La Medicina del Lavoro

Case report

Med. Lav. 2025; 116 (5): 17219
DOI: 10.23749/mdl.v116i5.17219

Occupational Asthma Due to Subtilisin: The Power of Specific Inhalation Challenge*

Filippo Liviero†, Laura Fabris, Marco Biasioli, Francesco Favretto, Paola Mason
Department of Cardiac-Vascular-Thoracic Sciences and Public Health, University of Padova, Padova, Italy

Keywords: Enzyme Allergy; FeNO Monitoring; Type-2 Inflammation; Workplace Sensitization; Respiratory Biomarkers; Endotyping; Healthcare Workers; Bronchial Provocation Test; Allergen Exposure; Negative Skin Tests

Summary

We report the first confirmed case in Italy of occupational asthma caused by subtilisin in a healthcare worker involved in cleaning surgical instruments. The diagnosis was confirmed through a specific inhalation challenge (SIC) performed one year after the last exposure and after stopping inhaled corticosteroid therapy. An immediate reaction was observed after three minutes of exposure to diluted Neogiozym ™, with a 30% decrease in FEV1. This case highlights the diagnostic importance of SIC even for high-molecular-weight (HMW) agents and emphasizes the need to reconsider occupational asthma as a complex, evolving disease influenced by both host and environmental factors. The patient exhibited a Type-2-high phenotype despite negative skin prick tests and normal IgE levels, reinforcing the value of dynamic, multi-marker assessment in occupational endotyping. This case supports the broader use of SIC in occupational settings where allergen-specific IgE testing is limited.

1. Introduction

The use of proteolytic enzymes in the detergent industry began in the 1960s. Subtilisins are serine proteases, widely used primarily because of their low substrate specificity and high stability. They are used in all types of laundry detergents, automatic dishwashing products, and disinfectants for cleaning medical-surgical instruments and devices. Their role is to break down protein-based stains [1], and since their introduction, subtilisins have been recognized as potent allergens linked to the development of occupational asthma [2]. The mechanism by which this high molecular weight (HMW) enzyme triggers allergic inflammation in the airways of susceptible individuals is not yet fully understood. Animal studies have shown that subtilisin is a strong inducer of type 2 immunity, activating both Th2-mediated responses and Innate Lymphoid Cells type 2 (ILC2), as well as promoting the production of specific IgE [3]. Asthmatic reactions described were immediate, with or without a delayed component [4]. We report a case of occupational asthma caused by subtilisin in a nurse.

2. Case Report

A 51-year-old woman, a former smoker since 2007 with a 1 pack-year history, had no family or personal history of respiratory or allergic diseases.

Received 29.05.2025 – Accepted 06.08.2025

*Poster presented at European Academy of Allergy and Clinical Immunology (EAACI) Congress 2025, 13 – 16 June 2025, Glasgow, United Kingdom

† Corresponding Author: Filippo Liviero; E-mail: filippo.liviero@unipd.it

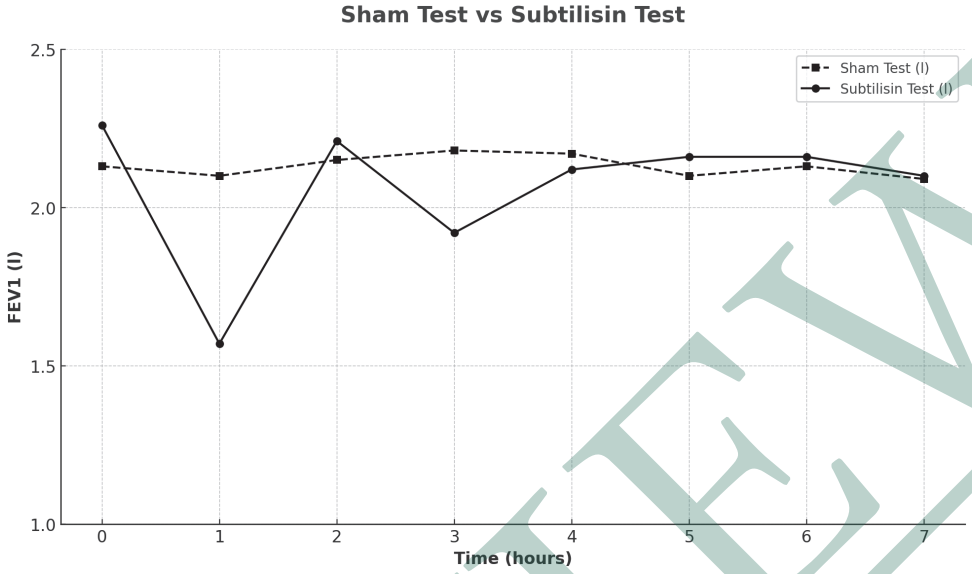

Figure 1. FEV1 trend during sham test and exposure to subtilisin.

She worked as a nurse in a hospital's medical department from 1998 to 2008, then in a surgical department from 2008 to 2023. Since June 2022, she was assigned to the digestive endoscopy department, where she was responsible for cleaning surgical instruments before sterilization using various detergents. The most used, Neogiozym ™, containing subtilisin, was prepared at a concentration of 0.2 mL diluted in 200 mL of water. Three months after starting this work, she developed dry cough and rhinitis, mainly with daytime rhinorrhea. No nocturnal awakenings or wheezing were reported. Her nasal symptoms worsened at work. In January 2023, she began experiencing nocturnal respiratory symptoms. She noticed slight symptom improvement during holidays. In May 2023, she underwent a non-specific inhalation challenge test with methacholine. The provocative dose causing a 20% fall in FEV1 (PD20 FEV1) was 91.99 µg, indicating severe bronchial hyperresponsiveness. Since then, she has been on inhaled therapy with budesonide/formoterol 320/9 mcg twice daily. Skin tests using the patch method with pure Neogiozym ™ (1 mL/1 mL) and diluted Neogiozym ™ (1 mL/10 mL), as well as prick tests for common inhalants and specific allergens to which cleaners are exposed, were negative; prick tests with pure and diluted Neogiozym ™ (1 mL/1 mL and 1 mL/10 mL) were also negative. A specific inhalation challenge (SIC) was performed in November 2024, when the nurse had not been exposed to subtilisin at work for a year, after discontinuing inhaled corticosteroids two weeks prior. Baseline FEV1 was 2.24 L (98% predicted), FVC was 2.85 L (106% predicted), and PD20 FEV1 was 732 µg; total IgE was 3.0 kUa/L. On the control day, with vapor exposure to water, FEV1 remained stable over 7 hours (see Figure 1). The following day, in a chamber maintained at 23–24°C, she inhaled vapor from diluted Neogiozym ™ (25 mL in 4 L of water). After three minutes, she started coughing, complained of chest tightness, and demonstrated a 30% decrease in FEV1 (1.57 L vs. a baseline of 2.26 L). She was monitored for seven hours post-exposure, and at the end of this period, her FEV1 measured 2.1 L (92.9% of baseline). At 24 hours post-SIC, PD20 was 838 µg. Additionally, fractional exhaled nitric oxide (FeNO) levels were monitored (see Figure 2): on the control day, before the sham test, the FeNO was 40 ppb; on the exposure day, before inhaling the specific vapor, it was 39 ppb. At the end of SIC, it rose to 45 ppb, and 24 hours later, it decreased to 33 ppb. The difference (between before and after- SIC levels), was not statistically significant.

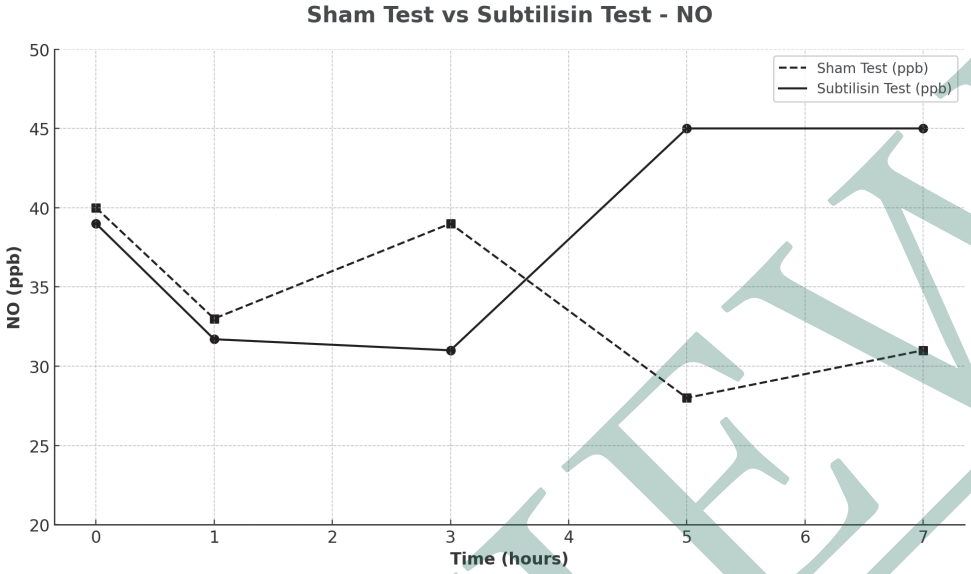

Figure 2. FeNO trend during sham test and subtilisin exposure.

3. Discussion

To our knowledge, this case report of occupational asthma caused by subtilisin is the first confirmed in Italy through SIC. It demonstrates the known potential of subtilisin to induce asthma. Further, it confirms that SIC remains the gold standard test for diagnosing occupational asthma, even in cases exposed to HMW agents. Whether occupational asthma is Type-2 high, Type-2 low, non-Type-2, or has a complex and mutant endotype remains a topic of debate. Historically and currently, the primary biomarkers analyzed to study the causal endotypes of occupational asthma include eosinophils and neutrophils in blood and/or induced sputum; total and/or specific IgE; FeNO; and cytokines and chemokines.

Asthma related to HMW agents is generally considered to be more Type-2 high, and this patient's initial phenotype supported this. She had work-related rhinitis before developing asthma symptoms, and her immediate response to SIC matched descriptions by Doyen et al. [5] as typical of asthma caused by HMW agents. Specifically, the decrease in FEV1 three minutes after exposure aligns with an IgE-mediated sensitization mechanism, confirming findings by Florsheim et al. [3]. The 24-hour pulmonary function monitoring showed no further decrease in FEV1, indicating no delayed-type reactions.

The rise in FeNO levels after exposure indicates eosinophilic inflammation, while the initial decrease in FeNO is linked to bronchoconstriction from subtilisin exposure, which reduced FeNO release, as described by Ferrazzoni et al. [6]. Notably, she had no blood eosinophilia or elevated total IgE. As Baur et al. [7] discussed in 2019, measuring specific IgE is often essential for pinpointing the exact cause of respiratory allergies, with testing for specific IgE to HMW allergens being a valuable diagnostic tool. Unfortunately, only a limited range of allergen-specific IgE tests are commercially available in the occupational field, and we could not develop an in-house test for this case. To address this, we performed a prick-by-prick skin test as described by Lemière et al. [4], which was negative. This result is hard to interpret—especially without specific IgE testing—but we speculate it's due to a mutant immune response. Like common asthma, occupational asthma should be considered a heterogeneous and complex condition, with the Th1/Th2 pattern shifting over time in response 1) to host–environment

interactions and 2) stimuli encountered during various occupational exposures.

Our case demonstrates that a negative skin prick test does not exclude occupational asthma caused by HMW agents, as our patient showed an immediate response at three minutes during SIC and an increase in FeNO after bronchoprovocation. In healthcare settings, workers handling disinfectants for medical and surgical instruments are exposed to numerous products. If they develop allergic symptoms—either respiratory or cutaneous—they are usually tested for sensitivity to these substances using prick tests, including specific allergens.

The SICremains the gold standard for diagnosing occupational asthma. While HMW allergens like subtilisin are known as potential triggers, confirmed cases through SIC are rare, especially in healthcare environments. Literature suggests SIC primarily when the suspected cause is a low-molecular-weight agent; however, our study highlights its importance for exposure to HMW agents as well. In conclusion, there is a growing need to combine different endotyping methods—analyzing occupational exposures, biomarkers, and genetics simultaneously—and this case report supports that combined approach.

FUNDING: This study was supported by the Ordinary Research Endowment (DOR) of the Department of Cardiac-Vascular-Thoracic Sciences and Public Health, University of Padua.

INSTITUTIONAL REVIEW BOARD STATEMENT: Ethical review and approval were waived for this study, as it describes a single case managed during routine clinical practice and does not involve prospective research.

INFORMED CONSENT STATEMENT: Written informed consent was obtained from the patient to publish this case report.

ACKNOWLEDGMENTS: We acknowledge the scientific contribution and support of the Occupational Medicine Working Group. Laboratory staff: Anna Bordin. Nurses: Maria Beatrice Galvan, Michela Tognon, and Maria Teresa Vangelista.

DECLARATION OF INTEREST: The authors declare no conflicts of interest.

AUTHOR CONTRIBUTION STATEMENT: F.L. and P.M. contributed to the conception and clinical management of the case. M.B., L.F., and F.F. were responsible for the collection of functional and clinical-anamnestic data. F.L. and P.M. contributed to the analysis and interpretation of pulmonary function data and FeNO trends. They also drafted the manuscript and critically revised the discussion section. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

DECLARATION ON THE USE OF AI: None.

REFERENCES

1. Azrin NAM, Ali MSM, Rahman RNZRA, Oslan SN, Noor NDM. Versatility of subtilisin: A review on structure, characteristics, and applications. *Biotechnol Appl Biochem*. 2022;69(6):2599–616.
2. Adisesh A, Murphy E, Barber CM, Ayres JG. Occupational asthma and rhinitis due to detergent enzymes in healthcare. *Occup Med*. 2011;61(5):364–9.
3. Florsheim E, Yu S, Bragatto I, et al. Integrated Innate Mechanisms Involved in Airway Allergic Inflammation to the Serine Protease Subtilisin. *J Immunol*. 2015;194(10):4621–30.
4. Lemière C, Cartier A, Dolovich J, Malo JL. Isolated Late Asthmatic Reaction After Exposure to a High-Molecular-Weight Occupational Agent, Subtilisin. *Chest*. 1996;110(3):823–4.
5. Doyen V, Gautrin D, Vandenplas O, Malo JL. Comparison of high- and low-molecular-weight sensitizing agents causing occupational asthma: an evidence-based insight. *Expert Rev Clin Immunol*. 2024;20(6):635–53.
6. Ferrazzoni S, Scarpa MC, Guarnieri G, Corradi M, Mutti A, Maestrelli P. Exhaled nitric oxide and breath condensate ph in asthmatic reactions induced by isocyanates. *Chest*. 2009;136(1):155–62.
7. Baur X, Akdis CA, Budnik LT, et al. Immunological methods for diagnosis and monitoring of IgE-mediated allergy caused by industrial sensitizing agents (IMExAllergy). *Allergy*. 2019;74(10):1885–97.