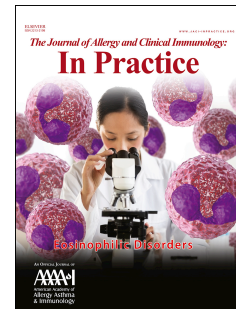


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Diagnostic Accuracy of Specific IgE Against Wheat and Rye in Flour-Induced Occupational Asthma

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Diagnostic Accuracy of Specific IgE Against Wheat and Rye in Flour-Induced Occupational Asthma

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ABSTRACT

BACKGROUND: Assessment of IgE-mediated sensitization to flour allergens is widely used to investigate flour-induced occupational asthma. The diagnostic efficiency of detecting specific IgE antibodies (sIgE) against wheat and rye flour, however, has not been thoroughly compared to other diagnostic procedures.

OBJECTIVE: We sought to evaluate the diagnostic accuracy of sIgE against wheat and rye compared to specific inhalation challenge (SIC) with flour as the reference standard.

METHODS: This retrospective multicenter study included 264 subjects who completed a SIC with flour in eight tertiary centers, of whom 205 subjects showed a positive SIC result.

RESULTS: Compared with SIC, sIgE levels ≥ 0.35 kU_A/L against wheat and rye provided similar sensitivities (84%-85%, respectively), specificities (71%-78%), positive predictive values (91%-93%), and negative predictive value (56%-61%). Increasing the threshold sIgE value to 5.10 kU_A/L for wheat and to 6.20 kU_A/L for rye provided a specificity $\geq 95\%$ and further enhanced the positive predictive value to 98%. Among subjects with a positive SIC, those who failed to demonstrate sIgE against wheat and rye (n=26) had significantly lower total serum IgE level and blood and sputum eosinophil counts, and a lesser increase in post-challenge fractional exhaled nitric oxide as compared to the subjects with detectable sIgE.

CONCLUSION: High levels of sIgE against wheat and/or rye flour strongly support a diagnosis of flour-induced occupational asthma without the need for performing a SIC. The absence of detectable sIgE against wheat and rye in subjects with a positive SIC seems to be associated with lower levels of T_H2 biomarkers.

Word count: 248 words

HIGHLIGHT BOX

What is already known about this topic? Although cereal flour remains the predominant cause of occupational asthma, information is scarce regarding the diagnostic performance of specific IgE antibodies against wheat and rye flour as compared with specific inhalation challenge with flour.

What does this article add to our knowledge? High levels of serum specific IgE against wheat/rye strongly support a diagnosis of flour-induced occupational asthma without requiring additional investigations. However, the absence of sIgE against wheat/rye does not allow for ruling out flour-induced occupational asthma.

How does this study impact current management guidelines? These findings may assist clinicians in identifying subjects who need additional investigations before establishing or excluding a diagnosis of flour-induced asthma.

Keywords: Occupational asthma; rye flour; specific inhalation challenge; specific IgE antibodies; wheat flour.

82 Abbreviations used

83	CI:	Confidence interval
84	FeNO:	Fractional exhaled nitric oxide
85	FEV ₁ :	Forced expiratory volume in one second
86	IQR:	Interquartile range
87	NPV:	Negative predictive value
88	NSBH:	Nonspecific bronchial hyperresponsiveness
89	OA:	Occupational asthma
90	PPV:	Positive predictive value
91	ROC:	Receiver operating characteristics
92	SIC:	Specific inhalation challenge
93	sIgE:	Specific IgE antibodies

94

95 INTRODUCTION

96 Flour remains the most prevalent cause of sensitizer-induced occupational asthma (OA),
97 accounting for about one third of cases in European countries (1). An accurate diagnosis of
98 flour-induced OA – often referred to as baker's asthma – is crucial for implementing appropriate
99 interventions aimed at minimizing the long-term adverse health and socioeconomic impacts of
100 the disease (2, 3). Assessment of IgE-mediated sensitization to flour allergens through skin-
101 prick testing or a serum specific IgE (sIgE) antibody assay is key for establishing a diagnosis
102 of flour-induced OA because facilities for performing safe and accurate specific inhalation
103 challenges (SICs) with occupational agents are not widely available (3, 4). Skin-prick tests with
104 commercial extracts of wheat and rye have provided variable but generally low sensitivity (40%
105 to 78%) for identifying flour-induced OA compared with SIC because of the highly variable
106 protein and antigen content of commercially available cereal flour extracts (5-7). Yet
107 information is scarce regarding the efficiency of determining sIgE antibodies against wheat and
108 rye flour in diagnosing flour-induced OA (6-8).

109 The aim of this study was to assess the accuracy of sIgE against wheat and rye in predicting
110 the result of a SIC with flour used as the reference standard in a large multicenter cohort of
111 subjects evaluated for possible OA related to flour exposure. We also aimed at further
112 characterizing the subjects who developed a positive SIC response to flour without detectable
113 sIgE to wheat and rye flour.

METHODS

Study design and population

This retrospective, observational study was conducted among consecutive subjects who completed a SIC procedure with flour in eight European tertiary centers between 2006 and 2018 (Appendix E1 in this article's Online Repository). A total of 264 subjects with available sIgE antibody data for both wheat and rye flour were eligible for this analysis (see Table E1 in this articles' Online Repository).

Ethics

This retrospective study was approved by the local Institutional Review Board of each participating center. The central database held at the Strasbourg University was approved by the *Comité Consultatif sur le Traitement de l'Information en Matière de Recherche dans le Domaine de la Santé* and the *Commission Nationale de l'Informatique et des Libertés*.

Data collection

Anonymized information on demographic, clinical, occupational, and functional characteristics of the subjects at the time of the diagnostic evaluation was entered in a standardized Excel spreadsheet at each participating center (1). Information was also collected on total serum IgE level, sIgE against bakery allergens (see below), and baseline blood eosinophil counts as well as induced sputum cell counts and fractional exhaled nitric oxide concentrations (FeNO) measured at baseline and 24 hours after challenge exposure to flour, when available.

Procedures

Assessment of specific IgE antibodies. The level of serum sIgE against wheat and rye flour was measured at the time of the diagnostic evaluation in each center using a commercial assay (ImmunoCAP, Thermo Fisher Scientific [Phadia], Uppsala, Sweden) according to the manufacturer's recommendations. The results of sIgE against a panel of allergens that are reported to be involved in baker's asthma were also collected, if available. These allergens

included fungal alpha-amylase, other cereals (barley, oat, corn, and rice), buckwheat, lupine, soybean, sesame seeds and storage mites.

Measurements of nonspecific bronchial hyperresponsiveness: The level of nonspecific bronchial hyperresponsiveness (NSBH) was measured using different bronchoprovocation procedures at the participating centers. The level of NSBH was categorized *a posteriori* as “absent”, “mild”, or “moderate-to-severe” based on the available recommendations or a consensus Delphi approach among investigators (1). The bronchoprovocation methods and threshold values used for defining the level of NSBH are detailed in Appendix E2 and Table E2 of this article’s Online Repository. A twofold or greater increase in the post-challenge level of NSBH was considered significant (4).

Specific inhalation challenges. The mode of selection of the subjects for performing a SIC with flour varied between centers (Appendix E1 of this article’s Online repository). A SIC with flour was completed by all subjects referred for the investigation of flour-related asthma symptoms in 5 of the 8 participating centers, which included the vast majority (235 out of 264, 89%) of the subjects considered in this analysis.

The SIC methodology conformed with international recommendations in terms of safety precautions, “placebo” challenge, and duration of functional monitoring (4). Asthma medications were withdrawn according to their duration of action and ICS were withheld three to five days before the SIC procedure.

Investigators were asked to provide information on the methodology and materials used for performing SICs with flour. Challenge exposure to flour was carried out using a “realistic” approach aimed at reproducing workplace exposure conditions by tipping flour from one tray to another or by dusting with pressured air without measurement of the level of airborne dust or concentration of flour allergens. The subjects were challenged with wheat flour (n=33), rye flour (n=23), or a mixture of wheat and rye flours used at work (n=208). These mixtures most often contained “dough improvers” and sometimes other cereals (barley, oat), but the detailed

constituents of the mixtures were most often unknown as already reported in UK bakeries (9). According to international recommendations (4), the duration of exposure to flour was gradually increased while spirometry was measured after each exposure and monitored for at least 6 hours after the end of the challenge. The median (IQR) cumulative duration of challenge exposure to flour was 30 min (5-60) in subjects with a positive SIC and 240 min (180-240, $P<0.001$) in those with a negative SIC.

SIC results were interpreted according to standardized criteria. A SIC result was considered positive when $\geq 15\%$ fall in forced expiratory volume in one second (FEV_1) was recorded at any time during the post-challenge monitoring period (4). A negative SIC was defined by changes in FEV_1 of less than 15% and the absence of a twofold or greater increase in the post-challenge level of NSBH compared with the baseline value. An isolated increase in post-challenge sputum eosinophils $\geq 3\%$ in the absence of NSBH was categorized as “occupational eosinophilic bronchitis” and considered as a positive SIC result in this analysis (10).

Data analysis

Continuous variables were presented as medians and interquartile ranges (IQR) and categorical variables as percentages. Comparisons between groups of subjects were made using the chi-squared or Fisher's exact test for categorical variables and non-parametric tests for numerical variables.

Receiver operating characteristics (ROC) curves were used to determine the diagnostic performance of sIgE levels against wheat and rye as compared to SIC with flour, which was considered the reference standard. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), area under the curve, and Youden index (i.e. sensitivity + specificity – 1) with their 95% confidence intervals (CIs) were determined for the following threshold values: 1) the usual sIgE cut-off value of 0.35 kU_A/L; 2) the “optimal” cut-off yielding the highest Youden index; and 3) the minimal threshold value that provided a specificity $\geq 95\%$.

190 Statistical analysis was performed using the R software version 3.4.1 (www.r-project.org,
191 Vienna, Austria). A *P* value <0.05 was considered significant.

RESULTS

Characteristics of the subjects

Among the 264 subjects included in this analysis, 205 (78%) showed a positive SIC result. Most subjects (81%) were bakers, while 8% were flour mill workers employed in various jobs, 5% bread shop sellers, 4% pastry makers, and 2% pizza makers. The main characteristics of the subjects are summarized in Table I.

Sensitivity, specificity, and predictive values of specific IgE antibodies against wheat and rye

The results of the ROC curves for wheat and rye flour sIgE are detailed in Table II. The “optimal” cut-off values for sIgE against wheat (0.50 kU_A/L) and rye (0.32 kU_A/L) that were associated with the highest Youden index values were very close to the usual cut-off of 0.35 kU_A/L and were not further considered in this analysis. The results of sIgE for wheat and rye showed a high concordance. Only nine subjects had sIgE $\geq$ 0.35 kU_A/L against wheat flour but not against rye flour, and seven demonstrated a positive sIgE level against rye flour only. Using the threshold value of 0.35 kU_A/L, the sensitivities of sIgE to wheat and rye as compared with SIC results were 84% and 85% respectively, but with low NPVs of 56% and 61%, respectively. A sIgE level $\geq$ 0.35 kU_A/L against wheat and rye showed moderate specificities of 71% and 78%, respectively, but yielded high PPVs of 91% and 93%. The minimal cut-off sIgE values that provided a specificity $\geq$ 95% for predicting the development of a positive SIC response were 5.10 kU_A/L for wheat and 6.20 kU_A/L for rye, which yielded PPVs of almost 100%, but with low sensitivities of 40% and 42%, respectively.

We also performed a ROC analysis in two selected subsets of the study population: 1) the subjects who were evaluated in centers where all subjects completed a SIC whatever the results of immunological tests or monitoring of peak expiratory flow rates and 2) the subjects who had a clinical diagnosis of asthma at the time of referral to the tertiary center. The sensitivity, specificity, PPV, and NP of sIgE against wheat and rye in these two subsets of

subjects did not differ from the estimates derived from the whole population (Table E3 in this article's Online Repository).

Characteristics of subjects with a positive SIC but undetectable sIgE against wheat and rye flour

Among the 205 subjects with a positive SIC, 26 (12.7%) failed to show sIgE against wheat and rye. When compared to those who had a positive SIC and detectable sIgE against wheat or rye flour, the 26 subjects with a positive SIC but undetectable sIgE antibodies against wheat and rye reported slightly less often work-related rhinitis and required a longer duration of challenge exposure to flour to elicit an asthmatic reaction during the SIC. These two subsets of subjects did not differ, however, in the duration of work-related asthma symptoms, interval of time elapsed since last workplace exposure at the time of the diagnostic evaluation, or level of baseline NSBH (Table III). Interestingly, subjects with undetectable sIgE against wheat and rye exhibited significantly lower total serum IgE levels and baseline blood and eosinophil counts, and a lesser increase in post-challenge FeNO (Table IV). Their sputum eosinophil count also was lower after challenge exposure to flour, although the median (IQR) post-challenge increase in sputum eosinophils (4.5% [1.0-7.0]) did not differ from the value observed for those with detectable sIgE against wheat and/or rye (6.0% [2.7-13.3]).

Specific IgE antibodies against other bakery allergens

sIgE antibodies against potential bakery allergens other than wheat and rye flour were available in a minority of subjects with the exception of sIgE against fungal alpha-amylase which was measured in 226 (85.6%) of the 264 subjects included in this analysis (Table V). Of note, values ≥ 0.35 kU_A/L for sIgE against alpha-amylase were detected in 27 of 179 (15%) tested subjects with a positive SIC to flour but in none of the 47 subjects with a negative SIC. Thus, a sIgE level ≥ 0.35 kU_A/L against alpha-amylase provided a PPV of 100% but a very low NPV of 24% (Table II). Most subjects (89.4%) with a positive SIC and positive sIgE antibodies

against wheat or rye flour also showed sIgE reactivity to at least one other cereal flour (i.e., barley, oat, corn, and/or rice).

Among the 26 subjects with a positive SIC with flour but undetectable sIgE against wheat and rye, one showed an isolated high sIgE level against alpha-amylase and another subject exhibited significant sIgE levels against cereals other than wheat and rye (i.e., barley, oat, corn, and rice) as well as buckwheat, soybean, and sesame seeds (Table V).

Sensitivity, specificity, and predictive values of nonspecific bronchial hyperresponsiveness

NSBH was absent at baseline in 29 (49%) subjects with a negative SIC and in 53 (26%) of those with a positive SIC. The diagnostic accuracy of NSBH alone or in combination with sIgE against wheat and rye is detailed in Table VI. The assessment of baseline NSBH provided lower sensitivity (74%), specificity (49%), PPV (83%), and NPV (35%) compared with the determination of sIgE against wheat or rye. Using the presence of either baseline NSBH or a sIgE level ≥ 0.35 kU_A/L against wheat or rye in identifying a positive SIC response increased the sensitivity from 84%-85% with sIgE alone to 98% and the NPV from 56%-61% to 80%, but with a lower specificity (34%) and PPV (84%) compared to sIgE alone. On the other hand, combining the presence of NSBH and a positive sIgE result to wheat or rye flour only slightly increased the specificity of sIgE assessment alone from 71%-78% to 85%, but decreased the sensitivity to 63%. Applying this combination of both NSBH and sIgE did not substantially improve the PPV (93%) compared with sIgE determination alone (91%-93%), but further reduced the NPV to 40%.

DISCUSSION

Diagnostic accuracy of sIgE antibodies

This study indicates that a high serum level of sIgE antibodies against wheat and/or rye offers a high PPV for a positive SIC response to flour in subjects evaluated for work-related asthma symptoms. In addition, raising the cut-off value for sIgE antibodies against wheat to ≥ 5.10 kU_A/L or rye to ≥ 6.20 kU_A/L allowed for further increasing the specificity to 95% or more and the PPV to almost 100% (Table II). However, the low NPVs (56%-61%) of sIgE against either wheat or rye indicate that a sIgE concentration below the cut-off value does not allow for ruling out a diagnosis of flour-induced OA without performing further objective testing, including a SIC or the assessment of work-related changes in functional indices (i.e., peak expiratory flow rates, NSBH) and/or in inflammatory markers (i.e., sputum eosinophils, FeNO) (2, 3). Of note, the sensitivity and specificity of sIgE against wheat or rye in our multicenter cohort (Table II) were in line with previous studies reporting sensitivity rates of 62% to 87% and specificities of 68% to 77% using the usual sIgE cut-off value (6, 7). Our findings are also consistent with a systematic review reporting a pooled sensitivity of 78% (95% CI, 68-86%) for sIgE against wheat and rye (8). Remarkably, our data show that the determination of sIgE against the fungal alpha-amylase should be systematically performed because a positive sIgE level for this flour additive was highly specific for a positive SIC result and further increased the likelihood of flour-induced OA.

Although SIC with the suspected occupational agent is still acknowledged as the “reference standard” for diagnosing sensitizer-induced OA (3, 4, 11), this procedure is rarely performed due to the unavailability of appropriate facilities for conducting the tests safely and accurately (4). The findings of this study have substantial clinical implications as they show that the determination of sIgE antibodies against wheat, rye, and alpha-amylase can accurately assist clinicians in a stepwise diagnostic triage approach (3). A high sIgE level against these allergens strongly supports a diagnosis of flour-induced OA and would eliminate the need for performing a SIC or other diagnostic procedures. Establishing the diagnosis of asthma by the

documentation of NSBH (or reversible airflow obstruction) is generally recommended as a key step in the investigation of work-related asthma symptoms because the absence of NSBH allows for reasonably ruling out a diagnosis of OA (2). However, in their large retrospective study Pralong and coworkers (12) have emphasized the importance of measuring NSBH when the subjects are still exposed at work because NSBH may improve rapidly and even return to normal after cessation of exposure to the causal agent. In our cohort, 45% of the subjects were assessed after having been away from the offending exposure for one month or more. The sensitivity (74%) and specificity (49%) of NSBH (Table VI) were lower than those provided by sIgE against wheat and rye, but were consistent with the figures (80% and 47%, respectively) reported by Pralong and coworkers (12). In their study, about half of the subjects were also away from work at the time of NSBH assessment. In our population of workers investigated for possible flour-induced OA in tertiary centers, the assessment of baseline NSBH failed to substantially improve the diagnostic accuracy of sIgE measurement. Nevertheless, combining the presence of either NSBH at baseline or a positive sIgE result to wheat or rye flour increased the sensitivity in identifying flour-induced OA to 98% and the NPV to 80%.

Discordance between a positive SIC and undetectable sIgE against wheat and rye flour

As far as we know, this study represents the first attempt at identifying the subjects' characteristics associated with a positive SIC response to flour despite the absence of detectable sIgE against wheat and rye. In this cohort, 26 (12.7%) of the 205 subjects who developed an asthmatic reaction upon challenge exposure to flour failed to demonstrate serum sIgE reactivity to wheat and rye. Remarkably, these subjects exhibited significantly lower total serum IgE levels and baseline blood and sputum eosinophil counts, and a smaller increase in post-challenge FeNO, characteristics that suggest a low baseline "T_H2 status". Nevertheless, they showed a significant increase in sputum eosinophils after flour exposure (Table IV). This subset also required a significantly longer duration of challenge exposure to flour to elicit an asthmatic reaction compared with those who showed positive sIgE antibodies, likely reflecting an attenuated degree of specific bronchial responsiveness to flour allergens.

Another potential explanation for the absence of detectable sIgE against wheat and rye would be that these subjects were selectively sensitized to cereals other than wheat and rye (i.e. barley, oat, corn, and rice), non-cereal ingredients (i.e., buckwheat, soybean, sesame, and lupine flours), or “improver” enzymes (13). Although sIgE antibodies against these allergens were not systematically available in this retrospective cohort, only one of the 26 subjects without detectable sIgE against wheat and rye demonstrated significant sIgE-mediated sensitization to cereals other than wheat and rye as well as sIgE antibodies against non-cereal flours (Table V). It has been documented that a substantial fraction (from 1% to 23%) of bakers develop IgE-mediated sensitization to “improver” enzymes, predominantly fungal alpha-amylase, that are incorporated into flour to improve bread processing and the quality of the end-products (9, 14-17). However, the contribution of these enzymes to the development of flour-induced OA remains uncertain because the vast majority of bakers sensitized to these enzymes are concomitantly sensitized to wheat (9). In addition, no sIgE assay for these enzymes is commercially available, with the exception of fungal alpha-amylase. In this cohort, sIgE against fungal alpha-amylase was assessed in 87% of the subjects with a positive SIC and were detected in 15% (Table V). Only one subject showed a selective sensitization to alpha-amylase without sIgE antibodies against wheat and rye.

It is also possible that some relevant allergens might be missing in the wheat/rye extracts currently used to assess sIgE, which could explain the absence of detectable sIgE similar to what has been observed for the natural rubber latex allergen *Hev b 5* (18). To date, 28 wheat allergens have been identified and characterized (www.allergen.org, last accessed on February 10, 2024), but none reached the status of a major allergen (19). Nevertheless, Bittner et al. (20) reported positive sIgE binding to at least one of six newly identified wheat allergens in 21 (49%) of 43 subjects with documented bakers' asthma who had negative results with the commercial wheat flour ImmunoCAP. To identify potentially relevant allergens that could improve the sensitivity of current sIgE assays, sIgE reactivity to wheat and rye allergens should be further characterized in the subset of subjects who develop a positive bronchial response

to flour without evidence of sIgE against cereal flour extracts. A prospective comparison between sIgE assays and skin-prick tests with native cereal flours would also be useful to determine whether flour extracts currently used in sIgE assay are lacking relevant allergens.

Several other factors might theoretically account for the observed discordance between the absence of serum sIgE level against wheat and rye and a positive bronchial response to flour. The lower level of total serum IgE in those with undetectable sIgE against wheat and rye may support the hypothesis that in some cases, circulating sIgE levels declined after avoidance of exposure in some subjects. The time-course of sIgE against high-molecular-weight occupational agents has rarely been investigated (21, 22). A study by Lemière et al. (22) suggested that subjects with a short duration of exposure to the sensitizing agent are more likely to have a significant decrease in sIgE against the causal agent after removal from exposure. However, in our study, disease duration and the proportion of subjects removed from workplace exposure at the time of the diagnostic evaluation did not differ by positive versus negative sIgE results. The possibility of “local sIgE responses” to wheat and rye allergens at the level of the target organ without systemic evidence of IgE sensitization (i.e., entopy) (23) would require the determination of sIgE antibodies in sputum samples. Finally, non-IgE-dependent immunological mechanisms might be involved in flour-induced OA. Results from *in vitro* and animal model experiments indicate that protein allergens, especially those with proteolytic activity, can interact directly with epithelial cells and the innate immune system, leading to the stimulation of innate lymphoid cells type 2, the release of type-2 cytokines and eosinophilic airway inflammation (24). However, further investigation using flour allergens would be required to explore this hypothesis.

Limitations of the study

The strengths of this study are the large sample size and its international, multicenter design. However, several potential limitations deserve further consideration.

The diagnostic algorithm for performing a SIC with flour varied between centers (Appendix E1 of this article’s Online repository). However, the vast majority (89%) of the subjects included in

this analysis were recruited from 5 tertiary centers where a SIC with flour was completed by all subjects whatever the results of other tests. The diagnostic accuracy of sIgE against wheat and rye in this subset of the population was similar to the whole population. Nevertheless, this may not exclude a selection bias – though unquantifiable – toward the referral to tertiary centers of subjects with a high clinical suspicion of flour-induced OA. Accordingly, caution should be applied in interpreting the predictive values of sIgE against wheat and rye because these estimates were obtained in a population with a high suspicion of flour-induced OA and a high prevalence (i.e., pre-test probability) of positive SIC results (78%). However, our data suggest that using high threshold values of sIgE antibodies against wheat and rye (Table II) may allow for increasing the specificity and PPV for diagnosing flour-induced OA.

Specific IgE antibodies were assayed in each center at the time of the SIC and the between-laboratory agreement could not be assessed due to the retrospective design of the study. However, the same technique and reagents were used in each center (ImmunoCAP, Thermo Fisher Scientific [Phadia], Uppsala, Sweden).

Another potential limitation of this retrospective study is the lack of a systematic assessment of sIgE against all potential bakery allergens. Nevertheless, our data suggest that IgE sensitization to allergens other than wheat/rye and alpha-amylase that could account for a positive SIC with flour is a rare occurrence (i.e. only one out of 26 subjects). In addition, those with a positive SIC to flour in the absence of detectable sIgE against wheat and rye shared common airway inflammation biomarkers characteristics suggesting a low “T_H2 status”, although biomarkers data were available in only a small fraction of the cohort. An important limitation resulted from our inability to reliably compare sIgE data with the results of skin-prick tests since these tests were performed using various commercially available flour extracts and/or with native flour. However, there is a lack of standardization of allergen contents of commercially available flour extracts (25).

Conclusion

The findings indicate that a high serum level of sIgE antibodies against wheat and/or rye and/or fungal alpha-amylase strongly supports a diagnosis of flour-induced OA without the need for performing a SIC. Nevertheless, the level of sIgE antibodies against these allergens has a low NPV, so that a negative sIgE result does not allow for ruling out flour-induced OA. In tertiary care clinics, the determination of sIgE against wheat, rye and fungal alpha-amylase may therefore assist clinicians in identifying subjects who need additional investigations before establishing or excluding a diagnosis of flour-induced asthma. Despite the limitations inherent to the retrospective design of the study, the data indicate that flour-induced OA may be identified by a positive SIC in the absence of detectable sIgE against wheat and flour in subjects with a low T_H2 status.

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483 **Table I. Baseline characteristics of subjects with positive compared to negative**
 484 **inhalation challenge with flour**

Characteristic	Missing values	Negative SIC with flour (n=59)	Positive SIC with flour (n=205)	P value
Age, yr	0/0	39 (28-45)	39 (31-48)	0.407
Sex, male, n (%)	0/0	41 (69.5)	181 (88.3)	<0.001
Body mass index, kg/m ²	0/2	25.4 (22.2-29.7)	27.0 (23.9-29.4)	0.207
Smoking status:	1/2			0.057
Current smoker, n (%)		21 (36.2)	43 (21.2)	
Ex-smoker, n (%)		12 (20.7)	45 (22.2)	
Never-smoker, n (%)		25 (43.1)	115 (56.6)	
Atopy, n (%) [*]	0/0	38 (64.4)	130 (63.4)	0.889
History of asthma preexisting to flour exposure, n (%)	0/0	9 (15.2)	27 (13.2)	0.681
Duration of exposure before onset of work-related asthma symptoms, mo	2/1	81 (18-227)	108 (58-204)	0.119
Duration of work-related asthma symptoms at work, mo	1/2	21 (9-42)	35 (16-72)	0.002
Removal from exposure for more than 1 mo at the time of the SIC, n (%)	1/2	21 (36.2)	98 (48.3)	0.104
Treatment with inhaled corticosteroid, n (%)	0/2	34 (57.6)	138 (68.0)	0.140
Inhaled corticosteroid daily dose, µg [†]	0/2	400 (0-1000)	500 (0-1000)	0.155
Use of a short-acting beta ₂ -agonist, n (%)	0/2	40 (67.8)	140 (69.0)	0.865
Short-acting beta ₂ -agonist ≥1 once a day, n (%)	3/3	18 (32.1)	74 (36.6)	0.535
Clinical diagnosis of asthma at the time of the SIC [‡]	0/2	43 (72.9)	174 (85.7)	0.0214
Coexisting conditions:				
Work-related rhinitis, n (%)	0/0	51 (86.4)	189 (92.2)	0.176
Work-related urticaria, n (%)	0/0	5 (8.5)	15 (7.3)	0.782
Work-related contact dermatitis, n (%)	0/0	15 (25.4)	27 (13.2)	0.023
Baseline spirometry:				
FVC, % pred	0/0	98 (92-104)	100 (92-110)	0.150
FEV ₁ , % pred	0/0	90 (81-100)	90 (79-98)	0.710
FEV ₁ /FVC, %	1/0	78 (68-83)	75 (67-79)	0.008
FEV ₁ /FVC <0.70, n (%)	1/0	17 (29.31%)	63 (30.73%)	0.835
Baseline level of NSBH [§] :	0/4			0.004
Absent, n (%)		29 (49.2)	53 (26.4)	
Mild, n (%)		17 (28.8)	81 (40.3)	
Moderate-to-severe, n (%)		13 (22.0)	67 (33.3)	
Serum total IgE level, kU/L	7/74	102 (56-332)	179 (82-453)	0.159
Blood eosinophils, cells/µL	8/32	211 (121-345)	265 (158-410)	0.137
Baseline FeNO, ppb	31/96	14 (11-24)	25 (15-41)	0.009
Baseline sputum eosinophils, %	39/111	1.0 (0.9-2.2)	1.6 (1.0-4.0)	0.263
Baseline sputum neutrophils, %	39/111	58.5 (42.9-72.1)	60.4 (41.5-78.8)	0.576

485 **Legend:** Data are presented as n (%) for proportions and median (interquartile range) for continuous variables. *FeNO*,
 486 fractional exhaled nitric oxide; *FEV₁*, forced expiratory volume in one second; *FVC*, forced vital capacity; *NSBH*, nonspecific
 487 bronchial hyperresponsiveness; *SIC*, specific inhalation challenge with flour. *P* values in boldface are statistically significant.

488 ^{*} Atopy defined by the presence of a positive skin-prick test to at least one common allergen.

489 [†] Daily dose of inhaled corticosteroid expressed as µg beclomethasone dipropionate equivalent.

490 [‡] Clinical diagnosis of asthma at the time of referral for the investigation of possible flour-induced occupational asthma
 491 through SICs in tertiary centers defined by the use of any asthma medication.

492 [§] See Appendix E2 and Table E2 of this article's Online Repository for the threshold values used for grading the level of
 493 NSBH.

494

Table II. Sensitivity, specificity, and predictive values of specific IgE levels in predicting a positive inhalation challenge with flour

	Cut-off sIgE value (kU _A /L)	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Youden Index % (95% CI)
Wheat sIgE	Usual: ≥ 0.35	84 (78-89)	71 (58-82)	91 (86-95)	56 (44-67)	55 (49-61)
	Optimal: $\geq 0.50^*$	82 (76-87)	78 (65-88)	93 (88-96)	55 (44-66)	60 (54-66)
	Specificity $\geq 95\%$: $\geq 5.10^\dagger$	40 (33-47)	97 (88-100)	98 (92-100)	32 (25-39)	37 (31-42)
Rye sIgE	Usual: ≥ 0.35	85 (80-90)	78 (65-88)	93 (88-96)	61 (49-72)	63 (57-69)
	Optimal: $\geq 0.32^*$	86 (81-91)	78 (65-88)	93 (89-96)	62 (50-73)	64 (58-70)
	Specificity $\geq 95\%$: $\geq 6.20^\dagger$	42 (36-50)	97 (88-100)	98 (92-100)	33 (26-40)	33 (27-39)
Wheat OR rye sIgE	Usual: ≥ 0.35	87 (82-92)	69 (56-81)	91 (86-94)	61 (49-73)	56 (49-63)
α -amylase sIgE	Usual: ≥ 0.35	13 (9-19)	100 (94-100)	100 (87-100)	24 (20-31)	32 (26-38)

Legend: NPV, negative predictive value; PPV, positive predictive value; sIgE, specific IgE antibodies. The area under the ROC curve was 0.85 (95% CI: 0.81-0.90) for wheat-sIgE and 0.86 (95% CI: 0.82-0.91) for rye-sIgE.

* Optimal cut-off value based on the highest Youden Index (i.e., sensitivity + specificity – 1) derived from the receiver operating characteristics (ROC) curve.

† Minimal sIgE cut-off value providing a specificity $\geq 95\%$ for a positive inhalation challenge with flour derived from the receiver operating characteristics (ROC) curve.

504 **Table III. Clinical and functional characteristics of subjects with a positive inhalation**
 505 **challenge with flour but undetectable sIgE antibodies to wheat and rye**

Characteristic	Missing values	Positive SIC with flour		P value
		Without sIgE to wheat and rye (n=26)	With sIgE to wheat and rye (n=179)	
Age, yr	0/0	42 (34-52)	38 (31-47)	0.144
Sex, male, n (%)	0/0	22 (84.6)	159 (88.8)	0.517
Body mass index, kg/m ²	0/2	27 (25-30)	27 (24-29)	0.654
Smoking habits:	0/2			0.382
Current smoker, n (%)		4 (15.4)	39 (22.0)	
Ex-smoker, n (%)		4 (15.4)	41 (23.2)	
Never-smoker, n (%)		18 (69.2)	97 (54.8)	
Atopy, n (%) [*]	0/0	20 (76.9)	110 (61.4)	0.126
History of asthma preexisting to flour exposure, n (%)	0/0	5 (19.2)	22 (12.3)	0.351
Duration of exposure before onset of work-related asthma symptoms, mo	0/1	120 (48-197)	108 (58-204)	0.831
Duration of work-related asthma symptoms at work, mo	0/2	49 (21-112)	32 (16-66)	0.258
Removal from exposure for more than 1 mo at the time of the SIC	0/2	10 (38.5)	60 (33.5)	0.355
Inhaled corticosteroid use, n (%)	0/2	17 (65.4)	121 (68.4)	0.761
Daily dose of inhaled corticosteroid, µg [†]	0/2	500 (0-1,000)	500 (0-1,000)	0.406
Inhaled short-acting β ₂ -agonist use, n (%)	0/2	18 (69.2)	122 (68.9)	0.975
Inhaled short-acting β ₂ -agonist ≥1 once a day	0/3	8 (30.8)	66 (37.5)	0.506
Clinical diagnosis of asthma at the time of the SIC [‡]	0/2	21 (80.8)	153 (86.4)	0.5462
Coexisting conditions:				
Work-related rhinitis, n (%)	0/0	21 (80.8)	168 (93.8)	0.036
Work-related urticaria, n (%)	0/0	0	15 (8.4)	0.225
Work-related contact dermatitis, n (%)	0/0	3 (11.5)	24 (13.4)	0.999
Baseline spirometry:	0/0			
FVC, % pred		100 (90-110)	100 (93-110)	0.896
FEV ₁ , % pred		82 (77-94)	91 (80-98)	0.134
FEV ₁ /FVC, %		72 (65-77)	75 (68-79)	0.121
FEV ₁ /FVC <0.70, n (%)		12 (46.1)	51 (28.5)	0.068
Baseline level of NSBH [§] :	1/3			
Absent n (%)		5 (20.0)	48 (27.3)	0.663
Mild, n (%)		10 (40.0)	71 (40.3)	
Moderate-to-severe, n (%)		10 (40.0)	57 (32.4)	
Post-challenge change in NSBH:	12/62			
Pre/post-SIC NSBH ratio		1.6 (1.3-2.0)	1.88 (1.0-4.0)	0.660
Pre/post-SIC NSBH ratio ≥2, n (%)		4 (28.6)	57 (48.7)	0.153
Duration of exposure to flour during the SIC, min	0/0	60 (45-143)	20 (5-60)	<0.001
Pattern of bronchial response to SIC:				0.154
Isolated early, n (%)		14 (53.8)	111 (62.)	
Isolated late, n (%)		2 (7.7)	6 (3.4)	
Dual, n (%)		8 (30.8)	59 (33.0)	
Occupational eosinophilic bronchitis [¥] , n (%)		2 (7.7)	3 (1.7)	

506 **Legend:** Data are presented as n (%) for proportions and median (interquartile range) for continuous variables. FEV₁, forced
 507 expiratory volume in one second; FVC, forced vital capacity; NSBH, nonspecific bronchial hyperresponsiveness; SIC, specific
 508 inhalation challenge with flour. Values in boldface are statistically significant.

- 509 * Atopy defined by the presence of a positive skin-prick test to at least one common allergen.
510 † Daily dose of inhaled corticosteroid expressed as μg beclomethasone dipropionate equivalent.
511 ‡ Clinical diagnosis of asthma at the time of referral for the investigation of possible flour-induced occupational asthma
512 through SICs in tertiary centers defined by the use of any asthma medication.
513 § See Appendix E2 and Table E2 of this article's Online Repository for the threshold values used for grading the level of
514 NSBH.
515 ¶ Duration of exposure to flour required to elicit a positive SIC response.
516 ¥ Defined by a $\geq 3\%$ increase in sputum eosinophil count in the absence of changes in FEV_1 after challenge exposure to flour
517 and absence of NSBH both at baseline and after challenge exposure to flour.
518

Table IV. Inflammatory biomarkers in subjects with a positive inhalation challenge with flour and undetectable sIgE antibodies to wheat and rye

Characteristic	Positive SIC with flour		P value
	Without sIgE to wheat and rye (n=26)	With sIgE to wheat and/or rye (n=179)	
Serum total IgE level, kU/L	(n=18)	(n=113)	
	91 (48-203)	192 (86- 462)	0.027
Blood eosinophils, cells/ μ L	(n=19)	(n=154)	
	158 (54-200)	292 (170-418)	<0.001
Baseline FeNO, ppb	(n=10)	(n=99)	
	23 (18-31)	26.00 (15-42)	0.733
Post-SIC change in FeNO, ppb	(n=8)	(n=86)	
	5 (2-18)	28 (7-50)	0.030
Baseline sputum eosinophils:	(n=9)	(n=72)	
%	1.0 (0-1.5)	2.0 (1.0-5.3)	0.016
Post-challenge sputum eosinophils:	(n=9)	(n=72)	
%	4.5 (1.0-8.0)*	12.2 (5.4-19.0) [†]	0.026
Post-SIC change, %	4.5 (1.0-7.0)*	6.0 (2.7-13.3) [†]	0.161
Baseline sputum neutrophils:	(n=10)	(n=84)	
%	54.0 (46.2-66.2)	60.8 (40.8-79.0)	0.672
Post-challenge sputum neutrophils:	(n=9)	(n=74)	
%	57.0 (44.0-67.0)	55.7 (35.0-68.5)	0.612

Legend: Data are presented as median (interquartile range). *FeNO*, fractional exhaled nitric oxide; *SIC*, specific inhalation challenge with flour. Values in boldface are statistically significant.

* Comparison between pre- and post-challenge values: $P=0.022$ using the Wilcoxon signed-rank test for paired samples).

[†] Comparison between pre- and post-challenge values: $P<0.001$ using the Wilcoxon signed-rank test for paired samples).

526 **Table V. sIgE antibodies to bakery allergens other than wheat and rye.**

Allergen	Subjects with a positive SIC with flour		Subjects with a negative SIC with flour (n=59) (No. positive sIgE/no. tested)
	Without sIgE to wheat and rye (n=26) (No. positive sIgE/no. tested)	With sIgE to wheat/rye (n=179) (No. positive sIgE/no. tested)	
Alpha-amylase	1/20 (5.0)*	26/159 (16.4)	0/47
Other cereals:			
Barley	1/23 (4.3)	131/145 (90.3)	7/47 (14.9)
Oat	1/20 (10.0)	88/118 (74.6)	5/33 (15.2)
Corn	1/8 (12.5)	57/93 (61.3)	3/29 (10.3)
Rice	1/8 (12.5)	41/65 (63.1)	12/55 (21.8)
At least one other cereal	1/23 (4.3) [†]	135/151 (89.4)	11/54 (20.4)
Buckwheat	1/12 (8.3) [†]	33/88 (37.5)	6/34 (17.6)
Soybean	1/12 (8.3) [†]	46/108 (42.6)	4/40 (10.0)
Sesame seeds	1/10 (10.0) [†]	44/88 (50.0)	7/29 (24.1)
Lupine	0/0	1/3 (33.3)	0/1
Storage mites			
<i>Acarus siro</i>	4/13 (30.8)	33/90 (36.7)	15/39 (38.5)
<i>Lepidoglyphus destructor</i>	5/14 (35.7)	29/92 (31.5)	14/37 (37.8)
<i>Tyrophagus putrescentiae</i>	5/14 (35.7)	37/85 (43.5)	11/35 (31.4)
<i>Glycyphagus domesticus</i>	4/9 (44.4)	27/77 (35.1)	18/39 (46.2)
At least one storage mite	5/15 (33.3) [‡]	47/99 (47.5)	17/38 (44.7)
House dust mites:			
<i>Dermatophagoïdes pteronyssinus</i>	5/12 (41.7)	60/121 (49.6)	24/44 (54.5)
<i>Dermatophagoïdes farinae</i>	4/12 (33.3)	46/92 (50.0)	22/38 (57.9)

527 **Legend:** Data are presented as numbers and % of available data. SIC, specific inhalation challenge with flour.

528 * One subject showed a high sIgE level to alpha-amylase alone.

529 [†] One subject exhibited significant sIgE levels (>0.60 kU_A/L) against cereals other than wheat and rye (i.e., barley, oat, corn,
530 and rice) as well as buckwheat, soybean, and sesame seeds.

531 [‡] Five subjects demonstrated sIgE antibodies against at least one storage mite; all of them were also sensitized to house
532 dust mites.

533

Table VI. Sensitivity, specificity, and predictive values of baseline nonspecific bronchial hyperresponsiveness in predicting a positive inhalation challenge with flour

	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Youden Index % (95% CI)
Baseline NSBH	74 (67-80)	49 (36-62)	83 (77-88)	35 (25-46)	23 (20-26)
Wheat sIgE*	84 (78-89)	71 (58-82)	91 (86-95)	56 (44-67)	55 (49-61)
Rye sIgE*	85 (80-90)	78 (65-88)	93 (88-96)	61 (49-72)	63 (57-69)
NSBH or [sIgE against wheat or rye]*	98 (94-99)	34 (22-47)	84 (78-88)	80 (59-93)	32 (18-28)
NSBH and [sIgE against wheat or rye]*	63 (56-70)	85 (73-93)	93 (88-97)	40 (32-50)	48 (42-54)

Legend: 95% CI, 95% confidence interval; NPV, negative predictive value; NSBH, presence of nonspecific bronchial hyperresponsiveness; PPV, positive predictive value; sIgE, specific IgE antibodies.

* Using the usual cut-off sIgE ≥ 0.35 kU_A/L