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ORIGINAL ARTICLE

Impact of cross-reactive carbohydrate determinants on wood dust sensitization

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Clinical & Experimental Allergy

Summary

Background Occupational wood dust exposure can induce allergy and may be one cause of respiratory health problems among woodworkers.

Objective The objective was to determine the prevalence and quantitative level of specific immunoglobulin E (sIgE) to beech and pine wood in exposed workers. Wood sensitization was specified with regard to cross-reactivity and was correlated to the reported symptoms.

Methods Danish workers ($n = 701$) were investigated for sIgE to beech and pine. Wood samples from workplaces were analysed and coupled to ImmunoCAPs. Workers sensitized to wood were tested for cross-reactive carbohydrate determinants (CCDs) and environmental allergens. IgE binding was specified for glycogenic vs. proteinogenic epitopes by inhibition tests.

Results The prevalence of wood sensitization among all workers was 3.7%. There was no association between sensitization prevalence or sIgE concentrations and self-reported allergic symptoms. Beech- and pine-sensitized workers showed a high prevalence of CCD sensitization (73%). However, workers with a single sensitization to wood had no sIgE to CCDs. Specifying IgE epitopes demonstrated that sera of workers reporting allergic symptoms recognized proteinogenic IgE-epitopes on wood allergens, whereas workers without allergic symptoms had primarily sIgE-epitopes to glycogenic structures. Although 96% of the wood-sensitized workers were atopic, no significant correlation was found between wood sensitization and sIgE to beech and birch pollen, but an association was found between sIgE against CCDs and pine pollen.

Conclusion Sensitization prevalence to beech and pine wood measured by tailored ImmunoCAPs was not correlated to allergic symptoms. We recommend the application of CCD tools to assess the relevance of individual wood sensitization.

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Introduction

More than three million workers in the European Union are occupationally exposed to wood dust [1]. Since 1995, wood dust is classified as a human carcinogen by the International Agency for Research on Cancer. Additionally, numerous epidemiological studies have identified wood dust as a risk factor for asthma or asthma-like symptoms [2–14] and nasal impairment diseases [4, 8, 9, 12], consistent with both an allergic and a non-allergic origin.

IgE-mediated sensitization and wood dust exposure measurements have been investigated intensively for western red cedar [2,13,14], obeche [15–20] and pine wood [21,22]. In western red cedar, an association between sIgE to plicatic acid [a non-proteinogenic, low-molecular-weight (LMW) substance of 440 Da] and occupational asthma was shown [13, 14]. In fact the clinical relevance of an IgE-mediated way of respiratory diseases especially by western red cedar-exposed subjects is discussed controversely [23].

Contrary to LMW substances from western red cedar were proteins described as allergens in the tropical obeche wood elucidating occupational asthma [15,17,19,20]. The major allergen was identified as class I chitinase of 38 kDa

[19] along with other IgE-binding proteins of 28, 20 and 14 kDa [15, 20]. In pine wood, proteins were identified as allergens [21] with molecular weights of 27 and 29 kDa and in the range of 43–59 kDa. However, only a weak association was observed between sIgE-mediated sensitization to pine wood dust and respiratory effects based on occupational wood dust exposure in pine wood-processing workers [21, 22].

Hence, no 'universal' wood allergen exists that could be used as a screening tool to study wood dust sensitization; individual wood allergen extracts must be prepared and tested for each wood species. Commercially available wood dust extracts are rare and standardization of wood allergen extract is difficult. Factors such as growing conditions and geographical origin as well as drying and storage strategies may influence the proteinogenic allergen content of the prepared allergen extracts. Because of these limitations, it is inherently complicated to measure sIgE-mediated wood sensitization. From recent publications based on cross-reactive carbohydrate determinants (CCDs), it is known that *in vitro* tests, in particular, to plant allergens should be re-investigated for clinically irrelevant results [24]. The term CCD includes an asparagine-linked carbohydrate moiety, which are the most abundant environmental immune determinants [25]. About 20% of allergic patients contain sIgE to plant glycans but as shown recently without clinical relevance [26, 27].

The purpose of our study was to determine the prevalence of beech and pine wood sIgE sensitization among a random sample of woodworkers and controls using characterized wood dust extracts. We further tried to clarify the association between the reported symptoms and wood dust sensitization as well as to discriminate between proteinogenic and glycogenic sIgE binding to pine and beech wood allergens. Additionally, sensitization to relevant environmental allergen mixture was analysed and taken into account.

Methods

Woodworker collective

A total of 701 serum samples were selected from two Danish studies [28] to investigate IgE-mediated sensitization to pine and/or beech wood dust.

The study population is described in detail elsewhere [10, 29]; but in brief, the 1997 Study included 54 furniture factories situated in Viborg County. The study population was workers employed in the woodworking, assembly or stock departments. Additionally, three factories (two producing refrigerators and one producing hearing aids) in the same area were selected as reference. The 2003 Study included 52 furniture factories, where 38 factories also participated in the 1997 Study. Additionally, two of the three references were included. Totally 1377 (68%) woodworkers and 297 (63%) references participated in both 1997 and 2003, together with 1104 only participating in 2003 (78% participation rate). From this population of 2481 woodworkers and 297 references, we collected a blood sample from 1506 woodworkers and 195 references. The reason for not having a blood sample drawn was due to closing of the factory ($n = 155$), absence on the day of the blood sampling ($n = 646$) or refusing to have a blood sample drawn ($n = 276$).

From the pool of 1701 blood samples, a random sample of 399 subjects, a random sample of 100 subjects suffering from rhinitis and all 298 persons with asthma symptoms were collected for further analysis of specific IgE (sIgE) to pine and beech dust.

Asthma symptoms were defined if the subject answered yes to at least one group A question (physician-diagnosed asthma, current asthma, ever had asthma or ever wheezed) and two or more group B questions (use of asthma drugs, ever had chest tightness, ever wake up with chest tightness, wake up wheezing, morning cough, wake up coughing, wheeze when exposed to cold air or pollen or animals or wheeze when exercise) or at least two group A questions [30].

Rhinitis was defined as answering yes to at least two nasal symptoms (rhinorrhoea, nasal itching, nasal congestion or sneezing) at least 2 days a week during the last year.

Wood dust extract preparation

Two workplace samples of each pine and beech wood, stored between 0.5 and 2.5 years and dried under different conditions until the water content was between 8% and 18%, were sawed to dust with a circular saw, collected and stored for 2 weeks at 4 °C.

Wood dust was mixed 1 : 12.5 (w/v) with phosphate-buffered saline (PBS; pH 7.4) containing 0.5 mM Pefablock (Serva, Heidelberg, Germany) and 2% polyvinylpolypyrrolidone (PVPP; Sigma, Deisenhofen, Germany) and was gently stirred for 6 h at 4 °C, centrifuged (12.000 g, 4 °C, 45 min) and the supernatant containing soluble wood dust proteins was dialysed against distilled water (Spektra/Por®, 3.5 kDa MWCO, Serva) for 38 h. Remaining wood dust pellets were solubilized with 50% EtOH in PBS 1 : 10 (w/v) and stirred again at 4 °C overnight. Ethanolic supernatant was dialysed for 22 h as described above. Aqueous and ethanolic extracts were completely pooled and lyophilized. Protein concentration was quantified with Biorad protein assay® (Biorad, Munich, Germany) using albumin bovine fraction V (Serva) as a standard.

Electrophoresis

Wood extract proteins were separated by SDS-PAGE using pre-cast NuPAGE gels (Novex, San Diego, CA, USA) in a

Novex Xcell II mini-cell unit according to the manufacturer's instructions. Silver staining of proteins was performed with small modifications as described by Blum et al. [31].

Biotinylation and coupling of wood dust proteins to Streptavidin-ImmunoCAPs

Wood dust extracts were biotinylated for 2 h with a fivefold molar excess of D-biotinoyl-ε-aminocaproic acid *N*-hydroxysuccinimide ester (NHS-Biotin) in 10 mM carbonate buffer as described by Sander et al. [32], assuming an average molecular weight of 26 kDa for beech and 36 kDa for pine wood proteins. The final protein concentration for biotinylation was calculated for beech as 0.5 mg protein/mL and for pine as 1 mg protein/mL. Biotinylated wood extracts (50 μL) were loaded on Streptavidin-ImmunoCAPs (o221, Phadia, Uppsala, Sweden), as described by Sander et al. [32]. Quality control was performed with sera of pine and beech wood-sensitized workers as a positive control and sera of non-sensitized woodworkers and ImmunoCAP diluent as negative controls.

Determination of specific immunoglobulin E

All 701 sera were tested for sIgE to pine and beech wood extracts with ImmunoCAP 250 (Phadia) using the specific tailored ImmunoCAPs. Samples with sIgE $\geqslant 0.35\ kU_A/L$ to any wood allergen were additionally tested for sIgE to the three commercially available CCDs, horseradish peroxidase (HRP; Ro400), bromelain (k202) and $MUXF^3$ (Ro214), as well as to an inhalative atopy screening test mixture (sx1; containing house dust mite, cat dander/hair, dog dander, *Cladosporium herbarum*, pollen of timothy, rye grass, birch and mugwort) and to beech (t5), pine (t213) and birch (t3) pollen. Allergen-sIgE values $\geqslant 0.35\ kU_A/L$ were again considered positive.

ImmunoCAP inhibition

To differentiate between proteinogenic and glycogenic sIgE epitopes, sIgE binding to beech and/or pine wood was analysed in a competitive inhibition assay with the glycogenic substance HRP as an inhibitor. HRP served as a proxy for CCDs. Each serum sample (50 μL) with an sIgE concentration to beech and pine $> 0.50\ kU_A/L$ was mixed with 10 μL of HRP solution (4.5 μg/μL) and incubated for 1 h at room temperature. Subsequently, sIgE binding was measured in the wood dust solid phase. As a positive control (100% binding, 0% inhibition), the same volume of PBS instead of HRP solution was taken. The reduction of IgE binding to wood allergen induced by HRP incubation was calculated as the difference between PBS control and HRP inhibition.

Statistical analysis

sIgE values were presented as mean value±standard deviation. For a correlation between sIgE results of different allergens, the squared Pearson correlation coefficients (r^2) of the log transformed values were calculated. Analysis of data (ANOVA) was performed as two-tailed Tukey's multiple comparison test with GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA). To avoid 'multiple testing artefacts,' *P*-values < 0.001 were stated as significant.

Results

Analysis of wood dust allergen profile and selection of suitable test allergens

The protein contents of two beech (B) and two pine (P) wood samples extracted under aqueous (a) and ethanolic (e) conditions were analysed by SDS-PAGE (Fig. 1). Protein patterns showed conspicuous differences. In beech wood samples Ba1 and Be1 the protein profile had dominant protein bands at 7, 8, 12, 16, 28 and 35 kDa, whereas in beech wood samples, Ba2 and Be2 only faint protein bands were detectable at 12 and 28 kDa. Similar results were observed for pine wood. Pine wood Pa2 and Pe2 exhibited protein bands in the range of 7, 24, 28 and 40 kDa, while in pine wood samples Pa1 and Pe1, no protein was detectable. For ImmunoCAP coupling, the total fraction of aqueous and ethanolic beech wood extract 1 (Ba1 and Be1) and pine wood extract 2 (Pa2 and Pe2) were pooled.

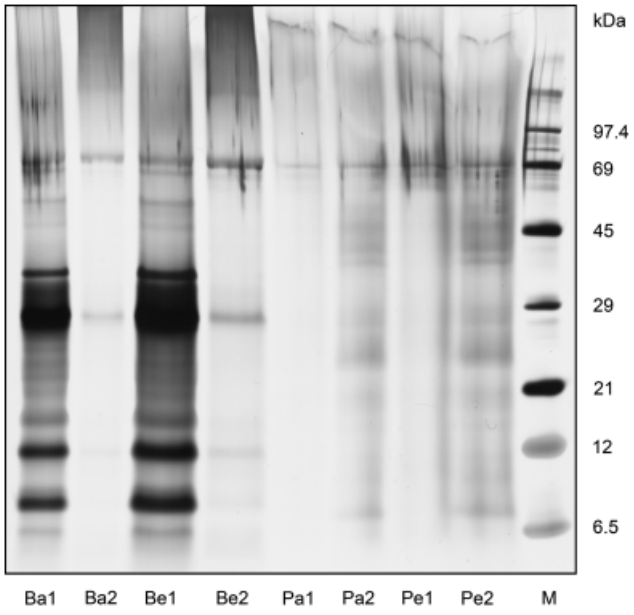

Fig. 1. SDS-PAGE silver stain of beech (B) and pine (P) wood samples from two different workplaces [1, 2], extracted under aqueous conditions (a) and subsequently with 50% ethanol (e). M, molecular weight standard.

Prevalence and quantitative levels of specific immunoglobulin E levels to beech and pine wood dust

sIgE concentrations $\geqslant 0.35\,kU_A/L$ to beech and/or pine wood dust were measured in 26 (3.7%) of the 701 sera (Table 1) using the tailored ImmunoCAP. Twenty-three of 26 were positive for beech wood, and 18 of 26 had sIgE to pine wood. Sensitization according to the reported symptoms was distributed as follows: in the group with asthma symptoms (A), five of 176 (2.8%) workers had sIgE to beech and/or pine wood; in the group with rhinitis symptoms (R), five of 127 (3.9%); in the group reporting asthma and rhinitis (A+R), six of 122 (4.9%); and in the group without reported symptoms (non-sym), 10 of 276 (3.6%).

Regarding the quantitative level of sIgE to beech or pine wood in all wood-sensitized workers (Table 1), the mean sIgE concentration for beech wood was 2.2 and $1.5\,kU_A/L$ for pine wood. No significant differences were observed between the symptomatic groups with regard to sIgE concentration for pine or beech.

Prevalence and quantitative specific immunoglobulin E levels to cross-reactive carbohydrate determinants in wood dust-sensitized workers

sIgE to the three CCD tools, HRP, bromelain and MUXF^3 was detected in 19 of 26 wood dust sIgE-positive sera (Table 2). The prevalence of glycogenic epitope recognition was 73% accordingly. Sensitization prevalence to CCDs was comparable across all groups and the quantitative level of sIgE to each of the three CCDs was always higher than the wood dust sIgE levels (Table 2). sIgE to HRP and bromelain were measured in 18 of all 26 serum samples (69%). In 17 of these sera, sIgE to both CCDs, HRP and bromelain was present. Additionally an sIgE response was measured exclusively to one CCD component, in one serum to bromelain and in another serum to HRP.

sIgE to the purified glycan structure MUXF^3 was measured only in 15 of 26 sera.

Table 1. Wood dust-specific IgE responses in woodworkers

Group	Number of wood positives*	Number Mean value±SD sIgE* to beech wood	Number Mean value±SD sIgE* to pine wood
All	26 (3.7%)	23	18
(n = 701)		2.2±2.7 kU_A/L	1.5±3.4 kU_A/L
A	5 (2.8%)	5	4
(n = 176)		2.7±3.9 kU_A/L	1.4±1.3 kU_A/L
R	5 (3.9%)	4	5
(n=127)		1.8±2.2 kU_A/L	1.2±0.8 kU_A/L
A+R	6 (4.9%)	4	5
(n=122)		1.8±1.1 kU_A/L	1.0±0.6 kU_A/L
Non-sym	10 (3.6%)	10	4
(n=276)		2.3±3.1 kU_A/L	2.4±3.4 kU_A/L

*sIgE $\geqslant 0.35\,kU_A/L$.
SD, standard deviation.

Specification of immunoglobulin E binding to wood allergens by ImmunoCAP inhibition

Analysis of the IgE profiles of sensitized workers showed that 15 out of 26 (58%) were double sensitized to beech and pine wood (Table 3). Additional sIgE to HRP was found in 14 of these workers. Seven workers were mono-sensitized to beech wood (Table 3, serum no. 11, 14, 17, 21, 23, 24 and 26), and three were mono-sensitized to pine wood (Table 3, serum no. 5, 9 and 15). In contrast to sera of mono-sensitized workers with allergic symptoms, which did not recognize HRP, only beech or pine wood-sensitized workers of the non-symptomatic group had additional sIgE to HRP (Table 3, serum no. 20, 23, 24 and 26).

To assess whether sIgE binding was conducted by proteinogenic or by glycogenic epitopes, inhibition tests with HRP as a CCD component were performed in 13 sera. In nine of 13 samples, the IgE binding to beech wood (Table 3) was reduced >80% by HRP, indicating that the sIgE binding in these samples was completely based on glycogenic structures. Intermediate inhibition of 53% and 63%, respectively, was measured in two sera (Table 3, sample no. 1 and 7). Both sera contained probable proteinogenic as well as glycogenic IgE epitopes which result in a notable but not complete reduction of sIgE binding. In two additional sera (Table 3, sample no. 3 and 12), no inhibition by HRP pre-incubation was measured, indicating that the IgE-mediated binding was exclusively based on proteinogenic epitopes. In all three sera of the non-symptomatic group, sIgE binding to beech was completely inhibited by HRP.

Similar results were obtained for pine wood allergens (Table 3). Complete reduction of sIgE binding by HRP was observed in eight of 13 tested sera (62%); these were classified as 'predominantly glycogenic epitope recognition'. Intermediate inhibitions of 27%, 64% and 33%, respectively, were measured in three sera (Table 3, samples 1, 4 and 7), indicating a mixture of glycogenic and proteinogenic epitopes. sIgE binding in samples 3 and 12 was again not affected by HRP inhibition. Likewise, in beech wood, in all three sera (no. 23, 24 and 26) of the non-symptomatic group, sIgE binding to pine was completely inhibited by HRP.

Prevalence and quantitative specific immunoglobulin E levels to environmental and pollen allergens in wood dust-sensitized workers

To investigate further potential sensitizations, wood dust-positive samples were tested on beech, pine and birch

Table 2. CCD-, sx1- and pollen-specific IgE responses in wood dust-sensitized workers

	Number Mean value±SD (kU_A/L)						
Group	sIgE* to HRP	sIgE* to bromelain	sIgE* to $MUXF^3$	sIgE* to sx1	sIgE* to beech pollen	sIgE* to pine pollen	sIgE* to birch pollen
All	18	18	15	25	24	16	22
($n = 26$)†	5.2±6.7	4.5±6.8	4.4±6.6	23.2±25.7	8.7±12.6	4.0±5.6	14.2±24.3
A	4	3	3	4	4	2	4
($n = 5$)†	3.4±3.8	2.7±2.9	0.9±0.5	13.6±9.2	2.8±3.6	1.1±0.9	7.3±9.6
R	3	3	3	5	5	3	5
($n = 5$)†	3.3±3.5	3.5±3.9	2.8±2.8	4.9±3.6	3.0±2.9	3.1±2.4	2.6±1.2
A+R	3	4	3	6	6	3	6
($n = 6$)†	8.5±11.9	7.2±12.2	7.7±11.7	57.6±20.1	23.6±20.1	7.4±10.5	34.6±38.3
Non-sym	8	8	6	10	9	8	7
($n = 10$)†	5.5±7.1	4.5±6.1	6.1±6.8	9.9±11.3	6.3±7.4	3.8±5.0	8.7±14.3

*sIgE $\geqslant 0.35\ kU_A/L$,

†sIgE to pine and/or beech wood $\geqslant 0.35\ kU_A/L$, SD: standard deviation, CCD: cross-reactive carbohydrate determinant, HRP, horseradish peroxidase; $MUXF^3$, purified glycan structure of bromelain; sx1, inhalative atopy screening test 1, including allergens from house dust mite, cat dander/hair, dog dander, *Cladosporium herbarum*, pollen of timothy, rye grass, birch, mugwort.

pollen as well as using an inhalative atopy screening mixture (sx1) (Table 2). Wood dust-sensitized workers had sIgE to the sx1 mixture in 25 of 26 cases. Only in the asthma group, a serum was negative to sx1. This serum was exclusively positive to pine wood and showed no sIgE reactivity to CCDs, but did show sIgE to pine and beech pollen. Among the 26 wood dust-sensitized workers, the prevalence of beech pollen sensitization was 92%, followed by birch pollen with 85% and pine pollen with 62%. The sIgE concentrations to sx1 reached the highest value of all investigated allergens with a mean value of $23.2\ kU_A/L$, followed by birch pollen ($14.2\ kU_A/L$) and beech pollen ($8.7\ kU_A/L$). The mean sIgE concentration to pine pollen was $4.0\ kU_A/L$.

Correlation of beech and pine wood sensitization to cross-reactive carbohydrate determinants, environmental and pollen allergens in wood dust-sensitized workers

IgE-mediated sensitization to beech wood was significantly correlated to pine wood with $r^2 = 0.77$ and P-value < 0.0001 (Table 4). The correlation among all three CCD components was high and significant ($r^2 = 0.95$, $P < 0.0001$). Correlation in the pollen and environmental allergen group was only observed between beech and birch pollen ($r^2 = 0.70$, $P < 0.0001$).

sIgE to beech and pine wood was significantly correlated to all tested CCD components and pine pollen, but not to birch and beech pollen. There was no correlation between sx1 and any other allergens or CCDs. In contrast, sIgE to pine pollen was significantly correlated to beech and pine wood sIgE and to all CCD sIgE.

Table 3. Specific IgE profiles and HRP inhibition to beech and pine wood in woodworkers

					Inhibition by HRP on	
Group	Number	sIgE to beech wood (kU_A/L)	sIgE to pine wood (kU_A/L)	sIgE to HRP (kU_A/L)	Beech wood (%)	Pine wood (%)
A ($n = 5$)	1	**1.60**	**0.59**	**2.63**	63	27
	2	**5.04**	**2.50**	**8.86**	**98**	**97**
	3	**0.67**	**1.31**	**1.38**	0	0
	4	**0.51**	**0.55**	**0.59**	**86**	**64**
	5	0.13	**1.27**	0.26	–	–
R ($n = 5$)	6	**2.32**	**0.53**	0.14	–	–
	7	**0.40**	**0.38**	**0.78**	53	33
	8	**1.68**	**1.46**	**2.08**	**99**	**99**
	9	0.08	**0.84**	0.32	–	–
	10	**2.83**	**1.83**	**7.07**	**100**	**99**
A+R	11	**0.64**	0.16	0.04	–	–
($n = 6$)	12	**1.16**	**0.50**	**1.49**	0	0
	13	**0.95**	**0.45**	**1.74**	**97**	**94**
	14	0.96	0.06	0.06	–	–
	15	0.16	**1.24**	0.10	–	–
	16	**9.56**	**3.20**	**22.20**	**99**	**97**
Non-sym	17	**0.41**	0.06	0.01	–	–
($n = 10$)	18	**6.31**	**2.79**	**15.5**	**99**	**99**
	19	**8.46**	**3.13**	**16.7**	**99**	**99**
	20	**0.35**	0.20	0.64	–	–
	21	**0.55**	0.03	0.00	–	–
	22	**5.12**	**3.35**	**8.61**	**99**	**98**
	23	**0.35**	0.17	**0.54**	–	–
	24	**0.39**	0.10	**0.79**	–	–
	25	**0.50**	**0.50**	**0.40**	–	–
	26	**0.62**	0.32	**1.08**	–	–

HRP, horseradish peroxidase.

Table 4. Pearson's correlation of beech and pine wood sensitization to CCDs, sx1 and pollen allergens in wood dust-sensitized workers

	Beech wood	Pine wood	HRP	Bromelain	MUXF^3	sx1	Beech pollen	Pine pollen	Birch pollen
Beech wood	**1.00**								
Pine wood	**0.77**	**1.00**							
	***P*<0.0001**								
HRP	**0.95**	**0.60**	**1.00**						
	***P*<0.0001**	***P*<0.0001**							
Bromelain	0.89	**0.64**	**0.95**	**1.00**					
	P<0.0001	***P*<0.0001**	***P*<0.0001**						
MUXF^3	**0.76**	**0.76**	**0.92**	**0.95**	**1.00**				
	***P*<0.0001**	***P*<0.0001**	***P*<0.0001**	***P*<0.0001**					
sx1	0.06	0.10	0.04	0.03	0.02	**1.00**			
	P = 0.1238	*P* = 0.0568	*P* = 0.1706	*P* = 0.2224	*P* = 0.0221				
Beech pollen	0.10	0.01	0.06	0.13	0.03	0.11	**1.00**		
	P = 0.0561	*P* = 0.3076	*P* = 0.0972	*P* = 0.0372	*P* = 0.0221	*P* = 0.0479			
Pine pollen	**0.83**	**0.59**	**0.92**	**0.96**	**0.98**	0.02	0.13	**1.00**	
	***P*<0.0001**	***P*<0.0001**	***P*<0.0001**	***P*<0.0001**	***P*<0.0001**	*P* = 0.2102	*P* = 0.0364		
Birch pollen	0.01	0.11	0.05	0.01	0.03	0.12	**0.70**	0.04	**1.00**
	P = 0.3232	*P* = 0.0925	*P* = 0.1913	*P* = 0.3084	*P* = 0.2445	*P* = 0.0799	***P*<0.0001**	*P* = 0.2073	

r^2-values are listed; *P*-values <0.0001 were stated as significant and indicated in bold.
CCDs, cross-reactive carbohydrate determinants; MUXF^3, purified glycan structure of bromelain; sx1, inhalative atopy screening test 1.

Discussion

Wood dust contains a variety of agents that may induce asthma. Proteins, as high-molecular-weight agents, typically elicit the IgE-mediated responses, which were investigated in this study. The prepared wood extracts showed considerable differences in protein content. For pine wood, it was demonstrated that storage time was closely associated with ongoing protein degradation [21, 33]. This may explain the considerably lower protein content in our older pine wood sample (P1) compared with the younger one (P2). In beech wood samples, storage time was not linked to protein degradation. Other reasons, such as wood drying, may be more relevant to protein degradation. By coupling proved allergen amounts (B1 and P2) to ImmunoCAPs, it was possible to prepare tailored diagnostic tools for characterized sIgE testing to wood. Nevertheless, it should be taken into consideration that IgE-binding epitopes of beech and pine wood could be destroyed by the biotinylation of wood proteins for ImmunoCAP coupling.

The sensitization prevalence to pine wood (2.6%) was quite low in the Danish cohort, but was in line with an earlier study of Danish woodworkers that reported a sensitization rate of 2.9% [21]. Comparable studies from pine wood-exposed workers in Sweden [22] and Poland [34] showed sensitization rates in the same frame with 2.2% and 2.3%, respectively. IgE-mediated sensitization to beech wood (3.3%) was in the same range as pine wood. These results confirmed suggestions that the sIgE-mediated allergic potential of wood dust is limited [23]. In addition, our data showed that, considering questionnaire-reported allergic symptoms and wood dust sensitization, neither a qualitative nor a quantitative association with sIgE to wood dust was present.

Hence, more sophisticated tools than sIgE measurement alone are required to verify the diagnosis of relevant wood dust sensitization; sIgE binding was verified in this study by HRP inhibition. Proteinogenic epitopes were only recognized in sera of the symptomatic groups, whereas sera of non-symptomatic workers bound solely to glycogenic structures. This possible association between proteinogenic epitopes and allergic symptoms was further supported by the fact that mono-sensitized workers (sIgE $>0.70\,kU_A/L$) without CCD recognition were found exclusively in the symptomatic group.

To evaluate other major sources of cross-reactivity, atopic status was investigated by means of positive sIgE to sx1 as well as sIgE to beech, pine and birch pollen. Ninety-six percent of wood dust-positive workers had additional sIgE to inhalable atopy screening mixture (sx1) with sIgE concentrations up to 10 times higher than sIgE to beech or pine wood. But there was no significant correlation between sIgE concentration to wood dust and sx1 or beech/birch pollen implicating a potential cross-reactivity based on homolog proteins like Bet v 1 and homologues or Bet v 2, which were recently identified in the majority of *Fagales* pollen [35]. In contrast, a highly significant correlation was observed among wood, CCDs and pine pollen indicating that the primary reason for cross-reactivity among wood dust and other allergen sources may be due to CCDs. In fact, there was only one woodworker exclusively sensitized to pine wood and not to other allergens or CCDs. This worker reported

asthmatic symptoms during work and was likely the only person with true allergic wood dust-induced problems.

In conclusion, sIgE determination to beech and pine wood as screening for workplace-related allergic respiratory diseases must be standardized using tailored diagnostic tools to display reliable wood dust sensitization. The prevalence of beech and pine wood dust sensitization was low, and association between reported symptoms and sIgE was poorly correlated. However, wood dust can induce IgE-mediated allergy, and workers with a clear clinical suspicion of wood dust sensitization should be examined comprehensively. Additional specification of sIgE binding to wood by CCD inhibition allows a discrimination between proteinogenic-based (indication for symptomatic or clinical relevance) and glycogenic-based (indication for non-symptomatic or clinical irrelevance) sensitization and may therefore be useful for the diagnosis of occupational allergy in the wood-processing industry.

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