=230	18 (8)	0.2163	1.242	0.0067	0.215	2.16 (0.72 – 6.48)	0.168	1.64 (0.35 – 7.82)	0.534
- Natural rubber latex (rhevb5/ rhevb602), n (%), n=644	8 (1)	0.7106	2.035	0.0152	**0.002**	3.30 (0.82 – 13.35)	0.094*	10.30 (2.37 – 44.87)	**0.002**
- Storage mite (*Lepidoglyphus destructor*), n (%), =434	73 (17)	0.5299	1.699	0.0064	**0.043**	2.73 (1.54 – 4.86)	**0.001**	3.90 (1.83 – 8.31)	**<0.001**
- Spider mite, n=203	34 (17)	0.2761	1.318	0.0184	**0.005**	3 (1.32 – 6.80)	**0.009**	1.9152 (0.57 – 6.42)	0.292
Low molecular weight agents (LMW)									
- Chlorhexidine, n (%), n=332	3 (1)	0.3797	1.462	0.0033	0.299	5.93 (0.53 – 66.22)	0.148	7.33 (0.64 – 84.20)	0.110
- Ortho-phthalaldehyde (OPA), n (%), n=644	22 (3)	0.3497	1.419	0.0099	**0.012**	4.13 (1.75 – 9.76)	**0.001**	2.65 (0.75 – 9.38)	0.131
Allergen-specific IgE (kU/L) class (n=1582)									
- Class 0 (< 0.35), n (%)	1297 (82)	0	1			1		1	
- Class I (0.35–<0.70), n (%)	85 (5)	0.1070	1.113	0.0679	0.178	2.14 (1.33 – 3.45)	**0.002**	2.36 (1.13 – 4.93)	**0.022**
- Class II (0.70–<3.50), n (%)	98 (6)	0.1837	1.202	0.0679	**0.013**	2.67 (1.73 – 4.12)	**<0.001**	2.78 (1.44 – 5.3542)	**0.002**
- Class III (3.50–<17.50), n (%)	59 (4)	0.5410	1.718	0.0679	**<0.001**	4.75 (2.80 – 8.07)	**<0.001**	6.79 (3.58 – 12.87)	**<0.001**
- Class IV (17.50–<50), n (%)	28 (2)	0.7006	2.015	0.0679	**<0.001**	8.26 (3.77 - 18.13)	**<0.001**	6.64 (2.72 – 16.21)	**<0.001**
- Class V and VI (≥50, n (%)	15 (1)	1.3754	3.957	0.0679	**<0.001**	NC	**–**	29.88 (10.31 – 86.59)	**<0.001**

NC: not calculable

*Borderline significant

Each OR: represents a separate regression model

Occupational risk factors associated with elevated FeNO

In multivariate linear and logistic regression models after adjusting for confounders (age, sex, current smoking status, atopy and recent chest infection) (**Table 4**) increased employment duration in the industry (factory) was positively associated with FeNO (β = 0.041, p=0.036). Occupational exposure to dust particulate concentration in current job negatively related to FeNO in both the simple linear regression (β = -0.0037, p=0.006) and logistic regression analysis. Exposure to LMW agents only explained 18% of FeNO ≥50 ppb variability in the negative direction (OR 0.56, 95% CI: 0.30 – 1.06).

Immunological factors associated with elevated FeNO

In both the multiple linear and logistic regression analysis (**Table 5**), sensitization (elevated serum IgE) to any occupational allergen was significantly associated with elevated FeNO≥25 ppb, (OR 2.85, 95% CI: 1.99 – 4.07) and high FeNO ≥50 ppb (OR 2.99, 95% CI: 1.82 – 4.91). Overall, allergen-specific sensitization explained 20% (adjR^2 0.1998, p<0.001) of FeNO variability, spider mite 35% (adjR^2 0.3529, p=0.064), rye 26% (adjR^2 0.2594, p<0.001) and wheat 25% (adjR^2 0.2513, p<0.001).

In multiple linear regression models, workers sensitized to any allergen (β = 0.2680), wheat (β = 0.5388), rye (β = 0.5446), and natural rubber latex (β = 0.5984, p=0.005) were associated with higher FeNO levels. Similarly, in logistic regression models, sensitization to wheat (OR 5.39), rye (OR 4.96) and garlic (OR 2.77, 95% CI: 0.98 – 7.83) was consistently significantly associated with FeNO≥25 ppb and FeNO ≥50 ppb. Furthermore, increase in serum IgE class levels were consistently positively associated with FeNO in a dose-dependent manner; FeNO ≥25 ppb, Class I (OR 1.73, 95% CI: 0.97 – 3.06), Class II (OR 1.74, 95% CI: 1.01 – 2.99), Class III (OR 6.23, 95% CI: 3.04 – 12.76) and Class IV – VI (OR 8.63, 95% CI: 3.90 – 19.06). Similar association was observed with FeNO ≥ 50 ppb.

Table 4: Occupational factors associated with levels of fractional exhaled nitric oxide, in multiple linear and logistic regression models

		FeNO (natural log)				FeNO ≥25 ppb (n=491)		FeNO ≥50 ppb (n=137)	
Subject characteristics	**Prevalence (%)**	**β**	**GM**	**Adjusted R^2**	**p-value**	**OR adj (95% CI)**	**p-value**	**OR adj (95% CI)**	**p-value**
Employment history in years (n=2043)									
Employment duration in current job, n=1959	4 (1.5 – 10)	0.0022	1.002	0.1476	0.357	1.01 (1 - 1.02)	0.171	1 (0.98 - 1.03)	0.928
Employment duration in factory/workplace	8 (3 – 16)	0.0041	1.004	0.1715	**0.036**	1 (0.99 – 1.01)	0.731	1 (0.98 - 1.02)	0.914
General dust particulate concentration (n=994)									
Dust particulate concentration in current job (mg/m^3)	4.7 (1 – 5.8)	-0.0037	0.996	0.0076	**0.006**	0.99 (0.98 – 1.00)	0.079*	1.01 (1.00 – 1.02)	0.257
- ≥0.5 mg/m^3, n (%)	867 (87)	-0.1013	0.904	0.1675	0.153	0.50 (0.30 – 0.85)	**0.010**	1.12 (0.51 – 2.48)	0.778
- ≥1 mg/m^3, n (%)	630 (63)	-0.0342	0.966	0.1656	0.561	0.96 (0.64 – 1.43)	0.834	0.43 (0.22 - 0 .83)	**0.012**
- ≥5 mg/m^3, n (%)	278 (28)	-0.0559	0.946	0.1657	0.498	0.52 (0.26 – 1.04)	0.064*	0.56 (0.18 – 1.73)	0.316
- ≥10 mg/m^3, n (%)	143 (14)	0.0819	1.085	0.1655	0.611	0.68 (0.21 – 2.22)	0.519	3.06 (0.73 – 12.81)	0.127
Dominant exposure agent group (n= 2043)									
- HMW and LMW	859 (42)	0	1			1		1	
- HMW only, n (%)	741 (36)	0.0140	1.014	0.1694	0.733	1.09 (0.80– 1.47)	0.594	1.34 (0.83 – 2.17)	0.233
- LMW only, n (%)	443 (22)	-0.0340	0.967	0.1694	0.504	0.93 (0.65 – 1.33)	0.684	0.56 (0.30 – 1.06)	0.074*

*Borderline significant

Dominant exposure group - HMW group: baking, poultry and spice milling industry; LMW group: wood industry; HMW and LMW group: health care and fruit farming industry

Each OR represents a separate regression model adjusted for age, sex, smoking status (current smoker), atopy and recent chest infection

Table 5: Immunological factors associated with levels of fractional exhaled nitric oxide, in multiple linear and logistic regression models.

Subject characteristics		FeNO (natural log)				FeNO ≥25 ppb (n=491)		FeNO ≥50 ppb (n=137)	
	Prevalence (%)	β	GM	AdjR^2	p-value	OR adj (95% CI)	p-value	OR adj (95% CI)	p-value
Immunological profile									
Occupational allergen-specific IgE									
- Occupational allergen-specific serum IgE (sIgE ≥0.35 kU/L) to any allergen, n (%), n=1582	285 (18)	0.2680	1.307	0.1998	**<0.001**	2.85 (1.99 – 4.07)	**<0.001**	2.99 (1.82 – 4.91)	**<0.001**
High molecular weight agents (HMW)									
- Wheat, n (%), n= 504	116 (23)	0.5388	1.714	0.2513	**<0.001**	5.39 (3.15 – 9.22)	**<0.001**	3.58 (1.68 – 7.64)	**0.001**
- Rye, n (%), n=354	100 (28)	0.5446	1.724	0.2594	**<0.001**	4.96 (2.69 – 9.12)	**<0.001**	4.43 (1.82 – 10.79)	**0.001**
- Fungal α-amylase, n (%), n=361	10 (3)	0.0755	1.078	0.1823	0.748	1.18 (0.30 – 4.68)	0.811	1.99 (0.46 – 8.59)	0.356
- Chilli pepper, n (%), n=150	7 (5)	0.2622	1.300	0.1727	0.352	2.12 (0.41 – 11.13)	0.373	NC	NC
- Garlic, n (%), n=150	29 (19)	0.1804	1.198	0.1751	0.257	2.77 (0.98 – 7.83)	0.056*	9.42 (1.45 – 61.35)	**0.019**
- Sunflower seed, n (%), n=230	29 (13)	-0.0654*	0.937	0.1111	0.666	0.68 (0.22 – 2.17)**	0.517	0.40 (0.08 – 2.09)**	0.279
- Chicken meat, serum, feathers, n (%), n=230	8 (4)	0.0574*	1.059	0.1105	0.829	1.44 (0.25 – 8.37)**	0.584	2.86 (0.44 – 18.43)**	0.270
- Chicken droppings, n (%), n=230	14 (6)	0.2532*	1.288	0.1171	0.190	2.02 (0.55 – 7.42)**	0.291	0.63 (0.07 – 5.65)**	0.680
- Mould mix, n (%), n=230	18 (8)	0.1402*	1.151	0.1128	0.427	1.61 (0.48 – 5.36)**	0.438	1.17 (0.22 – 6.29)**	0.855
- Natural rubber latex (rhevb5/ rhevb602), n (%), n=644	8 (1)	0.5984	1.819	0.0978	**0.005**	1.97 (0.47 – 8.19)	0.350	7.96 (1.72 – 36.88)	**0.008**
- Storage mite (*Lepidoglyphus destructor*), n (%), n=434	73 (17)	0.0836	1.087	0.3435	0.551	1.62 (0.54 – 4.91)	0.393	2.33 (0.49 – 11.06)	0.289
- Spider mite, n=203	34 (17)	0.0632	1.065	0.3529	0.064*	3.18 (1.07 – 9.44)	**0.037**	1.38 (0.28 – 6.96)	0.694
Low molecular weight agents (LMW)									
- Chlorhexidine, n (%), n=332	3 (1)	0.3159	1.371	0.0913	0.369	4.08 (0.35 – 47.22)	0.261	5.69 (0.40 – 79.92)	0.198
- Ortho-phthalaldehyde (OPA), n (%), n=644	22 (3)	0.2230	1.250	0.0907	0.108	2.51 (1.01 – 6.24)	**0.048**	1.71 (0.46 – 6.33)	0.419
Allergen-specific IgE (kU/L) class (n=1582)									
- Class 0 (< 0.35), n (%)	1,297 (82)	0	1			1		1	
- Class I (0.35–<0.70), n (%)	85 (5)	0.0300	1.030	0.2236	0.723	1.73 (0.97 – 3.06)	0.061*	1.35 (0.54 – 3.39)	0.522
- Class II (0.70–<3.50), n (%)	98 (6)	0.0716	1.074	0.2236	0.372	1.74 (1.01 – 2.99)	**0.045**	2.07 (0.95 – 4.49)	0.066*
- Class III (3.50–<17.50), n (%)	59 (4)	0.6019	1.826	0.2236	**<0.001**	6.23 (3.04 – 12.76)	**<0.001**	6.00 (2.70 – 13.33)	**<0.001**
- Class IV – VI (≥17.50), n (%)	43 (3)	0.6864	1.987	0.2236	**<0.001**	8.63 (3.90 – 19.06)	**<0.001**	5.98 (2.71 – 13.18)	**<0.001**

NC: not calculable, * OR borderline significant

Each OR adj: represents a separate regression model adjusted for age, sex, smoking status (current smoker), atopy and recent chest infection

**OR not adjusted for the presence of a recent chest infection due to the unavailability of data

AdjR^2: represents a separate multiple linear regression model adjusted for age, sex, smoking status (current smoker), atopy and recent chest infection

DISCUSSION

The findings of this study provide an in depth insight into the major factors into the determinants of fractional exhaled nitric oxide (FeNO) and its association with occupational respiratory allergy and asthma among workers in diverse occupations**.** Specifically, it showed that allergen specific sensitization is a major determinant of elevated FeNO in a dose-dependent manner. Furthermore, differentiating between exposure of workers to HMW or LMW agents was not an important determinant of elevated FeNO.

In this study of workers from different industries with diverse exposures in the southern African region, the median FeNO was 15 ppb (IQR: 10 – 24), which is similar to that reported by Angel et al in a study of workers exposed to both HMW and LMW agents including isocyanates. However, Sacks et al, 2020 reported much higher (30 ppb) levels among workers in indoor cannabis growing facilities.[34] The prevalence of elevated FeNO ≥25 ppb in the current study was 17%, which is lower than that reported in banana plantation workers (20%) and office workers (25%) exposed to indoor office dust containing endotoxin and (1,3)-β-glucans. This lower prevalence could be due to a healthy worker effect due to the cross-sectional nature of the current study.[13,15]

In the investigation of individual host factors that were correlated with FeNO, our study found that male sex was significantly associated with elevated FeNO ≥25 ppb. This is in contrast to previous studies in bakeries [35] and pathology workers that reported no significant differences in FeNO between sexes.[36] However, Engel et al. reported that males were less likely to have FeNO increase of ≥13 ppb after a specific challenge test (SIC).[7] Sex appears to be an inconsistent determinant of elevated FeNO. This also appears to be the case for age as a determinant in adult workers, where there was no significant correlation with FeNO, as has also been reported by Shiryaeva et al. [37]

More consistent host determinants that were also replicated in the current study related to the findings for smoking as well as atopy. Our findings demonstrated that workers with a history smoking (current, ex or passive) had lower FeNO levels. These findings are consistent with various previous studies. [1,3,4,7,11,15,16,35] However, atopy was significantly positively associated with elevated FeNO levels. These findings are consistent with previous epidemiological studies.[1–4,16] Interestingly both smoking status and atopy, each explained 6% of variability in FeNO in the simple linear regression models, signifying their importance as major host determinants.

In this current study, specific occupational allergen sensitization was a strong determinant of FeNO as explained by the high percentage (10-35%) of variability of individual allergen sensitisation

explained in the adjusted models. The associations for elevated FeNO ≥25 ppb were strongest for workers sensitized to wheat, rye, garlic, latex, spider mite and ortho-phthalaldehyde (OPA). Furthermore, the associations for high FeNO (≥50 ppb) were much stronger for those sensitized to high molecular weight proteins such as wheat, rye, garlic and latex . These findings are consistent with the study by Wild et al, which also reported elevated FeNO in highly sensitised apprentices compared to non-sensitised apprentices.[14] As mentioned earlier, dose-dependent manner of the allergen-specific IgE of the entire group in relation to elevated or high FeNO level suggests that the degree of sensitisation could be a proxy for the level of occupational allergen exposure, which is driving the IgE-mediated response resulting in Th2 inflammation as has been shown in other studies of specific inhalation challenge tests.[38–41]

With regard to occupational factors, the findings of this current study demonstrated that differentiating between exposure to HMW or LMW agents was not an important determinant of elevated FeNO. Previous studies have reported that exposure to HMW agents was associated with elevated FeNO,[1,3,12] whilst exposure to isocyanates was also associated with increased FeNO and an increased odds (OR 1.63) of having FeNO ≥13.0 ppb after a SIC.[1,3] On the other hand, a study of Greek furniture workers reported no association between baseline and longitudinal FeNO levels and wood-dust exposure.[42] Recently, a clinical case series of 455 occupational asthma patients by van Kampen et al, concluded that FeNO was increased in IgE-mediated inflammation, irrespective of the molecular weight of the causative agent. The study suggested that it was the pathophysiological mechanism and not the molecular weight of the agent that was responsible for inducing FeNO.[19] The differences observed in previous study findings could be due to exposure misclassification.

Another occupational factor that was found to be significant determinant of FeNO in this current study was duration of employment in the factory (workplace), which could be related to prolonged exposure to occupational allergen inducing an inflammatory response. However, worker duration of employment in current job did not demonstrate a significant association. The inconsistency in these findings could be explained by the healthy worker effect associated with job changes within the factory (workplace). This could also explain the negative associations observed between FeNO and dust particulate concentration exposures in all these individual cross-sectional studies across different sectors. The findings are also contradictory to various clinical studies using specific bronchial challenge with the specific putative sensitiser.[38–41] These difference in the study findings could therefore be attributed to the fact that crude dust particulate levels were used rather than the specific allergen exposure concentrations. Since dust particulate may be composed of diverse components, including exposure metrics of specific allergen concentrations would have been more

informative. Furthermore, it is possible that higher endotoxin levels could also play a role, however detailed exposure levels for all these sectors were not available.

Finally, this is the first study that reports on the host and occupational determinants of FeNO across diverse setting and geographical regions in southern Africa. A major strength of this study was that FeNO measurement was done in a similar standardised manner according to ERS/ATS guidelines across all sectors. Furthermore, pooling of participant data from the six individual studies enhanced the statistical power to investigate a number of factors and their relative contribution in determining elevated FeNO levels. However, one of the major limitation of this study is the cross-sectional design of the individual studies, which did not allow the investigation of temporal relationships between occupational exposure and FeNO.

CONCLUSION

This study confirms that atopy and smoking are important host determinants of elevated FeNO. Furthermore, allergen-specific sensitisation was the major and strongest determinant of elevated FeNO. There was a consistent association between sensitization to wheat, rye, garlic, latex, spider mite and OPA. Furthermore, a dose-response relationship was demonstrated between increasing level of sensitization classes and FeNO. However, categorization of workers according to their exposure to HMW or LMW was not an important determinant of FeNO. Further epidemiological studies using stronger designs such as cohort studies would be useful to investigate the association between specific allergen exposures and FeNO levels. In this way, temporal responses to occupational determinants and their relationship with FeNO can be better assessed. Gaining deeper insights into understanding and identifying the risk factors for increased FeNO in working populations will increase its applicability and use in the screening and surveillance of groups at risk of developing occupational respiratory allergy and asthma.

REFERENCES

1. Oțelea MR, Fell AKM, Handra CM, Holm M, Filon FL, Mijakovski D, et al. The value of fractional exhaled nitric oxide in occupational diseases – a systematic review. Journal of Occupational Medicine and Toxicology. 2022;17(14):1–12.
2. Nerpin E, Olivieri M, Gislason T, Olin AC, Nielsen R, Johannessen A, et al. Determinants of fractional exhaled nitric oxide in healthy men and women from the European Community Respiratory Health Survey III. Clinical & Experimental Allergy. 2019;49(7):969–79.
3. Coman I, Lemière C. Fractional Exhaled Nitric Oxide (FeNO) in the Screening and Diagnosis Work-Up of Occupational Asthma. Curr Treat Options Allergy. 2017;4(2):145–59.
4. Iavicoli I, Fontana L, Leso V, Macrini MC, Pelclova D. Fractional Exhaled Nitric Oxide and Nanomaterial Exposure in Workplaces. Curr Med Chem. 2020;27(42):7200–12.
5. Högman M, Bowerman C, Chavez L, Dressel H, Malinovschi A, Radtke T, et al. ERS technical standard: Global Lung Function Initiative reference values for exhaled nitric oxide fraction (FENO50). Eur Respir J. 2024 Jan 1;63(1):1–10.
6. Engel J, van Kampen V, Gering V, Hagemeyer O, Brüning T, Raulf M, et al. Non-invasive tools beyond lung function before and after specific inhalation challenges for diagnosing occupational asthma. Int Arch Occup Environ Health. 2019;92(7):1067–76.
7. Engel J, van Kampen V, Lotz A, Abramowski J, Gering V, Hagemeyer O, et al. An increase of fractional exhaled nitric oxide after specific inhalation challenge is highly predictive of occupational asthma. Int Arch Occup Environ Health. 2018;91(7):799–809.
8. Bjermer L, Alving K, Diamant Z, Magnussen H, Pavord I, Piacentini G, et al. Current evidence and future research needs for FeNO measurement in respiratory diseases. Vol. 108, Respiratory Medicine. W.B. Saunders Ltd; 2014. p. 830–41.
9. Mfune P, Jeebhay MF. Determinants of fractional exhaled nitric oxide in workers at risk of developing occupational respiratory allergy and asthma: A review. Current Allergy & Clinical Immunology. 2024 Mar 1;37(1):31–41.
10. Louis R, Satia I, Ojanguren I, Schleich F, Bonini M, Tonia T, et al. European Respiratory Society guidelines for the diagnosis of asthma in adults. European Respiratory Journal. 2022;60.
11. Pignatti P, Visca D, Loukides S, Märtson AG, Alffenaar JWC, Migliori GB, et al. A snapshot of exhaled nitric oxide and asthma characteristics: experience from high to low income countries. Pulmonology. 2021;28(1):44–58.
12. van Kampen V, Brüning T, Merget R. Serial fractional exhaled nitric oxide measurements off and at work in the diagnosis of occupational asthma. Am J Ind Med. 2019;62(8):663–71.

13. Lim FL, Hashim Z, Than LTL, Md Said S, Hashim JH, Norbäck D. Respiratory health among office workers in Malaysia and endotoxin and (1,3)-b-glucan in office dust. International Journal of Tuberculosis and Lung Disease. 2019;23(11):1171–7.
14. Wild P, Mével H, Penven E, Zmirou-Navier D, Barbaud A, Bohadana A, et al. FeNO levels increase with degree of sensitisation in apprentices at risk of occupational asthma. International Journal of Tuberculosis and Lung Disease. 2017;21(11):1194–200.
15. Werthmann D, van Wendel de Joode B, Cuffney MT, Reich BJ, Soto-Martinez ME, Corrales-Vargas A, et al. A cross-sectional analysis of medical conditions and environmental factors associated with fractional exhaled nitric oxide (FeNO) in women and children from the ISA birth cohort, Costa Rica. Environ Res. 2023;233:1–8.
16. Beretta C, Rifflart C, Evrard G, Jamart J, Thimpont J, Vandenplas O. Assessment of eosinophilic airway inflammation as a contribution to the diagnosis of occupational asthma. Allergy: European Journal of Allergy and Clinical Immunology. 2018;73(1):206–13.
17. Suojalehto H, Suuronen K, Cullinan P, Lindström I, Sastre J, Walusiak-Skorupa J, et al. Phenotyping Occupational Asthma Caused by Acrylates in a Multicenter Cohort Study. J Allergy Clin Immunol Pract. 2020;8(3):971-979.e1.
18. Mason P, Scarpa MC, Guarnieri G, Giordano G, Baraldi E, Maestrelli P. Exhaled nitric oxide dynamics in asthmatic reactions induced by diisocyanates. Clinical and Experimental Allergy. 2016;46(12):1531–9.
19. van Kampen V, Migueres N, Doyen V, Deckert A, de Blay F, Vandenplas O, et al. Phenotyping Occupational Asthma Caused by Platinum Salts Compared to Other Low-molecular-weight Agents. J Allergy Clin Immunol Pract. 2023;17(10):1076–93.
20. Vinnikov D, Tulekov Z, Blanc PD. Fractional exhaled NO in a metalworking occupational cohort. Int Arch Occup Environ Health. 2022;95(3):701–8.
21. Al Badri FM, Baatjies R, Jeebhay MF. Assessing the health impact of interventions for baker's allergy and asthma in supermarket bakeries: a group randomised trial. Int Arch Occup Environ Health. 2020;93(5):589–99.
22. Baatjies R, Jeebhay MF. Sensitisation to cereal flour allergens is a major determinant of elevated exhaled nitric oxide in bakers. Occup Environ Med. 2013 May;70(5):310–6.
23. Ndlovu V, Dalvie MA, Jeebhay MF. Asthma associated with pesticide exposure among women in rural Western Cape of South Africa. Am J Ind Med. 2014;57(12):1331–43.
24. van Der Walt A, Baatjies R, Singh T, Jeebhay MF. Environmental factors associated with baseline and serial changes in fractional exhaled nitric oxide (FeNO) in spice mill workers. Occup Environ Med. 2016;73(9):614–20.

25. Ngajilo D, Singh T, Ratshikhopha E, Dayal P, Matuka O, Baatjies R, et al. Risk factors associated with allergic sensitization and asthma phenotypes among poultry farm workers. Am J Ind Med. 2018;61(6):515–23.
26. Chamba P, Baatjies R, Jeebhay MF. Respiratory symptoms, airway inflammation and lung function of workers in the Mozambiquan Wood Processing Industry [PhD Thesis]. [Cape Town]: University of Cape Town; 2023.
27. Mwanga HH, Baatjies R, Singh T, Jeebhay MF. Work-related allergy and asthma associated with cleaning agents in health workers in Southern African tertiary hospitals. Am J Ind Med. 2022;65(5):382–95.
28. Burney PGJ, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. European Respiratory Journal. 1994;7(5):954–60.
29. Mwanga HH, Baatjies R, Singh T, Jeebhay MF. Asthma Phenotypes and Host Risk Factors Associated With Various Asthma-Related Outcomes in Health Workers. Frontiers in Allergy. 2021;2:1–12.
30. Baatjies R, Lopata AL, Sander I, Raulf-Heimsoth M, Batemane ED, Meijster T, et al. Determinants of asthma phenotypes in supermarket bakery workers. European Respiratory Journal. 2009 Oct;34(4):825–33.
31. American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med. 2005;171(8):912–30.
32. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An Official ATS Clinical Practice Guideline: Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Applications. Am J Respir Crit Care Med. 2011 May;184:602–15.
33. Högman M, Bowerman C, Chavez L, Dressel H, Malinovschi A, Radtke T, et al. ERS technical standard: Global Lung Function Initiative reference values for exhaled nitric oxide (F E NO). Eurpean Respiratory Journal [Internet]. 2023; Available from: https://doi.org/10.1183/13993003.00370
34. Sack C, Ghodsian N, Jansen K, Silvey B, Simpson CD. Allergic and respiratory symptoms in employees of indoor cannabis grow facilities. Ann Work Expo Health. 2020;64(7):754–64.
35. Olivieri M, Malerba M, Spiteri G, Torroni L, Biscardo CA, Valenza D, et al. Fractional exhaled nitric oxide levels in relation to work-related respiratory burden and sensitization to wheat flour and multigrain in bakers. Clin Transl Allergy. 2021;11(8):1–9.

36. Suzuki RA, Irokawa T, Ogawa H, Ohkouchi S, Tabata M, Togashi S, et al. Fractional exhaled nitric oxide (FeNO) and spirometry as indicators of inhalation exposure to chemical agents in pathology workers. J Occup Environ Med. 2017;59(5):467–73.
37. Shiryaeva O, Aasmoe L, Straume B, Bang BE. Respiratory symptoms, lung functions, and exhaled nitric oxide (FeNO) in two types of fish processing workers: Russian trawler fishermen and Norwegian salmon industry workers. Int J Occup Environ Health. 2015 Jan 1;21(1):53–60.
38. Allmers H, Chen Z, Barbinova L, Marczynski B, Kirschmann V, Baur X. Challenge from methacholine, natural rubber latex, or 4,4¢-diphenylmethane diisocyanate in workers with suspected sensitization affects exhaled nitric oxide [change in exhaled NO levels after allergen challenges]. Int Arch Occup Environ Health. 2000;73:181–6.
39. Piipari R, Piirilä P, Keskinen H, Tuppurainen M, Sovijärvi A, Nordman H. Exhaled nitric oxide in specific challenge tests to assess occupational asthma. European Respiratory Journal. 2002 Dec 1;20(6):1532–7.
40. Baur X, Barbinova L. Latex allergen exposure increases exhaled nitric oxide in symptomatic healthcare workers. European Respiratory Journal. 2005 Feb;25(2):309–16.
41. Barbinova L, Baur X. Increase in exhaled nitric oxide (eNO) after work-related isocyanate exposure. Int Arch Occup Environ Health. 2006 May;79(5):387–95.
42. Paraskevaidou K, Porpodis K, Kontakiotis T, Kioumis I, Spyratos D, Papakosta D. Asthma and rhinitis in Greek furniture workers. Journal of Asthma. 2021;58(2):1–10.

SECTION D: APPENDICES

SUPPLEMENTARY TABLE

Appendix 1: Table 6 – Supplementary Table 1 (Table S1): Immunological profile of study population dataset by industry type

Subject characteristics	**Industry**						
	Total	**Baking**	**Health care**	**Poultry farming**	**Spice milling**	**Wood processing**	**Fruit farming**
N (% of total sample)	**(n= 2043)**	**n = 361 (18%)**	**n = 652 (32%)**	**n = 230 (11%)**	**n = 150 (7%)**	**n = 443 (22%)**	**n = 207 (10%)**
Occupational allergen-specific IgE							
- Occupational allergen-specific serum IgE (sIgE ≥0.35 kU/L) to any allergen, n (%), n=1582	285 (18)	113 (32)	26 (4)	56 (24)	31 (21)		59 (29)
High molecular weight agents (HMW)							
- Wheat, n (%), n= 504	116 (23)	102 (29)			14 (9)		
- Rye, n (%), n=354	100 (28)	100 (28)					
- Fungal α-amylase, n (%), n=361	10 (3)	10 (3)					
- Chilli pepper, n (%), n=150	7 (5)				7 (5)		
- Garlic, n (%), n=150	29 (19)				29 (19)		
- Sunflower seed, n (%), n=230	29 (13)			29 (13)			
- Chicken meat, serum, feathers, n (%), n=230	7 (3)			7 (3)			
- Chicken droppings, n (%), n=230	14 (6)			14 (6)			
- Mould mix, n (%), n=230	18 (8)			18 (8)			
- Natural rubber latex (rhevb5/ rhevb602), n (%), n=644	8 (1)		8 (1)				
- Storage mite (*Lepidoglyphus destructor*), n (%), n=434	73 (17)			29 (13)			44 (22)
- Spider mite, n=203	34 (17)						34 (17)
Low molecular weight agents (LMW)							
- Chlorhexidine, n (%), n=332	3 (1)		3 (1)				
- Ortho-phthalaldehyde (OPA), n (%), n=644	22 (3)		22 (3)				
Allergen-specific IgE (kU/L) class (n=1582)							
- Class 0 (< 0.35), n (%)	1297 (82)	241 (68)	618 (96)	174 (76)	119 (79)		145 (71)
- Class I (0.35–<0.70), n (%)	85 (5)	14 (4)	18 (3)	19 (8)	13 (9)		21 (10)
- Class II (0.70–<3.50), n (%)	98 (6)	30 (9)	7 (1)	21 (9)	16 (11)		24 (12)
- Class III (3.50–<17.50), n (%)	59 (4)	32 (9)	1 (0)	15 (7)	1 (1)		10 (5)
- Class IV – VI combined (≥50.00), n (%)	43 (3)	37 (10)	0	1 (0)	1 (1)		4 (2)

Appendix 2: Letter of approval from University of Cape Town Human Research Ethics Committee (HREC)

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

E-52 – Room46, E-Floor, Old Main Building
Groote Schuur Hospital
Observatory 7925
Email: hrec-submissions@uct.ac.za
Website: https://health.uct.ac.za/home/human-research-ethics

28 November 2023

HREC REF: 878/2023

Prof Mohamed Jeebhay
Department of Occupational Medicine
Room4.45 Level 4, Falmouth Building
Faculty of Health Sciences, UCT
Email: mohamed.jeebhay@uct.ac.za
Student: phiniasmfune@gmail.com / mfnphi004@wf.uct.ac.za

Dear Prof Jeebhay

PROJECT TITLE: DETERMINANTS OF FRACTIONAL EXHALED NITRIC OXIDE (FeNO) AMONG WORKERS AT RISK OF DEVELOPING OCCUPATIONAL RESPIRATORY ALLERGY AND ASTHMA IN DIFFERENT OCCUPATIONAL SETTINGS (MMED CANDIDATE DR PHINIAS MFUNE)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review and approval.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is only granted for one year until the 30 November 2024.

Please submit a progress report, using the standardised Annual Progress Report Forms (FHS016) **or** (FHS 017) if the study continues beyond the approval period. Please submit a Standard Closure form (FHS 010) when the study has been completed, this includes after publication or thesis submission and final completion.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Dr Phinias HK Mfune will also be involved in this study.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC REF 878/2023 in all your correspondence.

Yours sincerely

PROFESSOR MARC BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE

HREC REF NO. 878/2023

Federal Wide Assurance Number: FWA00001637. Institutional Review Board (IRB) number: IRB00001938 NHREC-registration number: REC-210208-007
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2020), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HREC REF NO. 878/2023

Appendix 3: Prototype Consent form (Health workers exposed to cleaning agents [English])

RISK FACTORS FOR WORK-RELATED ASTHMA IN HEALTH CARE WORKERS WITH EXPOSURE TO DIVERSE CLEANING AGENTS – 2014 .

HREC REF: 212/2013.

CONSENT FORM

1. **Title of research project**

Risk factors for work-related asthma in health care workers with exposure to diverse cleaning agents in two African health care settings – Groote Schuur Hospital and Muhimbili National Hospital

2. **Purpose of the research**

The University of Cape Town is conducting this important study of work related asthma in health care workers. This study is going to be done by researchers who are independent of the hospital. We will be studying health care workers working in the different departments of the hospital. It is hoped that this study will provide greater insight into the risk factors for work related asthma among health care workers and identify appropriate preventative strategies to be implemented in order to reduce the incidence of asthma among health care workers.

3. **Description of the research project**

If you agree to participate you will be asked to complete the following tests during working time:

a) **Complete a questionnaire**. A member of our study team will interview you in privacy to complete the questionnaire. You will be asked questions about any breathing or chest problems; current and previous employment history; and working with different products at the hospital.

b) **Blood test** You will also be asked to undergo a blood test to check for allergies to specific allergens. Ten ml (about two teaspoons) of blood will be drawn once by a nurse.

c) **Breathing tests** - You will be asked to blow several times into a machine which measures how well your lungs are working.

- You will be asked to repeat the breathing test after you first breathe in a small amount of a chemical substance (methacholine). Methacholine is a chemical that can cause the airways to become narrow. This test helps us to find out if you may have a breathing problem like asthma. You may be asked to

breathe in this substance and then blow into the machine several times. This test will not be done if you are pregnant or breastfeeding.

- You will also be asked to blow two times into a NIOX MINO machine, which measures nitric oxide produced by the airways. This machine is used to detect if a person has allergic airway inflammation which is present in asthma or rhinitis.

d) **Urine test**

- We will collect a urine sample to test for chlorhexidine and its products.

- Should you be uncertain of your menstrual pattern you will be offered a urine pregnancy test before we conduct the breathing test with methacholine.

4. **Confidentiality of information collected**

Your name will not appear on any reports or data collection forms. The records of blood tests, questionnaires and breathing tests will be kept completely confidential and will be seen only by members of the study team.

5. **Risks and discomforts of the research**

a) **From the questionnaire:** Some of the research questions may make you uncomfortable; however, we have trained our interviewer to be as sensitive when asking these questions. You may be concerned about confidentiality of the information that you provide; however, we have taken precautions to minimize this risk.

b) **From the blood tests**. You will feel a single needle stick when the blood is taken. Sometimes a small bruise may occur from the needle stick. The total amount of blood taken is quite small and your body will quickly replace it.

c) **From breathing tests:** There is a small chance that the initial breathing test could cause you to become light-headed or faint. Having you complete the test in a seated position under the observation of trained personnel greatly reduces the chance of your having such a problem. You will be given medicine (salbutamol) to breathe in that works to open your lungs. Although very rare, this medication can briefly cause a fast heartbeat, tremor, nervousness or chest pain. Part of the breathing test uses a chemical substance (methacholine) that can cause headache, cough, shortness of breath, chest tightness, wheezing, hoarse voice or a sore throat for a short time in some people. Very rarely it can cause severe breathing problems. Such breathing problems almost always can be treated

successfully immediately with a different medication (salbutamol), which you breathe in. You will only be given the chemical substance (methacholine) if your simple breathing test is normal. This greatly reduces the chance of having a serious problem. These tests will be carried out in a lung function laboratory with medical personnel knowledgeable in the treatment of such problems being immediately available. In a very rare instance the test resulted in a fatality (the individual had a number of methacholine challenge tests over a period of 2 weeks). You will have this test only once.

6. **Expected benefits to you and to others**

You may not receive any personal or direct benefit from participating in this research study. However, you will be given a written copy of all your test results along with an explanation of what they mean, unless you tell us that you do not wish to receive this. You may wish to show these to your doctor if you are having any problems. These tests will help determine if you have asthma or allergy to several substances used in the allergy test. What we learn from this study will help to protect you, and those working with different products in the hospitals in South Africa and other parts of the world. We will learn how best to monitor workers' health and how to reduce workers' exposure to different products.

7. **Costs to you resulting from participation in the study**

The study is offered at no cost to you. In the event a problem is discovered and you wish to be seen by a doctor for it, we can recommend to you who to see. However, the study cannot pay for these additional medical visits or treatments.

8. **Contact person**

You may contact one of the following persons for answers to further questions about the research, your rights, or any injury you may feel is related to the study.

University of Cape Town Researchers: Prof. Mohamed Jeebhay, Telephone No. (021) 406-6309
Dr Hussein Mwanga, Telephone No. 079 034 1280 / 084 331 2222 / 021 404 5428
University of Cape Town Human Research Ethics Committee (HREC):
Prof. Marc Blockman (021) 406-6492
University of Michigan Medical School Institutional Review Board (IRBMED)
Telephone No. 001-734-763-4768; E-mail: irbmed@umich.edu
2800 Plymouth Road, Building 520, Room 3214, Ann Arbor, Michigan 48105 (USA)

Appendix 4: Prototype consent form

RISK FACTORS FOR WORK-RELATED ASTHMA IN HEALTH CARE WORKERS WITH EXPOSURE TO DIVERSE CLEANING AGENTS – 2014

HREC REF: 212/2013

CONSENT FORM

STUDY NO. _____

9. **Consent of the participant**

I have read the information given above, or it has been read to me. I understand the meaning of this information, Dr./Mr./Ms. _____

has offered to answer any questions concerning the study. By signing this form, I hereby consent to participate in the study. I also understand that I am free to withdraw from the study at any time without penalty.

10. **Documentation of the consent**

One copy of this signed document will be kept together with our research records for this study. A copy of the information sheet about the study will be given to you to keep.

_____ _____

Printed name of participant Signature, Mark, or Thumb Print

_____ _____

Interviewer's name (Print) Signature

DATE: _____

Appendix 5: Protype Lung Function Pre-Test Questionnaire (English)

RISK FACTORS FOR WORK-RELATED ASTHMA IN HEALTH CARE WORKERS WITH EXPOSURE TO DIVERSE CLEANING AGENTS – 2014

LUNG FUNCTION TESTS--Pretest Questionnaire

a. Survey Number _____ 1-3

b. Staff Number _____ 4-11

c. Interviewer's initials: _____ 12-13

d. Date of interview:

Day____Month_____Year_____ 14-21

1. Have you had any recent operation (in the last 12 months)?
Yes (1) No (2) 22

1.1 If ***Yes***, what type and how many months ago? 23
_____
_____

1.2 How many months ago? _____ 24

2. Have you had a heart attack or stroke in the last 3 months?
Yes (1) No (2) 25

3. Do you have epilepsy?
Yes (1) No (2) 26

If **YES** to ***any*** of the above no. 1-3, indicate to the person that the lung function tests will not be done.
Otherwise proceed with the questions below.

4. Are you being treated for Tuberculosis (TB)?
Yes (1) No (2) 27

4.1 If **Yes**, for how long? _____months _____weeks 28-31

5. Have you had the flu or sinusitis or a lung infection in the past 3 weeks? Yes (1) No (2) 32

If **YES**, to question no. 4 or 5, indicate to person that the tests will not be done today. Schedule another appointment in **three weeks** from the end of their **illness** or **three months** time from the start of their **TB medication**.

6.For women:

6.1 Are you Pregnant?
Yes (1) No (2) Don't know (3) ☐ 33

6.2 If **Don't know:** Would you like to perform a urinary pregnancy test to make sure?
Yes (1) No (2) ☐ 34

6.3 Are you Breastfeeding? Yes (1) No (2) ☐ 35

If **Pregnant**, indicate to the person that the spirometry and methacholine tests will **NOT** be done today, but proceed with the exhaled nitric oxide test.

If **Breastfeeding**, proceed with spirometry test with post-bronchodilator. Otherwise proceed with the questions below.

ALCOHOL CONSUMPTION

7. Do you drink alcohol? Yes (1) No (2) ☐ 36

7.1 If **Yes**, when have you last consumed alcohol? ☐ 37
1-2 hours ago (1)
1 day ago (2)
1 week ago (3)

7.2 How much alcohol did you consume? Units: _____ ☐☐ 38-39
(1 unit = 1 beer, 1 glass of wine, 1 tot of spirits)

GREEN VEGETABLE CONSUMPTION

8. How often do you eat the following vegetable products?

Type of product		**Daily**	**1 to 3 times a week**	**1 to 3 times a month**	**Never**		
8.1. Green salad		1	2	3	4	☐	40
8.2. Spinach & other green leafy vegetables		1	2	3	4	☐	41

9. When did you last consume green salad and/or spinach/ other green leafy vegetables? ☐ 42
1-2 hours ago (1)
1 day ago (2)
1 a week ago (3)

PHYSICAL ACTIVITY

10. Do you exercise?

11. When was the last time you exercised?

1-2 hours ago	(1)	☐	43
1 day ago	(2)		
1 week ago	(3)		

SPIROMETRY/LUNG FUNCTION TEST

12. Have you ever had a spirometry/lung function test?

Yes (1) No (2) ☐ 44

12.1 If **yes,** when last did you blow into a lung function machine?

1-2 hours ago	(1)	☐	45
1 day ago	(2)		
1 week ago	(3)		
> a week ago	(4)		

RECENT ORAL INTAKE

13. Did you have anything to eat or drink in the last hour?

Yes (1) No (2) ☐ 46

14. Have you smoked in the last hour? Yes (1) No (2) ☐ 47

If **YES** to no. 13 or 14, reschedule nitric oxide test for at least 1 hour later the same day or for another date.

15. Have you had asthma in the past? Yes (1) No (2) ☐ 48

16. Do you have asthma now? Yes (1) No (2) ☐ 49

17. Are you taking any medicine(s) from a doctor or clinic at the moment for your chest OR any heart condition OR for your eyes? Yes (1) No (2) ☐ 50

17.1 If **YES**, what are you taking and when did you last take them?

Names:	Hours since last dose:		
_____	_____	☐☐	51-52
_____	_____	☐☐	53-54
_____	_____	☐☐	55-56
_____	_____	☐☐	57-58

If Beta-Blockers such as Atenolol, Carvedilol etc. are being

used—**DO NOT** perform **methacholine challenge test**.
Additionally, if anti-cholinesterase medication such as Pyridostigmine for Myasthenia gravis is being used —**DO NOT** perform **methacholine challenge test**.

If short-acting beta-2-agonist or anti-cholinergic inhalers have been used in the **last 4 hours** or long-acting MDI or theophylline have been used in **last 8 hours**, reschedule and counsel accordingly.

18. Do you currently have any of these symptoms?

18.1 chest tightness	Yes (1)	No (2)	☐	59
18.2 shortness of breath	Yes (1)	No (2)	☐	60
18.3 wheezing or whistling in your chest	Yes (1)	No (2)	☐	61
18.4 dry cough	Yes (1)	No (2)	☐	62

19. If **YES** to any of the above:
19.1 Are you feeling very unwell today?

Yes (1)	No (2)	☐	63

If **YES** to no. 19.1: Consult with the doctor on site whether to proceed with spirometry and methacholine challenge test.

END

Appendix 6: Protype FeNO data collection sheet

RISK FACTORS FOR WORK-RELATED ASTHMA IN HEALTH CARE WORKERS WITH EXPOSURE TO DIVERSE CLEANING AGENTS – 2014

EXHALED NITRIC OXIDE--Data Collection Sheet

Survey Number _____			☐☐☐ 1-3
Date: _____			
Time _____			
Ambient NO concentration (ppb) _____			☐☐☐ 4-6
Ambient temperature (degrees Celsius) _____			☐☐ 7-8
1. Subject's height (in centimetres) _____			☐☐☐ 9-11
2. Subject's weight (in kilograms) _____			☐☐☐ 12-14
3. Subject's blood pressure	systolic _____		☐☐☐ 15-17
	diastolic _____		☐☐☐ 18-20
4. Effort number (start) _____			☐☐☐ 21-23
4.1 FeNO measurement (ppb) 1st effort		_____	☐☐☐ 24-26
4.2 FeNO measurement (ppb) 2nd effort		_____	☐☐☐ 27-29
If there is a discrepancy of > 10 ppb between 1st & 2nd efforts, perform a 3rd effort			
4.3 FeNO measurement (ppb) 3rd effort		_____	☐☐☐ 30-32
5. FeNO measurement record attached?	Yes (1)	No (2)	☐ 33

Appendix 7: JOURNAL INSTRUCTIONS AND/OR GUIDELINES FOR THE AUTHORS

BMJ Journals

Occupational and Environmental Medicine – Instructions to authors:

https://oem.bmj.com/pages/authors#submission_guidelines

Submission guidelines

Please review the below article type specifications including the required article lengths, illustrations, table limits and reference counts. The word count excludes the title page, abstract, tables, acknowledgements, contributions and references. Manuscripts should be as succinct as possible. For further support when making your submission please refer to the resources available on the BMJ Author Hub. Here you will find information on writing and formatting your research through to the peer review process and promoting your paper. You may also wish to use the language editing and translation services provided by BMJ Author Services.

Original research

Please include the key messages of your article after your abstract using the following headings. This section should be no more than 3-5 sentences and should be distinct from the abstract; be succinct, specific and accurate.

- **What is already known on this topic –** summarise the state of scientific knowledge on this subject before you did your study and why this study needed to be done
- **What this study adds –** summarise what we now know as a result of this study that we did not know before
- **How this study might affect research, practice or policy –** summarise the implications of this study

This will be published as a summary box after the abstract in the final published article. When submitting an Original research article, you will be asked to select the most appropriate **Section Head** to help categorise your paper. Please see below for a brief description of each Section Head:

- **Workplace:** Papers focusing on workplace exposures (in the broadest sense) and health outcomes
- **Environment:** Papers focusing on environmental and indoor exposures and health outcomes

- **Exposure assessment:** Papers focusing solely on (methods of) exposure assessment in the occupational and general environment
- **Methodology:** Papers focusing on improving research methods and techniques relevant to occupational and environmental research
- **Practice:** Papers that inform the practice and policies of occupational and environmental health

Word count: up to 3,500

Structured abstract: up to 250 words; 'Objectives', 'Methods', 'Results', 'Conclusions'

Tables/Illustrations: up to 5

References: up to 40

Supplements

The BMJ Publishing Group journals are willing to consider publishing supplements to regular issues. Supplement proposals may be made at the request of:

- The journal editor, an editorial board member or a learned society may wish to organise a meeting, sponsorship may be sought and the proceedings published as a supplement.
- The journal editor, editorial board member or learned society may wish to commission a supplement on a particular theme or topic. Again, sponsorship may be sought.
- The BMJPG itself may have proposals for supplements where sponsorship may be necessary.

A sponsoring organisation, often a pharmaceutical company or a charitable foundation, that wishes to arrange a meeting, the proceedings of which will be published as a supplement.

In all cases, it is vital that the journal's integrity, independence and academic reputation is not compromised in any way. For further information on criteria that must be fulfilled, download the supplements guidelines. When contacting us regarding a potential supplement, please include as much of the information below as possible.

- Journal in which you would like the supplement published
- Title of supplement and/or meeting on which it is based
- Date of meeting on which it is based
- Proposed table of contents with provisional article titles and proposed authors
- An indication of whether authors have agreed to participate
- Sponsor information including any relevant deadlines
- An indication of the expected length of each paper Guest Editor proposals if appropriate

Appendix 8: PUBLISHED JOURNAL ARTICLE - Determinants of fractional exhaled nitric oxide in workers at risk of developing occupational respiratory allergy and asthma: A review

Allergies in the Workplace

DETERMINANTS OF FRACTIONAL EXHALED NITRIC OXIDE IN WORKERS AT RISK OF DEVELOPING OCCUPATIONAL RESPIRATORY ALLERGY AND ASTHMA: A REVIEW

Phinias Mfune,[1] Mohamed F Jeebhay[1,2]

[1] *Occupational Medicine Division, School of Public Health, University of Cape Town, South Africa*
[2] *Centre for Environmental & Occupational Health Research, School of Public Health, University of Cape Town, South Africa*

Email | phiniasmfune@gmail.com

ABSTRACT

Fractional exhaled nitric oxide (FeNO) is a non-invasive biomarker of eosinophilic airway inflammation. FeNO is elevated in allergic asthma and its production increases in the airways in the presence of Type-2 inflammation. Both individual and occupational factors are associated with increased FeNO in workers with occupational asthma. This review focuses on identifying the specific predictors of elevated FeNO in workers at risk of developing occupational respiratory allergy and asthma, with a specific focus on those settings where it is used for medical screening and surveillance. The review found that individual factors such as age and atopy are associated with increased FeNO, whereas smoking and inhaled steroid use are consistently associated with decreased FeNO levels. Occupational factors such as exposure to either high or low molecular weight agents, occupational allergic sensitisation, the nature of work, the duration of employment and the level of exposure to occupational allergen(s) are associated with elevated FeNO. More recent evidence suggests that it is the agent's ability to induce an IgE-mediated mechanism, and not its molecular weight or its nature, that is responsible for elevated FeNO levels in occupationally exposed workers. Stronger epidemiological study designs such as cohort studies are needed that offer better opportunities to investigate these relationships in a more refined manner.

Keywords: FeNO, occupational allergy, asthma, occupational respiratory allergy, sensitisation

INTRODUCTION

Fractional exhaled nitric oxide (FeNO) is a non-invasive biomarker of eosinophilic airway inflammation and is able to predict steroid treatment response in asthmatics. This biomarker is primarily used in asthma, but is also relevant to various other pulmonary disorders, both allergic and non-allergic. These include chronic allergic rhinitis, acute and chronic eosinophilic pneumonia, chronic obstructive pulmonary disease (COPD), bronchiolitis obliterans syndrome, hypersensitivity pneumonitis (HP), viral infection and interstitial lung disease.[1–8]

Since a number of respiratory diseases can also be caused by work, FeNO represents a safe and easy measure with which to assess their work-relatedness in an occupational setting.[1,2] However, FeNO may be lower among those who smoke, have bronchoconstriction or use steroid therapy.[8]

Nitric oxide (NO) is a gas produced in the airways and is detectable as fractional exhaled NO (FeNO). Its production increases in the airways when Type-2 inflammation is present.[4] Induced sputum and FeNO measurements are the most reliable non-invasive procedures with which to assess the presence of Type-2 airway inflammation.[1,4]

It is understood that FeNO levels relate more closely to the risk of disease exacerbation than to disease severity.[4] FeNO levels correlate with asthma exacerbations in severe asthmatic patients independently of blood eosinophils. Whereas FeNO is associated with increased sputum and blood eosinophil levels, it correlates moderately with the former and weakly with the latter.[3,8]

Apart from its usefulness in the diagnostic work-up of individuals with suspected asthma, the measurement of FeNO offers a useful tool in the screening and surveillance of working populations at risk of developing occupational asthma (OA) associated with exposure to respiratory sensitisers.[3,9] Most studies on the determinants of FeNO have generally focused on the general population as opposed to workers in the occupational setting.[1–3,10]

The aim of this review was to evaluate the individual or host and occupation-related determinants of elevated FeNO in workers at risk of developing occupational respiratory allergy and asthma. The findings of the review will contribute to obtaining better insights into FeNO levels in working populations and

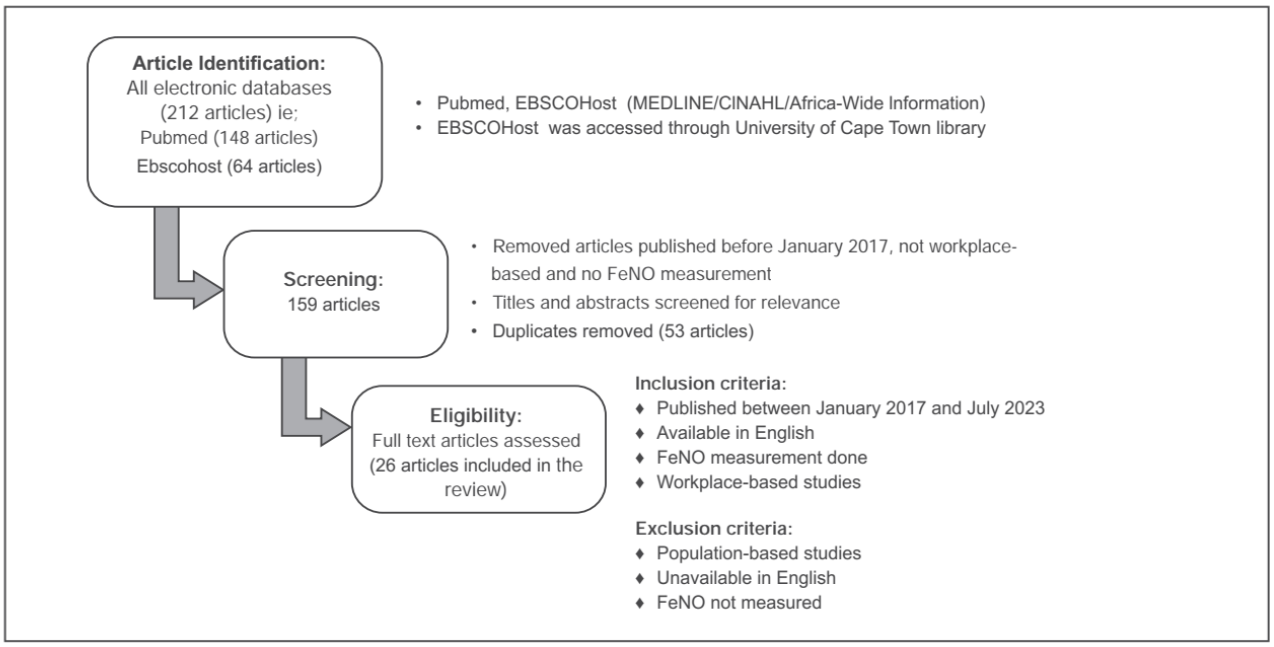

Figure 1: Flow diagram outlining the article search strategy

to assess their applicability in the screening and surveillance of high-risk occupational groups. This could contribute to improved preventive measures for workers at risk of developing occupational respiratory allergy and asthma.

The review focused on the research question: 'What are the determinants of elevated FeNO levels in workers at risk of developing occupational respiratory allergy and asthma in different occupational settings?'

The review included epidemiological and clinical studies of FeNO measurements in workers from different occupations that were published in English from January 2017 to July 2023. The literature search followed the PICO framework to search systematically for relevant articles in PubMed and EBSCOHost (MEDLINE/CINAHL/Africa-Wide Information) databases using specific search terms and identifying relevant articles cited in the references of the articles identified through the search. The following search terms were used:

> Workers OR occupation OR occupational OR work related OR occupational exposure OR industry OR baking OR wood processing OR spice milling OR spices OR health care OR health care worker OR poultry farming OR fruit farming OR spices OR flour OR dust OR alpha amylase OR α-amylase OR amylase OR latex gloves OR cleaning agents AND FeNO OR fractional exhaled nitric oxide OR high molecular weight agents OR low molecular weight agents" AND "Asthma OR sensitization OR atopy OR determinants.

The review finally included 26 epidemiological and clinical studies of FeNO measurements in workers from different occupations (see Figure 1).

DETERMINANTS OF FRACTIONAL EXHALED NITRIC OXIDE

Studies of the determinants of FeNO were broadly categorised into either observational epidemiological studies of high-risk working populations exposed to agents of high molecular weight (HMW) or low molecular weight (LMW) and clinical studies of patients with confirmed or suspected occupational asthma (OA). The epidemiological findings are summarised according to workers exposed to agents of HMW (see Table I), LMW (see Table II) or both HMW and LMW (see Table III). The findings observed in patients with either confirmed or suspected OA are presented in Table IV. The review identified various individual (host) and occupational factors associated with elevated FeNO in these various studies.

INDIVIDUAL (HOST) FACTORS

AGE

Workplace-based studies that analysed the effect of age on FeNO measurement are very limited, hence reference values for adults are still not available. Age is generally positively associated with FeNO in healthy individuals. In a population-based study on the determinants of FeNO in healthy men and women from the European Community Respiratory Health Survey (ECRHS III), Nerpin et al reported that men in the upper age quartile (60.3–67.6 years) had 19.2 ppb (95% CI 18.3–20.2) higher FeNO than those in the lowest quartile (39.7–48.3 years).[2] Similarly, women in the two highest age quartiles (54.6–60.2 and 60.3–67.6 years) had 15.4 ppb (14.7–16.2) and 16.4 ppb (15.6–17.1), respectively higher FeNO, compared to the lowest quartile. However, Shiryaeva et al, 2015 found that age was not significantly correlated with FeNO levels.[11]

SEX

Most studies did not report on the association between sex

TABLE I: EPIDEMIOLOGICAL STUDIES OF RISK FACTORS ASSOCIATED WITH ELEVATED FRACTIONAL EXHALED (FeNO) IN WORKERS EXPOSED TO HMW AGENTS

AUTHOR, YEAR	STUDY DESIGN	SUBJECTS (N)	EXPOSURE OR OCCUPATIONAL AGENT	FeNO, PPB MEAN (±SD); MEDIAN (IQR)	DETERMINANTS (INDIVIDUAL/HOST, OCCUPATIONAL)	PREDICTORS OF ELEVATED FeNO OR(CI), RR(CI)
Al Badri et al, 2020[7]	Group-randomised intervention (bakery mixer tub lid, dust- control training)	337 bakers	Cereal flours	*FeNO mean difference* Intervention 2.2 (±23) Controls 1.7 (±14.5) *FeNOΔ baseline >25* Intervention 16.9 (±35.2) Control 7.7 (±24.2)	***Individual:*** Current smoker ***Occupational:*** *FeNO change ≥10%* *Intervention group:* Bakers with WRONS Bakers without WRONS *Baseline FeNO > 25:* Bakers with WRONS Bakers without WRONS	OR 0.92 (0.56–1.52) OR 3.73 (1.22–11.42) OR 1.62 (0.93–2.81) OR 5.03 (1.79–14.16) OR 3.02 (1.35–6.77)
Olivieri et al, 2021[5]	Cross-sectional	174 bakers	Wheat, multigrain	*Sensitisation* Wheat 24.7 (20.1–30.3) Rye 29.3 (21.5–40.0) Alpha-amylase 22.3 (16.2–30.6) *Respiratory symptoms* WRR 25.4 (20.6–31.3) Wheeze 35.5 (25.9–48.6) Chest tightness 27.7 (18.5–41.7)	***Individual:*** Sensitisation Respiratory symptoms	ND ND
Sack et al, 2020[25]	Cross-sectional	45 cannabis workers (growing facility)	Cannabis dust	FeNO 30 (8–102) *Cross-week change* 3.78 (0.68–6.88) *Cross-shift change* −1.36 (−4.46–1.74)	***Individual:*** Respiratory Ocular Nasal	ND ND ND
Crivellaro et al, 2020[33]	Cross-sectional	142 bakery workers	Cereal flour dust	FeNO 23.7 (±25.0) *FeNO mean difference* Flour dust concentration >2 mg/m³ 8.60 (p = 0.06) Occupational exposure duration (yrs) (p = 0.22)	***Individual:*** Smokers ***Occupational:*** Dust concentration >2 mg/m³ Occupational exposure duration	ND ND ND
Ngajilo et al, 2018[21]	Cross-sectional	230 poultry workers (rearing, laying, hatchery, broiler, catching)	Poultry dust and bioaerosols	FeNO >50: 7%	***Individual:*** Current smoker Atopy ***Occupational:*** Casual worker Rearing department	OR 0.41 (0.13–1.33) OR 3.04 (1.11–8.34) OR 1.76 (0.26–11.94) OR 1.67 (0.31–8.89)
Van der Walt et al, 2016[19]	Cross-sectional	150 spice mill workers	Spice dust (chilli, garlic)	*FeNO baseline >50* Day1 preshift FeNO 67.74 (±1.37) Day1 post shift 60.31 (±1.62) Day2 preshift 64.11 (±1.57) *24-hour FeNOΔ ≥12% increase* Day1 preshift 14.09 (±2.07) Day2 preshift 20.12 (±2.07)	***Individual:*** **Sensitisation** *FeNO baseline >50* Chilli pepper Garlic Wheat ***Occupational:*** Work-related respiratory symptoms ***24-hour FeNOΔ ≥12% increase*** Spice dust particulate (>1.18–<3.78 mg/m³)	 OR 17.04 (2.30–126.03) OR 6.80 (1.35–34.20) OR 4.84 (0.88–26.74) OR 5.38 (1.0–28.94) OR 3.77 (1.01–28)
Lim et al, 2019[27]	Cross-sectional	695 office workers	Indoor office dust and biological exposures (endotoxin, (1,3)-β-glucans)	*FeNO >25* Office workers 25.8% Rhinoconjunctivitis 35.2% Doctor-diagnosed asthma 47.7%	***Occupational:*** Sieved dust (1,3)-b-glucan	OR 2.91 (1.31–6.47) OR 1.10 (1.01–1.20)

CI: confidence interval, FeNO: fractional exhaled nitric oxide, FeNOΔ: change in fractional exhaled nitric oxide, HMW: high molecular weight agents, ppb: parts per billion, IQR: interquartile range, OR: odds ratio, RR: relative risk, ND: not done, WRONS: work-related ocular-nasal symptoms, WRR: work-related rhinitis

TABLE II: EPIDEMIOLOGICAL STUDIES OF RISK FACTORS ASSOCIATED WITH ELEVATED FRACTIONAL EXHALED NITRIC OXIDE (FeNO) IN WORKERS EXPOSED TO LMW AGENTS

AUTHOR, YEAR	STUDY DESIGN	SUBJECTS (N)	EXPOSURE OR OCCUPATIONAL AGENT	FeNO, PPB MEAN (±SD): MEDIAN (IQR)	DETERMINANTS (INDIVIDUAL/HOST, OCCUPATIONAL)	PREDICTORS OF ELEVATED FeNO OR(CI), RR(CI)
Werthmann et al, 2023[6]	Cross-sectional	277 women living < 5 km from banana plantation	Pesticides, smoke, agricultural chemicals	FeNO >25: 20%	***Individual:*** Current smoker Pregnant Asthma Rhinitis Wheeze ***Occupational:*** Pesticide use Partners work in agriculture	 OR 0.28 (0.02–1.42) OR 0.89 (0.13–3.60) OR 1.60 (0.76–3.22) OR 3.67 (1.81–7.35) OR 4.50 (2.25–8.99) OR 1.46 (0.76–2.93) OR 1.61 (0.77–3.58)
Klusáčková et al, 2022[24]	Cross-sectional	35 workers (26 factory, 9 office work)	Polyurethane and glue containing diisocyanates (MDI, TDI, HDI)	*FeNO day1* exposed 23 (±10) non-exposed 17 (±11) *FeNO day2* exposed 21 (±10) non-exposed 16.6 (±8.50)	***Individual:*** Cough Dyspnoea ***Occupational:*** Isocyanate exposure	 ND ND ND
Vinnikov et al, 2022[22]	Cross-sectional	80 metal-working workers (33 machine operators, 26 welders/ assemblers, 21 administrative)	Welding, machining, assembling, metal coatings application (mild steel, higher nickel-content steel, and stainless steel)	Smoker (0–10/day) 35.8 (±8.9) Smoker (>10/day) 24.9 (±8.8) Welders/assemblers 43.5 (±49.3) Machine operators 25 (±15) Administrative 22 (±16)	***Individual:*** Smoker ***Occupational:*** Welders Machinists Administrators	 ND ND ND ND
Paraskevaidou et al, 2021[32]	Cross-sectional	83 furniture workers (35 chemical exposed, 23 wood dust, 25 office workers)	Chemicals, wood dust	Chemical exposed 14.42 (±9.13) Wood-dust exposed 15.24 (±6.93) Office workers 12.98 (±5.28)	***Individual:*** Smoker ***Occupational:*** Length of exposure (months)	 ND ND
Pelclova et al, 2018[31]	Cross-sectional	41 office workers (20 exposed, 21 unexposed)	Nanocomposite materials	Pre-shift 24 (6–50) Post-shift 17 (4–40) Unexposed 15 (5–35)	***Individual:*** Age Sex Respiratory symptoms	 ND ND ND
Wang et al, 2018[14]	Cross-sectional	264 diesel exhaust technicians, DET (n = 137), 127 unexposed (water utility authority)	Diesel exhaust (high levels PM2.5 and elemental carbon)	DETs 16.5 (8.0–43.0) Non-DETs 16.2 (8.0–N34.0) *DETs exposure:* (0.6–6.3) years, 17.4 (9.0–53.0) (6.3–9.3) years, 16.4 (8.0–33.0) (9.3–36.8) years, 15.9 (8.0–30.0)	***Individual:*** Smoker ***Occupational:*** DETs length of exposure (years)	 ND ND
Suzuki et al, 2017[13]	Cross-sectional	63 pathology technicians (33 exposed, 30 unexposed)	Formaldehyde	*Cross-week change in subjects:* *Asthmatics:* Post-work 47.5 (±39.7) *Non-asthmatics:* Post-work 17.5 (±11.0) *Cross-week change in Controls:* *Asthmatics:* Post-work 30.9 (±15.3) *Non-asthmatics:* Post-work 14.0 (±7.2)	***Individual:*** Asthmatics ***Occupational:*** Length of employment (months)	 ND ND

TABLE II: CONTINUED

AUTHOR, YEAR	STUDY DESIGN	SUBJECTS (N)	EXPOSURE OR OCCUPATIONAL AGENT	FeNO, PPB MEAN (±SD); MEDIAN (IQR)	DETERMINANTS (INDIVIDUAL/HOST, OCCUPATIONAL)	PREDICTORS OF ELEVATED FeNO OR(CI), RR(CI)
Vlaanderen et al, 2017[30]	Cross-sectional	50 nanotube workers (21 exposed) vs 29 unexposed (age and gender matched)	Carbon nanotubes	*FeNO difference (Lab exposure):* Low 0.25 High 0.08 Intermediate (operator) 0.88 EC exposure trend estimate –0 .020	***Individual:*** Smoker ***Occupational:*** Level of exposure	 ND ND

BMI: body mass index, CI: confidence interval, DETs: diesel engine testers, EC: elemental carbon, FeNO: fractional exhaled nitric oxide, HCW: health care worker, HDI: hexamethylene diisocyanate, IQR: interquartile range, LMW: low molecular weight agents, MDI: methylene diphenyl diisocyanate, MWCNT: multi-wall carbon nanotubes, ND: not done, OR: odds ratio, ppb: parts per billion, RR: relative risk, SIC: specific inhalation challenge test, SD: standard deviation

TABLE III: EPIDEMIOLOGICAL STUDIES OF RISK FACTORS ASSOCIATED WITH ELEVATED FRACTIONAL EXHALED NITRIC OXIDE (FeNO) IN WORKERS EXPOSED TO BOTH HMW AND LMW AGENTS

AUTHOR, YEAR	STUDY DESIGN	SUBJECTS (N)	EXPOSURE OR OCCUPATIONAL AGENT	FeNO, PPB MEAN (±SD); MEDIAN (IQR)	DETERMINANTS (INDIVIDUAL/HOST, OCCUPATIONAL)	PREDICTORS OF ELEVATED FeNO OR(CI), RR(CI)
Mwanga et al, 2022[18]	Cross-sectional	699 healthcare workers	Cleaning agents (ortho-phthaladehyde, chlorhexidine, natural rubber latex)	*Sensitisation* OPA: 1.49 (1.15 – 1.93) NRL: 1.82 (1.19 – 2.78) All: 1.57 (1.25 – 1.97)	***Individual:*** *Sensitisation:* *FeNO ≥ 25* OPA NRL All *FeNO ≥ 50* OPA NRL All	 OR 3.90 (1.74–8.75) OR 4.32 (1.15–16.29) OR 4.18 (2.03–8.59) OR 3.10 (1.01–9.50) OR 4.50 (0.91–22.43) OR 3.94 (1.52–10.21)
Wild et al, 2017[20]	Cohort study	441 apprentices	Baking, pastry-cooking, hairdressing	*Baseline:* Bakers 21.0 ± 23.1 Pastry makers 19.8 ± 17.3 Hairdressers 15.5 ± 15.0	***Individual:*** *Sensitisation to common inhalants:* Weak (1–2 positive SPT) High (≥3 positive SPT)	 OR 1.30 (1.14–1.49) OR 1.82 (1.58–2.10)
Shiryaeva et al, 2015[11]	Cross-sectional	266 (trawler fishermen, n=127; salmon processors, n=139)	Seafood processing, (slaughtering and fileting of fish)	Trawler fishermen 28.1 smoker 9.5 non-smoker 17.2 asthmatics 28.1 non-asthmatics 12.4 Salmon processors 12.6 smoker (men) 11.1 non-smoker (men) 11.5 asthmatics 12.6 non-asthmatics 10.9	***Individual:*** Smoker Asthma	 ND ND

CI: confidence interval, FeNO: fractional exhaled nitric oxide, FEV1: forced expiratory volume in 1 second, HMW: high molecular weight agents, IQR: interquartile range, LMW: low molecular weight agents, ND: not done, NRL: natural rubber latex, OPA: ortho-phthalaldehyde, OR: odds ratio, ppb: parts per billion, RR: relative risk, SPT: skin-prick test

and FeNO. However, various population studies have reported differences in FeNO levels between men and women. In the ECRHS III study, FeNO levels were 21.3% higher in men compared to women ($p < 0.001$).[2] However, an earlier study by Engel et al reported that male patients with OA were less likely (OR 0.69, CI 0.26–1.87) to have a ≥13 ppb FeNO increase after a specific inhalation challenge test.[12] In a study of bakers, no significant difference was reported between median FeNO in men of 18.1 ppb (95% CI 15.3, 21.4) compared to women 15.3 ppb (95% CI 11.7–20.3).[5] Similarly, no significant sex differences were found in pathology workers exposed to chemical agents.[13]


2a

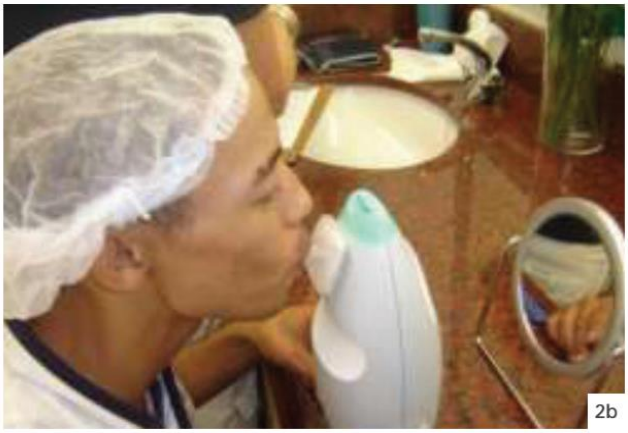
2b

Figure 2a-b: FeNO measurement in the workplace (courtesy of Drs Paulino Chamba and Roslynn Baatjies)

ANTHROPOMETRIC CHARACTERISTICS

In their study of bakers, Olivieri et al reported no significant correlation between FeNO and either height, weight or BMI.[5] Height contributed an 8% significantly higher FeNO in men and 5% in women.[2] Other studies have reported that BMI was not significantly associated with elevated FeNO levels.[2,14,15] Whereas the recent study of women working on banana plantations reported a positive association between BMI and elevated FeNO (OR 1.61, 0.89–2.95), the association was not significant.[6]

ATOPY AND SPECIFIC ALLERGIC SENSITISATION

It is well known that FeNO is higher in atopic individuals subjected to either occupational or environmental exposures.[1–3,10,15] In a study of patients with suspected OA due to HMW and LMW agents, Beretta et al reported that in subjects who undertook a positive specific inhalation challenge test (SIC), a FeNO >25 ppb was more likely to be observed in atopics (OR 2.9, 1.4–6.0).[16] A review of the determinants of FeNO in asthmatics also reported that levels are elevated in asthmatic patients, particularly in those who are atopic.[4] Engel et al also reported that male patients with a ≥13 ppb FeNO increase after specific inhalation challenge test were more likely (OR 1.86, 0.75–4.64) to be atopic.[12] In a clinical case series of workers with asthmatic reactions induced by isocyanates, the subjects demonstrated a delayed increase in FeNO 24 hours following exposure in the active (6.6 nL/s) SIC group, of 111.8 ppb, CI (68.5–236.0) compared to sham (3.3 nL/s) SIC of 58.6 ppb, CI (24.1–98.8).[17]

In health workers who frequently use cleaning agents, a strong positive association was observed between those with elevated FeNO ≥25 ppb and specific allergic sensitisation to either ortho-phthalaldehyde (OPA) (OR 3.10, 1.01–9.50) or natural rubber latex (NRL) (OR 4.32, 1.15–16.29).[18] Similarly, in a cross-sectional study of spice mill workers, atopy was strongly associated with sensitisation and FeNO >50 ppb.[19] Workers with FeNO >50 ppb were also more likely to be specifically sensitised to chilli pepper (OR 17.04, 2.30–126.03) or garlic (OR 6.80, 1.35–34.20).[19] Other studies have also shown that laboratory animal workers with a high specific IgE to laboratory animal allergens showed an increase in their FeNO levels when compared to those without specific IgE.[3] Similar findings were reported by Wild et al in apprentices.[20] Highly sensitised apprentices exhibited increased log odds of elevated FeNO compared to non-sensitised (log OR 1.42, 1.10–1.83).[20]

SMOKING STATUS

It is well known that smoking status is inversely related to FeNO levels in adults, including asthmatic individuals, such that it masks the elevated FeNO in asthmatics.[1,3,10] Studies also report significantly lower FeNO values among both acute and chronic smokers compared to non-smokers in the occupational context.[3,4]

In a group randomised trial assessing the impact of interventions for baker's allergy and asthma in supermarket bakeries, Al Badri et al showed that current smokers were more likely (OR 0.92, 0.56–1.52) to have reduced FeNO levels.[7] Similar observations were reported in poultry farmers, bakery workers and banana plantation workers.[5,6,21]

Some studies have been able to demonstrate an inverse dose–response relationship between smoking status and FeNO. Wang et al in their study on diesel exhaust engine testers demonstrated that cigarette smokers with a low internal dose had higher FeNO levels (18.7 ppb) than those with a medium (16.5 ppb) or high (14.4 ppb) internal dose of cigarette smoke.[14] Similar observations were also made in metal-working workers, in that subjects who smoked <10 cigarettes per day had a higher mean FeNO of 35.8 (±8.9 ppb) compared to those who smoked >10 cigarettes per day, having a mean FeNO of 24.9 (±8.8 ppb).[22]

In clinical studies of patients with suspected OA due to HMW and LMW agents, subjects with FeNO ≥25 ppb post-specific inhalation challenge were more likely (OR 2.5, 1.2–5.0) to have never smoked.[16] Similar studies among patients exposed to HMW, LMW and isocyanates, demonstrated that smokers were more likely (OR 1.25, 0.45–3.45) to have a ≥13 ppb increase in FeNO after SIC.[12]

INHALED CORTICOSTEROID USE

It is well known that inhaled corticosteroids can cause lower

TABLE IV: CLINICAL STUDIES OF PATIENTS WITH OA (OR SUSPECTED) AND RISK FACTORS ASSOCIATED WITH ELEVATED FRACTIONAL EXHALED NITRIC OXIDE (FeNO)

AUTHOR, YEAR	STUDY DESIGN	SUBJECTS (N)	EXPOSURE OR OCCUPATIONAL AGENT	FeNO, PPB MEAN (±SD); MEDIAN (IQR)	DETERMINANTS (INDIVIDUAL/HOST, OCCUPATIONAL)	PREDICTORS OF ELEVATED FeNO OR(CI), RR(CI)
Van Kampen et al., 2023[29]	Retrospective observational	455 with OA due to Pt salts (*n* = 14), HMW (*n* = 225), LMW (*n* = 216)	Platinum (Pt) salts, HMW, LMW agents	*Pt vs LMW agents* *Baseline:* Pt 30 (13–64) LMW 19 (11–31) HMW 25 (13–41) Isocyanates 17 (12–23) Persulfate 17 (11–26) *Post-challenge:* Pt 77 (44–142) LMW 23 (14–40) HMW 44 (24–84) Isocyanates 23 (15–43) Persulfate 21 (14–34)	***Individual:*** Sex (male) Inhaled SABA use ***Occupational:*** Length of exposure Isocyanates	 ND ND ND ND
Suojalehto et al, 2020[28]	Retrospective observational	598 with OA due to acrylates (*n* = 55), Isocyanates (*n* = 125), other LMW agents (*n* = 418)	Acrylates	*FeNO Baseline:* Acrylate OA 15 (10.0–29.0) Isocyanates OA 20 (12.0–35.0) Other LMW agents OA 19 (10.0–32.8) *Post-SIC FeNO*Δ*:* Acrylate OA 26.0 (8.2–38.0) Isocyanates OA 5.0 (2.0–16.0) Other LMW agents OA 3.0 (–1.0–10.0)	***Occupational:*** Acrylates Isocyanates Other LMW agents	 ND ND ND
Dubini et al, 2020[23]	Case series	5 bakers with OA	Wheat flour dust	*FeNO reduction:* After SLIT 12.20 (9.76–14.64)	***Individual:*** Sex Atopy ICS use ***Occupational:*** HMW agents	 ND ND ND ND
Engel et al, 2019[34]	Retrospective observational	122 with suspected OA (pulmonary responders vs non-responders)	HMW and LMW agents	FeNO increase ≥ 13 ppb post- SIC *Baseline: FeNO:* SIC(+) pulmonary response 24 (3–151) SIC(–) 14 (5–119) Post-SIC FeNO: *SIC positive pulmonary* response 54 (6–156) SIC(–) 16 (6–126)	***Individual:*** Sex (male) Smoker Atopy ICS use ***Occupational:*** HMW Isocyanates	 ND ND ND ND ND ND
Van Kampen et al, 2019[26]	Retrospective observational	41 with suspected OA	HMW and LMW agents	FeNO increase: At work 20.5 Off-work 16.0	***Individual:*** Smoker Atopy ***Occupational:*** HMW LMW	 ND ND ND ND
Engel et al, 2018[12]	Retrospective observational	148 with suspected OA (pulmonary responders, non-responders, doubtful)	HMW, LMW and isocyanates	*Total:* Baseline 15 (3–158) Post-SIC 21 (5–193) SIC(+): Baseline 23 (3–151) Post-SIC 41 (5–156) *SIC(–):* Baseline 14 (3–158) Post-SIC 17 (6–193)	***Individual:*** *FeNO ≥13 ppb after SIC* Sex (male) Smoker Atopy ICS use ***Occupational:*** HMW Isocyanates	 OR 0.69 (0.26–1.87) OR 1.25 (0.45–3.45) OR 1.86 (0.75–4.64) OR 0.53 (0.22–1.30) OR 0.11 (0.01–1.08) OR 1.63 (0.58–4.63)

TABLE IV: CONTINUED

AUTHOR, YEAR	STUDY DESIGN	SUBJECTS (N)	EXPOSURE OR OCCUPATIONAL AGENT	FeNO, PPB MEAN (±SD); MEDIAN (IQR)	DETERMINANTS (INDIVIDUAL/HOST, OCCUPATIONAL)	PREDICTORS OF ELEVATED FeNO OR(CI), RR(CI)
Beretta et al, 2018[16]	Prospective observational	240 with suspected OA (133 SIC(+) and 107 SIC(–))	HMW and LMW agents	*Positive SIC* PC20 ≤16 mg/mL 25.2 (10.9–46.0) PC20 >16 mg/mL 18 (17.0–35.3) *Negative SIC* PC20 ≤16 mg/mL 14 (8.7–33.0) PC20 >16 mg/mL 14.4 (7.8–22.2)	***Individual:*** *In SIC+ FeNO (≥25 ppb)* Never smoker Atopy ***Occupational:*** HMW	 OR 2.5 (1.2–5.0) OR 2.9 (1.4–6.0) OR: 7.2 (1.4–37.7)
Mason et al, 2016[17]	Case series	26 with suspected OA (17 SIC (+) vs 9 SIC(–))	Isocyanates (polyurethane)	*Positive SIC* *Baseline:* Active (6.6 nL/s) 58.6 (24.1–98.8) Sham (3.3 nL/s) 51.2 (25.2–120.3) *SIC+ 24 hrs* Active 111.8 (68.5–236.0) Sham 58.6 (24.1–98.8) *Negative SIC* *Baseline:* Active 17.6 (11–26.8) Sham 13.5 (10.9–19.6) *SIC(–) 24 hrs* Active 19.5 (12–26.8) Sham 17.6 (11–26.8) Atopic SIC+ 122.9 (89.4–156.4) Non-atopic SIC+ 54.1 (41.6–66.6)	***Individual:*** Atopy ***Occupational:*** SIC(+) SIC(–)	ND ND ND

CI: confidence interval, FeNO: fractional exhaled nitric oxide, HMW: high molecular weight agents, FeNO∆: change in fractional exhaled nitric oxide, ICS: inhaled corticosteroids, IQR: interquartile range, LMW: low molecular weight agents, ND: not done, NSBH: non-specific bronchial hyperresponsiveness, OA: occupational asthma, OR: odds ratio, PC20: provocative concentration of histamine causing a 20% fall in FEV1, Pt: platinum salts, RR: relative risk, SD: standard deviation, SIC (+): positive specific inhalation challenge test, SIC (–): negative specific inhalation challenge test, SLIT: sublingual immunotherapy treatment

FeNO in certain individuals.[4] Furthermore, FeNO levels decline after inhaled or systemic steroid treatment in steroid-sensitive asthmatic patients.[3,4]

In studies of suspected OA due to HMW and LMW agents, the use of inhaled corticosteroids was associated with reduced odds (OR 0.53, 0.22–1.30) of having a ≥13 ppb increase in FeNO post-SIC.[12] Other studies have reported that an increase in FeNO after occupational exposure, especially to HMW agents, may be blunted in patients receiving inhaled or oral steroid treatment.[3] Similarly, FeNO reduction was also reported after a sublingual specific immunotherapy treatment (SLIT) administered to bakers with OA and rhinitis caused by wheat flour.[23]

RESPIRATORY SYMPTOMS

Respiratory symptoms also correlate with increased FeNO in various occupational contexts. Cough, dyspnoea, rhinitis and ocular–nasal symptoms were associated with FeNO >50 ppb in isocyanate exposed car-factory workers.[24] In their study of banana plantation workers, Werthmann et al reported that women with elevated FeNO levels were more likely to report wheeze (OR 4.50, 2.25–8.99), rhinitis (OR 3.67, 1.81–7.35) or asthma (OR 1.60, 0.76–3.22).[6] In bakery workers, FeNO was higher in flour- and multigrain-exposed subjects having wheeze, chest tightness and shortness of breath. Workers having two asthma-related symptoms had significantly ($p < 0.001$) higher median (IQR) FeNO 35.9 ppb (25.6–50.2) than those with only one, 17.5 ppb (12.7–24.0), or no symptoms, 13.3 ppb (11.4–15.6).[5] In a cross-sectional study investigating the relationship of workplace exposures and health symptoms at an indoor growing facility, cannabis workers had 1.6 higher odds of having work-related nasal symptoms (OR 1.6, 95% CI 0.27–11) and 2.5-fold higher odds (OR 2.5, 95% CI 0.19–37) in individuals with medium and high cannabis-dust exposure.[25] Shiryaeva et al in their study of Russian trawler fishermen and Norwegian salmon industry workers found that asthmatic exhibited notably higher FeNO (28.1 ppb) compared to non-asthmatic trawler workers (12.4 ppb), while there was no significant difference in FeNO between asthmatic (12.6 ppb) and non-asthmatic salmon workers (10.9 ppb).[11]

PREGNANCY

The study of women working in banana plantations in Costa

Rica reported an inverse association between pregnancy and elevated FeNO (OR 0.89, 0.13–3.60), but the association was not significant.[6]

OCCUPATIONAL FACTORS

NATURE OF AGENTS

High molecular weight agents

Exposure to HMW agents has been reported previously to be associated with elevated FeNO.[3,26] In a cross-sectional study of bakers in a wheat and multigrain bakery, workers exposed to wheat, rye, oats, barley, yeast and alpha amylase had elevated baseline FeNO levels.[5] In the spice-milling industry, workers with work-related asthma symptoms were also more likely (OR 5.38, CI 1.01–28.95) to have baseline FeNO >50 ppb.[19]

In clinical studies, exposure to HMW agents at work have also been associated with elevated FeNO levels. Specific inhalation challenge tests with HMW agents inducing an IgE-mediated sensitisation were associated with a ≥12% increase in FeNO.[3] Similarly, subjects exposed to HMW agents on SIC were more likely (OR 7.2, 1.4–37.7) to have FeNO ≥25 ppb.[16] A study of 41 workers that measured FeNO longitudinally at home and at work demonstrated higher FeNO (20.5 ppb) at work compared to periods off work (16.0 ppb), especially among those exposed to flour dust.[26] In a study of 460 office workers by Lim et al, office workers exposed to sieved dust had increased odds of FeNO ≥25 (OR 2.91, 95% CI 1.31–6.47).[27]

Low molecular weight agents

In clinical studies of OA patients with exposure to LMW, agents were generally not associated with elevated FeNO.[26] LMW agents do not typically demonstrate significant longitudinal differences in FeNO (increase ≥20% or >6 ppb) 24 hours after SIC.[1] However, exposure to specific LMW agents may be associated with varying FeNO levels. In a multicentre retrospective observational study of 598 workers, Suojalehto et al observed that workers exposed to acrylate (15.0 ppb) and other LMW agents (19.0 ppb) had lower baseline FeNO compared to isocyanates (20.0 ppb).[28] Furthermore, SIC with isocyanates was associated with significantly increased FeNO levels and an increased odds (OR 1.63, 0.58–4.63) of having FeNO ≥13.0 ppb after SIC.[1,3,12]

Recent studies show that FeNO is increased in IgE-mediated inflammation, irrespective of the molecular weight of the causative agent.[29] Similarly to isocyanates, workers with platinum salt-induced OA were shown to have a significantly greater increase in FeNO post-challenge compared to HMW and other LMW agents.[29] The study suggested that it was the mechanism and not the molecular weight of the agent that was important for inducing FeNO since it is generally understood that OA caused by LMW platinum salts is related to an IgE-mediated mechanism.[29]

Epidemiological studies have also yielded some interesting results. In a cross-sectional study by Suzuki et al, pathology technicians with bronchial asthma and exposed to formaldehyde showed a significant increase in FeNO (26 ppb) compared to control subjects.[13] However, a study of workers exposed to multi-walled carbon nanotubes showed a depression of FeNO with increasing levels of exposure. The FeNO difference was smaller in the high-exposure group (0.0841 ppb) compared to the low-exposure group (0.2478 ppb).[30] Similar findings were reported by Iavicoli et al in their review of seven studies of FeNO and nanomaterial exposure in workplaces.[10] No significant differences were observed between mean FeNO in the exposed (1.20 ppb (±0.32)) and the control groups (1.28 ppb (±0.32)).[10] However, in a study of 41 workers exposed to nanocomposite materials, Pelclova et al reported post-shift FeNO reduction in the exposed group (pre-shift 24 ppb (6–50); post-shift 17 ppb (4–40)).[31]

Few studies have been reported in the wood and furniture manufacturing industry. A study of Greek furniture workers reported no association between baseline and longitudinal FeNO levels associated with wood-dust exposure, despite asthma and rhinitis being common among the chemical and wood workers.[32] The study showed no significant differences between wood- or chemical-exposed workers and office workers, and only 6% of the subjects had FeNO >25 ppb.[32] A limitation of that study was the lack of power and heterogeneity of work practices, ventilation systems, and proper personal protective equipment (PPE) use among the furniture workers. Coman et al previously also reported no difference in FeNO levels in workers who had ceased exposure to red cedar compared to those with continued exposure.[3]

NATURE OF WORK (OR TASK)

Changes in FeNO levels could also be attributed to the nature of the work. In the same workplace, FeNO levels may differ in individual workers depending on the work they perform. In their study of metal-working workers, for instance, Vinnikov D et al reported significantly higher mean FeNO levels in welders or assemblers of 44.3 ppb (±49.3) compared to machine operators with 25 ppb (±15) and office workers with 22 ppb (±16), despite their all working in the same area.[22]

Similar observations were made in poultry farm workers, where working in the rearing department was more likely (OR 1.67, 0.31–8.89) than other areas to be associated with FeNO >50 ppb.[21] The study also found that casual workers were more likely (OR 1.76, 0.26–11.94) to have FeNO >50 ppb compared to permanent workers.[21] This was attributed to various reasons, including workers using poor-quality PPE, being pressurised to take on manual and more hazardous work with limited or no training, being subjected to constant changes in their tasks, and casual workers lacking confidence in raising safety concerns with their supervisors or employers for fear of losing their jobs.[21]

LEVEL OF EXPOSURE

In a study of 150 spice-mill workers, those with higher spice-dust particulate exposures (>1.18–<3.78 mg/m^3) were almost fourfold more likely (OR 3.77, CI (1.01–28)) to have a 24-hour FeNO delayed increase of ≥12%.[19] Among banana-plantation workers with passive pesticide exposure (occupational and para-occupational exposure to agricultural chemicals) increased FeNO levels were found among those whose partners worked in agriculture (OR 1.61 (0.77–3.58)) and/or in banana plantations (OR 1.80, 0.93–3.48). Generally, women that used pesticides were more likely (OR 1.46, 0.76–2.93) to have elevated FeNO.[6]

In their study of assessing the impact of an intervention for baker's allergy and asthma in supermarket bakeries, Al Badri et al demonstrated that bakers with ocular–nasal symptoms were more likely (OR 3.73, CI (1.22–11.42)) to demonstrate a significant decline in FeNO of ≥10% if they belonged to the intervention group (bakery lids and training with lower flour-dust levels) compared to the control group (higher flour-dust levels).[7] However, Crivallaro et al did not find a significant FeNO change (mean difference −8.60 ppb p-value 0.06) with an increase in dust concentrations among workers occupationally exposed to flour dust.[33]

SPECIFIC INHALATION CHALLENGE TEST – ALLERGEN DOSE–RESPONSE RELATIONSHIP

In clinical studies, a case series of workers with asthmatic reactions induced by isocyanates, demonstrated a delayed increase in FeNO 24 hours following exposure in the active (6.6 nL/s) SIC test group of 111.8 ppb, CI (68.5–236.0) compared to sham (3.3 nL/s) SIC of 58.6 ppb, CI (24.1–98.8).[17] A SIC test with HMW agents inducing an IgE-mediated sensitisation were also associated with a ≥12% increase in FeNO.[3] Beretta et al also showed that patients exposed to HMW agents during SIC were sevenfold more likely (OR 7.2, CI (1.4–37.7)) to have FeNO ≥25 ppb.[16] Furthermore, subjects with a positive SIC test had a higher FeNO baseline (23.0 ppb) and post-specific inhalation challenge test (41.0 ppb) than the subjects with a negative SIC (14.0 ppb and 17.0 ppb) respectively.[12]

Similar findings were reported in a study assessing the diagnostic accuracy of non-invasive tests before and after SIC for the diagnosis OA; post-SIC FeNO was higher in SIC-positive 54 ppb (6–156) pulmonary responders compared to SIC-negative 16 ppb (6–126) pulmonary responders.[34]

CONCLUSION

Occupational respiratory allergy and asthma are associated with elevated FeNO levels. Certain individual (host)-related factors such as age and atopy are more consistently associated with higher FeNO ≥50 ppb or ≥12% increase after SIC. However, some individual-related factors such as smoking and ICS use are associated with lower (<25 ppb) FeNO levels. Whereas an elevated dose of exposure to either HMW or LMW inducing an IgE-mediated inflammatory response is associated with increased FeNO levels, workplace interventions, aimed at reducing exposure to sensitisers, are associated with a significant (≥10%) decrease in FeNO levels in exposed workers. Furthermore, there has been an increasing focus in recent years on the relationship between FeNO and LMW agents, given the inconsistent relationships observed in previous studies. The evidence also suggests that it is the agent's ability to induce an IgE-mediated mechanism, and not its molecular weight or its nature, that is responsible for elevated FeNO levels in occupationally exposed workers.

Future epidemiological studies need to use stronger study designs such as cohort studies in order to investigate the various factors that determine FeNO levels in different occupational settings, which can ultimately influence the development of occupational respiratory allergy and asthma. In this way, temporal responses to host-related and occupational determinants and their relationship with FeNO can be better assessed. Gaining deeper insights into understanding and identifying the risk factors for increased FeNO is important in the surveillance programmes of workers aimed at developing appropriate preventive measures to mitigate risk.

ACKNOWLEDGEMENT

We would like to acknowledge Drs Roslynn Baatjies and Paulino Chamba for their permission to use pictures from the CEOHR archives.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

This article has been peer-reviewed.

REFERENCES

1. Oțelea MR, Fell AKM, Handra CM, et al. The value of fractional exhaled nitric oxide in occupational diseases: A systematic review. J Occup Med Toxicol. 2022;17(14):1–12. https://doi.org/10.1186/s12995-022-00355-1.
2. Nerpin E, Olivieri M, Gislason T, et al. Determinants of fractional exhaled nitric oxide in healthy men and women from the European Community Respiratory Health Survey III. Clin Exp Allergy. 2019;49(7):969–979. https://doi.org/10.1111/cea.13394.
3. Coman I, Lemière C. Fractional exhaled nitric oxide (FeNO) in the screening and diagnosis work-up of occupational asthma. Curr Treat Options Allergy. 2017;4(2):145–159. https://doi.org/10.1007/s40521-017-0122-8.
4. Pignatti P, Visca D, Loukides S, et al. A snapshot of exhaled nitric oxide and asthma characteristics: Experience from high to low income countries. Pulmonology. 2021;28(1):44–58. https://doi.org/10.1016/j.pulmoe.2020.10.016.
5. Olivieri M, Malerba M, Spiteri G, et al. Fractional exhaled nitric oxide levels in relation to work-related respiratory burden and sensitization to wheat flour and multigrain in bakers. Clin Transl Allergy. 2021;11(8):1–9. https://doi.org/10.1002/clt2.12018.
6. Werthmann D, van Wendel de Joode B, Cuffney MT, et al. A cross-sectional analysis of medical conditions and environmental factors associated with fractional exhaled nitric oxide (FeNO) in women and children from the ISA birth cohort, Costa Rica. Environ Res. 2023;233:1–8. https://doi.org/10.1016/j.envres.2023.116449.
7. Al Badri FM, Baatjies R, Jeebhay MF. Assessing the health impact of interventions for baker's allergy and asthma in supermarket bakeries: A group randomised trial. Int Arch Occup Environ Health. 2020;93(5):589–599. https://doi.org/10.1007/s00420-019-01511-7.
8. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention: 2023 GINA Report [Internet]. 2023. Available from: www.ginasthma.org.
9. Louis R, Satia I, Ojanguren I, et al. European Respiratory Society guidelines for the diagnosis of asthma in adults. Eur Respir J. 2022;60. https://doi.org/10.1183/13993003.01585-2021.
10. Iavicoli I, Fontana L, Leso V, Macrini MC, Pelclova D. Fractional exhaled nitric oxide and nanomaterial exposure in workplaces. Curr Med Chem. 2020;27(42):7200–7212. https://doi.org/10.2174/0929867327666200320154545.
11. Shiryaeva O, Aasmoe L, Straume B, Bang BE. Respiratory symptoms, lung functions, and exhaled nitric oxide (FeNO) in two types of fish processing workers: Russian trawler fishermen and Norwegian salmon industry workers. Int J Occup Environ Health. 2015;21(1):53–60. https://doi:10.1179/2049396714Y.0000000089.
12. Engel J, van Kampen V, Lotz A, et al. An increase of fractional exhaled nitric oxide after specific inhalation challenge is highly predictive of occupational asthma. Int Arch Occup Environ Health. 2018;91(7):799–809. https://doi.org/10.1007/s00420-018-1325-4.
13. Suzuki RA, Irokawa T, Ogawa H, et al. Fractional exhaled nitric oxide (FeNO) and spirometry as indicators of inhalation exposure to chemical agents in pathology workers. J Occup Environ Med. 2017;59(5):467–473. https://doi.org/10.1097/JOM.0000000000000976.

14. Wang H, Duan H, Meng T, et al. Local and systemic inflammation may mediate diesel engine exhaust-induced lung function impairment in a Chinese occupational cohort. Toxicol Sci. 2018;162(2):372–382. https://doi.org/10.1093/toxsci/kfx259.
15. Mwanga HH, Baatjies R, Singh T, Jeebhay MF. Asthma phenotypes and host risk factors associated with various asthma-related outcomes in health workers. Front Allergy. 2021;2:1–12. https://doi.org/10.3389/falgy.2021.747566.
16. Beretta C, Rifflart C, Evrard G, et al. Assessment of eosinophilic airway inflammation as a contribution to the diagnosis of occupational asthma. Allergy: Eur J Allergy Clin Immunol. 2018;73(1):206–213. https://doi.org/10.1111/all.13265.
17. Mason P, Scarpa MC, Guarnieri G, et al. Exhaled nitric oxide dynamics in asthmatic reactions induced by diisocyanates. Clin Exp Allergy. 2016;46(12):1531–1539. https://doi.org/10.1111/cea.12798.
18. Mwanga HH, Baatjies R, Singh T, Jeebhay MF. Work-related allergy and asthma associated with cleaning agents in health workers in southern African tertiary hospitals. Am J Ind Med. 2022;65(5):382–395. https://doi.org/10.1002/ajim.23344.
19. Van Der Walt A, Baatjies R, Singh T, Jeebhay MF. Environmental factors associated with baseline and serial changes in fractional exhaled nitric oxide (FeNO) in spice mill workers. Occup Environ Med. 2016;73(9):614–620. https://doi.org/10.1136/oemed-2015-103005.
20. Wild P, Mével H, Penven E, et al. FeNO levels increase with degree of sensitisation in apprentices at risk of occupational asthma. Int J Tuberc Lung Dis. 2017;21(11):1194–1200. https://doi.org/10.5588/ijtld.16.0758.
21. Ngajilo D, Singh T, Ratshikhopha E, et al. Risk factors associated with allergic sensitization and asthma phenotypes among poultry farm workers. Am J Ind Med. 2018;61(6):515–523. https://doi.org/10.1002/ajim.22841.
22. Vinnikov D, Tulekov Z, Blanc PD. Fractional exhaled NO in a metalworking occupational cohort. Int Arch Occup Environ Health. 2022;95(3):701–708. https://doi.org/10.1007/s00420-021-01801-z.
23. Dubini M, Marraccini P, Brass DM, Patrini L, Riboldi L. Occupational asthma and rhinitis due to wheat flour: Sublingual specific immunotherapy treatment. Medicina del Lavoro. 2020;111(3):203–209.
24. Klusáčková P, Dušková Š, Mráz J, et al. Health effects of exposure to isocyanates in a car factory. Cent Eur J Public Health. 2022;30(1):32–36. https://doi.org/10.21101/cejph.a6805.
25. Sack C, Ghodsian N, Jansen K, Silvey B, Simpson CD. Allergic and respiratory symptoms in employees of indoor cannabis grow facilities. Ann Work Expo Health. 2020;64(7):754–764. https://doi.org/10.1093/annweh/wxaa050.
26. Van Kampen V, Brüning T, Merget R. Serial fractional exhaled nitric oxide measurements off and at work in the diagnosis of occupational asthma. Am J Ind Med. 2019;62(8):663–671. https://doi.org/10.1002/ajim.22996.
27. Lim FL, Hashim Z, Than LTL, et al. Respiratory health among office workers in Malaysia and endotoxin and (1,3)-b-glucan in office dust. Int J Tuberc Lung Dis. 2019;23(11):1171–1177. https://doi.org/10.5588/ijtld.18.0668.
28. Suojalehto H, Suuronen K, Cullinan P, et al. Phenotyping occupational asthma caused by acrylates in a multicenter cohort study. J Allergy Clin Immunol Pract. 2020;8(3):971–979.e1. https://doi.org/10.1016/j.jaip.2019.10.017.
29. Van Kampen V, Migueres N, Doyen V, et al. Phenotyping occupational asthma caused by platinum salts compared to other low-molecular-weight agents. J Allergy Clin Immunol Pract. 2023;17(10):1076–1093. https://doi.org/10.1016/j.jaip.2023.06.014.
30. Vlaanderen J, Pronk A, Rothman N, et al. A cross-sectional study of changes in markers of immunological effects and lung health due to exposure to multi-walled carbon nanotubes. Nanotoxicology. 2017;11(3):395–404. https://doi.org/10.1080/17435390.2017.1308031.
31. Pelclova D, Zdimal V, Komarc M, et al. Deep airway inflammation and respiratory disorders in nanocomposite workers. Nanomaterials. 2018;8(731):1–13. https://doi.org/10.3390/nano8090731.
32. Paraskevaidou K, Porpodis K, Kontakiotis T, et al. Asthma and rhinitis in Greek furniture workers. J Asthma. 2021;58(2):1–10. https://doi.org/10.1080/02770903.2019.1674328.
33. Crivellaro MA, Ottaviano G, Maculan P, et al. Upper and lower respiratory signs and symptoms in workers occupationally exposed to flour dust. Int J Environ Res Public Health. 2020;17(19):7075. https://doi.org/10.3390/ijerph17197075.
34. Engel J, van Kampen V, Gering V, et al. Non-invasive tools beyond lung function before and after specific inhalation challenges for diagnosing occupational asthma. Int Arch Occup Environ Health. 2019;92(7):1067–1076. https://doi.org/10.1007/s00420-019-01439-y.