

Original article

Only few workers exposed to wood dust are detected with specific IgE against pine wood

Background: Our aim was to investigate the frequency of pine allergy in woodworkers with respiratory symptoms and to identify high molecular weight allergens in pine wood extracts.

Methods: In a cross-sectional study we examined work-related respiratory symptoms in 2033 furniture workers and 474 controls by questionnaires. Clinical examination was performed in 365 wood dust exposed and 116 nonexposed subjects. Blood samples were collected for measuring pine-specific immunoglobulin (Ig)E by an immunoassay and Western blots.

Results: Eleven exposed and three nonexposed subjects had pine-specific IgE. In the group with clinically defined asthma eight persons (5.4%) had pine-specific IgE compared with six persons (1.8%) in the group without asthma ($P < 0.05$). In the groups with and without respiratory symptoms, 13 (3.8%) and one (0.7%) subject, respectively, had pine-specific IgE ($P = 0.06$). Western blots demonstrated pine-specific IgE to components in the molecular range of 14–100 kD in eight samples (all wood dust exposed). Five samples had pine-specific IgE against components in a 43–59 kD zone and against two bands at 27 and 29 kD that are candidates for major allergens.

Conclusion: Some workers in the Danish furniture industry are specific IgE sensitized against pine wood dust. Pine-specific IgE probably explains a minor part of the respiratory symptoms in workers exposed to pine wood dust.

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Occupational and work-aggravated asthma are often preventable. The prognosis of occupational asthma depends on early detection and isolation of the causative agent (1). A few case reports (2–5) and smaller population studies (6) have described sensitization to wood dust from pine, oak, beech, and other types of wood used in the Scandinavian wood industry. Pine wood is commonly used in the Danish furniture industry. No standardized allergenic extracts (for use *in vitro* or *in vivo*) are, to our knowledge, available for diagnosing allergy to wood dust, although allergic asthma can be related to components from pine wood and other types of wood (7).

Some studies have found an increased prevalence of asthma symptoms among workers in the wood industry (8). Workers in the Danish furniture industry working on pine have an increased frequency of wheezing (9). Exposure to pine wood dust has shown a relation between cross-shift decrease in Forced expiratory volume in 1 s (FEV₁) and a decrease in baseline FEV₁ in subjects with asthma symptoms (10).

Sensitization to red cedar is related to plicatic acid, a low molecular weight component (11, 12). Earlier studies

have suggested that specific immunoglobulin (Ig)E against albumin-conjugated plicatic acid may be involved in the immediate asthmatic reaction (13). In the last decade no publications have supported the findings that specific IgE against plicatic acid is an important factor in the development of red cedar asthma. Histamine release or other cell-mediated reactions may be involved in different mechanisms generating the work-related asthmatic response (14). To our knowledge, no reports have excluded or ruled out the possibility that sensitization to high molecular weight components (proteins) is responsible for some of the immediate asthmatic reactions to wood dust from red cedar.

In two reports (15, 16) specific IgE against obeche and beech wood extract has been identified in subjects with wood dust-related occupational asthma and rhinitis. Specific IgE against obeche was detected in immunoblotting and specific IgE against beech in ELISA. Both the results suggest an allergic mechanism may be involved in some subjects with respiratory diseases. Our aim in this study was in accordance with proposed guidelines (17) – to investigate the frequency of pine allergy in

woodworkers with respiratory symptoms, and to identify high molecular weight allergens in pine wood extracts. Furthermore, we wanted to investigate if the presence of pine-specific IgE was related to exposure to wood dust, to respiratory symptoms, or to allergic diseases.

Material and methods

Study design

A cross-sectional study among workers employed in 54 Danish furniture industries was performed in Viborg County. The control group consisted of workers not exposed to wood dust in three different factories, two producing refrigerators and one producing hearing aids. Work-related respiratory symptoms were examined in 2507 persons (2033 woodworkers and 474 controls), by questionnaires. A sample of 483 subjects participated in a clinical examination (9) (participation rate 75%). This part of the study was mainly designed to investigate the relation between self-reported respiratory symptoms and objective measures of respiratory impairment among woodworkers and controls. Consequently, the clinical cohort was randomly selected, but stratified by respiratory symptoms and by exposure (woodworkers and controls) in order to maximize the efficiency of the clinical investigations. As a result, it was not possible to investigate differences in overall prevalence (e.g. asthma symptoms, bronchial hyper-reactivity, and sensitization) among exposed and nonexposed subjects, as the clinical cohort did not represent an unselected random sample of the base cohort, although it was possible to investigate the prevalence in each symptom group.

All participants underwent skin prick tests, and blood samples were collected for measuring total IgE and pine-specific IgE. The subjects were divided into groups according to the presence of respiratory symptoms: (1) asthma symptoms $n = 131$ (wood dust exposed $n = 107$ and nonexposed $n = 24$), (2) both asthma and rhinitis symptoms $n = 112$ (wood dust exposed $n = 89$ and nonexposed $n = 23$), (3) rhinitis symptoms $n = 57$ (wood dust exposed $n = 37$ and nonexposed $n = 20$), (4) possibly asthma or rhinitis $n = 39$ (wood dust exposed $n = 26$ and nonexposed $n = 13$), (5) no symptoms $n = 142$ (wood dust exposed $n = 106$ and nonexposed $n = 36$). Clinical examinations and storage of blood samples were carried out at Skive Hospital. Blood samples were sent by mail to Slagelse Hospital for biochemical examinations and were received within 24 h after sampling. Two samples were lost during shipment. Eight samples were received 48 h after sampling. One sample was not analyzed due to a technical problem in an assay run. A total of 480 samples were examined in an immunoassay.

Preparation of pine wood extracts

Wood dust (139 g) was extracted overnight in 2.5 l isotonic phosphate-buffered saline (PBS, pH 7.6) and was filtrated through a 11-mm filter (Whatman®, no. 1) on a Büchner funnel. The extracts were filtered through a prefilter (Millipore, Bedford, MA) and 0.45 µm filter (Millipore) and up-concentrated $\times 100$ using ultra filtration polysulfone membrane (cutoff at 10 kD, Millipore, Bio-max High-flux) on a Minitan II system (Millipore).

Three types of wood extracts were prepared from sawdust: (1) dust from a pine tree cut 48 h before extraction, (2) dust from a pine tree cut 6 months before extraction, and (3) dust from pine wood delivered from a furniture factory. The stock extracts were frozen at -80°C until the extract was used in an immunoassay and

sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Detection of specific IgE against pine wood

A pine-specific IgE immunoassay was developed. Pine extract used in the immunoassay was prepared from saw dust of a pine tree cut 6 months before extraction. The assay consisted of a solid phase with a monoclonal anti-human-IgE antibody that is coupled to paramagnetic particles (Universal Solid Phase, ADVIA®, Centaur™, Bayer, Tarrytown, NY) used in combination with biotinylated pine extract and plasma samples. About 25 µl plasma and 50 µl biotinylated pine extract were added to 250 µl paramagnetic particles in a polystyrene tube and incubated for 30 min at room temperature. After incubation, the paramagnetic particles were separated on the side of the tube by a magnetic block, decanted and washed in 1 ml washing buffer (Magic Lite® SQ, ALK-Abellö, Copenhagen, Denmark). About 100 µl acridinium ester-labeled streptavidin (ADVIA®, Centaur™, IgE Universal Lite Reagents, Bayer) was added in order to bind any remaining biotinylated pine extract. After incubating for 30 min at room temperature the particles were separated and washed in 1 ml washing buffer and suspended in 100 µl distilled water. The suspension was counted in a Magic Lite Analyzer (Automatic Luminometer, LB 954, Berthold, Germany) and pine-specific IgE was expressed in relative light units (RLU). Plasma samples were analyzed in duplicates and results expressed as the mean (RLU) of the two determinations.

Biotinylation of pine extract

Approximately 5.1 mg NHS-LC-LC Biotin (Pierce, Rockford, IL) was dissolved in 2.5 ml pine extract and mixed carefully. After incubation for 2 h on ice, the extract/biotin solution was centrifuged. The supernatant was desalted on a desalting column (PD-10, Sephadex® G-25M, Amersham Pharmacia Biotech, Uppsala, Sweden). The eluate in PBS was stored at 4°C in 0.01% sodium azide and used within 3 weeks. The outcome of the biotinylation of pine extract was confirmed by streptavidin AP in SDS-PAGE and Western blots as described below. The biotinylated extract was diluted 1 : 5 in washing buffer before it was used in the pine-specific IgE assay.

SDS-PAGE and immunodetection

Pine extract was diluted 2 : 1 in sample buffer (NuPAGE™, Novex, San Diego, CA) and 10 ml was applied on a tris-glycine 10–20% TB precast gradient gel (BioWittaker Molecular Applications, Walkersville, MD). Electrophoresis was performed in an electrophoresis unit (Hoefer mini VE, Amersham Pharmacia Biotech) with 150 mA current for 45 min. Proteins were electrotransferred to nitrocellulose (NC) with 75 mA current for 2 h. Proteins were visualized on NC by colloidal staining. Pine-specific IgE immuno-blots were performed by incubation of plasma diluted 1 : 10 in 1% bovine serum albumin phosphate saline solution. Specific IgE was detected with a biotinylated antihuman IgE (the binding site) diluted 1 : 500 and alkaline phosphatase streptavidin (DAKO, Glostrup, Denmark) diluted 1 : 1000. 5-Bromo-4-chloro-3-indolyl phosphate was used as chromogenic substrate to visualize specific IgE binding. A total of 50 plasma samples were investigated, 14 samples positive in a pine-specific IgE immunoassay, and 36 randomly selected samples negative in a pine-specific IgE immunoassay.

Monitoring of respiratory impairment

Questionnaire

A modified American Thoracic Society (18) questionnaire with additional questions on allergy, coughing, asthma, family history of allergy, rhinitis, conjunctivitis, smoking, and occupational history including working tasks were handed out at the factories. In total, 2033 woodworkers (88%) and 474 control persons (82%) returned the questionnaire. Asthma symptoms were diagnosed, if the subject answered 'yes' to at least one group A question, and two or more group B questions, or at least two group A questions (19) Table 1. Possible asthma symptoms were diagnosed, if the subject answered 'yes' to one group A question, and no group B questions, or at least two group B questions. Rhinitis was diagnosed if the subject answered 'yes' to at least two nose symptoms (rhinorrhoea, nasal itching, nasal congestion, or sneezing) at least 2 days a week (20).

Bronchial responsiveness. Bronchial responsiveness was measured using the method of Yan et al. (21) Subjects whose FEV₁ dropped by 20% or more from baseline FEV₁ were considered as having bronchial hyper-responsiveness (BHR). A total of 461 persons performed a valid test.

Peak flow

All persons attending the clinical investigation with or without asthma symptoms (*n* = 425) were asked to perform a peak flow (PEF) monitoring. A total of 306 or 72% performed valid measurements. PEF recordings were performed using a Mini-Wright peak flow meter (Clement Clarke International, London, UK) for at least 2 weeks. The persons were asked to measure PEF every 3 h except during sleep. Serial PEF measurements were classified as acceptable when at least three measurements/day were performed. The recordings from the first day were discarded to avoid learning effect on the variability.

Bronchodilator-induced reversibility test

A test for reversibility of airway obstruction was carried out among persons who had a predicted baseline of FEV₁ < 60%, and among persons whose FEV₁ fell by 15% or more of baseline FEV₁ by bronchial histamine provocation test, totaling 84 persons. The subject was given five inhalations of 0.5 mg tebutaline and the airway response (FEV₁) was measured after 15 min. A positive test was interpreted as an increase in FEV₁ of > 12% from baseline

FEV₁, and an absolute increase of > 200 ml. In total, 63 persons had bronchodilator-induced reversibility (BDIR).

Diagnostic criteria of asthma

Asthma. Persons with asthma symptoms and either (1) BHR, or (2) BDIR, or (3) daily PEF variability of > 20%, on at least 2 days or (4) within variability for the whole PEF monitoring period of > 30%, or (5) wheezing or chest tightness by exposure to pollen or animals (22).

Atopy. Skin prick tests (double tests) were performed to evaluate immediate allergic reaction to a panel of 12 common inhalant allergens (Soluprick ALK-Abell ). The panel comprised birch, timothy, mugwort, horse, dog, cat, house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) and molds (*Alternaria alternata* and *Claudosporium herbarium*), extended with allergens from storage mites (*Acarus siro* and *Lepidoglyphus destructor*). Histamine hydrochloride (10 mg/ml) and the dilutant were used as positive and negative references, respectively. The extracts were placed on the skin of the forearm in two columns 50 mm apart. The skin was penetrated with a standard 1 mm lancet (ALK-Abell ), and after 15 min the tests were read. A positive test was defined when the mean of the longest diameter and the mid-point orthogonal diameter of the wheel was 3 mm, as recommended (23). Atopic status was diagnosed, if the person had one or more positive skin prick tests against 12 inhalant allergens. In total, 467 had valid skin prick tests performed.

Results

Selection of protein-based pine extract

The intensity of colloidal gold staining varied depending on the origin of the extract (Fig. 1). Extract from fresh wood showed a high protein content, whereas extract from wood dust from a pine tree cut 6 months before extraction contained less protein, but several bands. Pine wood dust from a furniture factory contained very small amounts of protein. Only three bands were identified. Previous experiments with pine wood extract from a furniture factory failed to generate any response in a pine-specific IgE assay (data not presented), which most likely was caused by the very low protein contents in the extract. Pine wood dust was primarily used in the extraction procedures, but as a consequence of the low protein content and the lack of IgE reactions, 6-month-old wood was used in the development of the specific IgE immunoassay.

A pine-specific IgE immunoassay

Plasma samples from 480 individuals were tested and were distributed in a bimodal distribution (Fig. 2). The total range of the results varied from 3400 to 213 500 RLU. One distribution was found in the range 9100–36 300 RLU and one > 40 000 RLU. The data in the range 9100–36 300 RLU showed a normal distribution (Kolgomorov–Smirnov coefficient = 0.63, *P* > 0.15), mean and standard deviation of 19 900 and 4300 RLU, respectively. A cutoff limit for the

Table 1. Questions on asthma

Group A	Group B
Have you been told by a doctor that you have asthma?	Do you ever have chest tightness?
Do you have asthma?	Do you wake in the night with chest tightness?
Have you ever had asthma?	Do you wake in the night wheezing?
Do you ever wheeze?	Do you cough when you wake up in the morning?
	Do you wake up because of coughing?
	Do you wheeze because of exposure to cold air?
	Do you wheeze when you exercise?
	Do you wheeze because of exposure to pollen?
	Do you wheeze because of exposure to animals?
	Do you use asthma drugs?

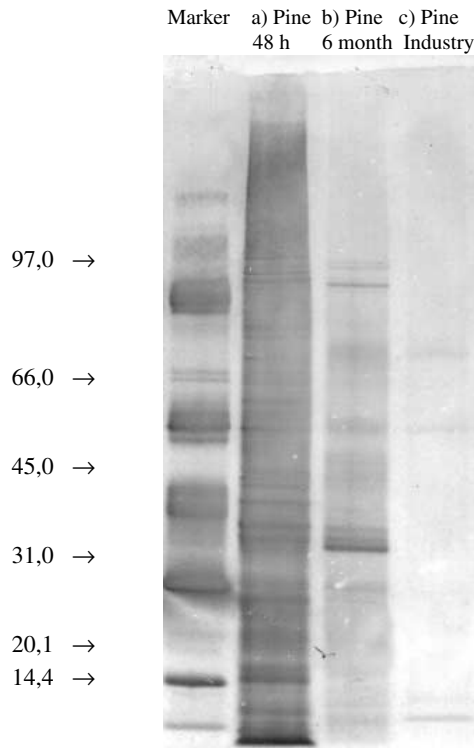


Figure 1. Pine extracts stained with a colloidal gold: (a) pine wood dust extracted 48 h after cutting time; (b) pine wood dust extracted 6 months after cutting, and (c) pine wood dust extract from the furniture industry.

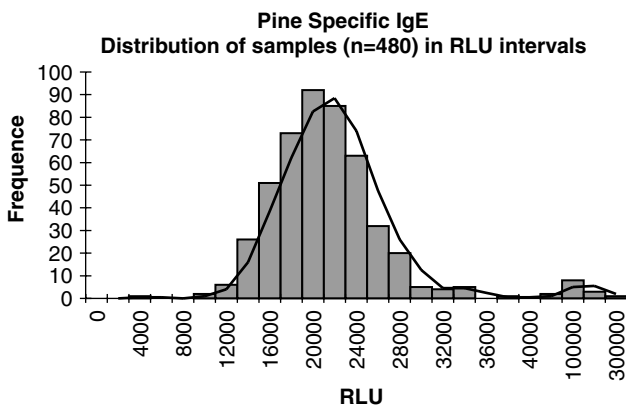


Figure 2. Distribution of pine-specific immunoglobulin E reactions from all samples ($n = 480$). The majority of samples (466 samples) is normally distributed with a mean value of 19 900 RLU. Mean and four standard deviation are shown. The other group ($n = 14$) deviates significantly from the 466 normal distributed samples.

classification of positive pine-specific IgE was defined as mean + $4 \times$ standard deviations, i.e. 36 700 RLU. A total of 14 samples were classified as positive with this definition.

Pine-specific IgE according to wood dust exposure

In Table 2, the relation between pine-specific IgE, wood dust exposure and respiratory symptoms is shown. There was no significant difference between the numbers of persons detected with pine-specific IgE among woodworkers and controls in the different symptom groups. Among the 19 controls with previous occupational wood dust exposure, one person with asthma symptoms had pine-specific IgE. Among persons with respiratory symptoms (asthma symptoms, or rhinitis) there was a tendency toward an increased number with pine-specific IgE, although not significant.

A total of seven woodworkers (64% of the sensitized) most frequently used pine wood in the production. The rest, four persons, used mixed wood types. Altogether, 4.2% of pine workers were sensitized, compared to with 2% of workers using predominantly other wood species (Fisher's exact test: $P = 0.23$).

Pine-specific IgE according to respiratory impairment

In Table 3, the relation between pine-specific IgE and respiratory impairment is presented. In the group with clinically defined asthma, significantly more had pine-specific IgE, 5.4% vs 1.8%. The same tendency was observed for BHR and respiratory symptoms, although not significant.

Pine-specific IgE according to atopy and pollen skin prick positive individuals

A total of 257 persons or 55% were classified as atopic. The proportions of samples detected with pine-specific IgE were not different among nonatopic and atopic individuals (seven positive in the atopic and nonatopic groups, respectively).

When individuals had skin prick test reactions to at least one of three common pollen allergens they were classified as pollen positive. Six of 123 (4.9%) pollen positive individuals and eight of 345 (2.5%) pollen-negative individuals had pine-specific IgE. The difference was not significant ($P = 0.26$ Fisher's exact test).

Demonstration of IgE-binding components in SDS-PAGE and immunoblotting

The pine-positive samples were examined in SDS-PAGE and immunoblotting, and several IgE-binding components were found (Fig. 3). Eight of 14 pine-positive plasma samples had specific IgE against several IgE-binding components in the molecular range of 14–100 kD (Table 4). Five samples had specific IgE against components in a 43–59 kD low intensity-stained zone. The same samples also identified two bands at 27 and 29 kD that appeared more sharp and heavily stained. Three samples identified single components at 14, 42, 70, and 85 kD that

Table 2. Number of persons (%) detected with specific IgE against pine in relation to respiratory impairment among 364 woodworkers, 19 controls with previous occupational exposure to wood dust and 95 controls. Asthma and rhinitis is defined as stated in the text.

Symptoms	Asthma	Asthma and rhinitis	Rhinitis	Possibly asthma and/or rhinitis	No symptoms
Number in groups	131	112	57	39	139
<i>Wood dust exposed</i>					
Yes (<i>n</i> = 364)	5 (4.7%)	3 (3.4%)	2 (5.4%)	0	1 (0.9%)
Yes, previously (<i>n</i> = 19)	1 (25%)	0	0	0	0
No (<i>n</i> = 95)	1 (4.8%)	1 (6.3%)	0	0	0
Total in group	7 (5.3%)	4 (3.6%)	2 (3.5%)	0 (0.0%)	1 (0.7%)

were not identified by any other samples. The IgE-binding components in the 43–59 kD zone, and the two bands at 27 and 29 kD are candidates for major allergens based on the observed frequency of positive reactions to pine extracts.

Six samples were negative in Western blot – three of the samples were from the wood dust exposed and three from the nonexposed group. The eight samples that were positive in Western blot were all from the wood dust-exposed group ($P = 0.11$ Fisher's exact test).

In order to control whether the results in Western blots to pine could be related to pollen sensitization, we randomly selected 36 samples that were pine-negative, of which six were pine-positive. One sample was positive to four bands and five samples were positive to one band with very low staining intensity (data not shown). When we compared the frequency of positive pollen skin prick tests in 50 Western blots we found significantly more pollen-positive reactions among the pine-positive samples ($P = 0.025$ Fisher's exact test). In 14 positive samples, nine had a positive pollen skin prick test; in 36 pine-negative samples, nine had a positive pollen skin prick test. The relatively high frequency of samples with a sensitization to pollen and positive reaction to pine in Western blot could be caused by cross-reactions to similar structures on carbohydrate determinants in pine and pollen extracts. Specific IgE cross-reactions against components with profilin-like structure, or other pollen-related structures in the pine extract, were outside the scope of this investigation and not investigated further.

Discussion

In the present study we have demonstrated few cases of specific IgE antibodies against wood dust from pine. Allergy to wood dust has been demonstrated earlier from other types of wood (2–5, 15, 24), but no evidence has, to our knowledge, been published, identifying high molecular weight allergens from pine wood dust.

Åhman et al. (6) examined IgE-mediated wood allergy in 127 Swedish woodwork teachers and 111 reference subjects by radioallergosorbent test (RAST) and found one pine-positive subject. No visualization, e.g. Western

blot, was demonstrated, and the procedure of allergen extraction differed from the present investigation. Further, the positive pine wood IgE was found in one of the control persons, the origin of which was doubtful.

In Western blots we found evidence for major antigens in the 27–59 kD zone in several IgE-positive patients. In IgE-negative patients we found only very weak-stained bands in different regions compared with IgE-positive patients. These reactions were more common in pollen-positive persons, and could be regarded as cross-reactivity to similar structures on carbohydrate determinants in pine and pollen extracts. Five of eight positive patients had specific IgE against components in the molecular range of 43–59 kD and two components at 27 and 29 kD determined by Western blots. These antigens may be major allergens and could be important in the future production of standardized pine extracts for *in vitro* and *in vivo* diagnostic use. The protein contents of pine extract differ depending on the source of pine wood used in the extraction. We used 6-month-old pine wood in our immunoassay because only very little protein was present in extracts of wood from furniture factories. It has been shown that protein content in pine wood decreases in a logarithmic scale (25), which may be the reason for the very low protein content in the wood dust emanating from the factories. This low protein content may be the reason for the negative findings of positive reactions when dust from the factories was used as allergen. Although antigens may change in time, we are convinced that the IgE reactions under consideration indicate pine wood allergy.

The frequency of sensitization to pine wood was low. However, we found a positive relation between respiratory impairment and sensitization, indicating that sensitization may explain a minor part of respiratory impairment found in the study. This is in concordance with earlier results (9, 10). Difference in atopic status could not explain the increased number of persons with respiratory impairment sensitized to pine.

We found no significant difference between exposed and control persons according to prevalence of positive pine-specific IgE. There was a tendency toward more sensitized persons among pine workers than in other woodworkers, although not significant. Because of the low number of sensitized persons we could not investigate

Table 3. Pine-specific IgE related to respiratory impairment among 365 woodworkers and 118 controls from the furniture industry (F.I.) and control industry (C.I.)

Pine-specific IgE (%)	Respiratory symptoms		Bronchial hyper-reactivity		Clinical defined Asthma	
	Yes (n = 338)	No (n = 142)	Yes (n = 63)	No (n = 398)	Yes (n = 148)	No (n = 335)
Workers in groups	13 (3.9%)	1 (0.7%)	4 (6.3%)	10 (2.5%)	8 (5.4%)*	6 (1.8%)
Pine-specific IgE (%)	F.I. (n = 257) 10 (3.9%) 3† (3.7%) C.I. (n = 81) 3† (3.7%)	F.I. (n = 106) 1 (0.9%) 0 C.I. (n = 36) 0	F.I. (n = 48) 3 (6.3%) 1† (6.7%) C.I. (n = 15) 1† (6.7%)	F.I. (n = 299) 8 (2.7%) 2 (2.0%) C.I. (n = 99) 2 (2.0%)	F.I. (n = 120) 6 (5.0%) 2† (7.1%) C.I. (n = 28) 2† (7.1%)	F.I. (n = 245) 5 (2.0%) 1 (1.1%) C.I. (n = 90) 1 (1.1%)

Respiratory symptoms: asthma and/or rhinitis symptoms. Bronchial hyperreactivity and clinically defined asthma as stated in the text. One person in the control industry was previously exposed to wood dust from previous work (†).

* $P < 0.05$ between group 'Yes' and 'No' (chi-square test).

the dose-response relationship between wood dust concentration and prevalence of positive pine-specific IgE among woodworkers. In this study, we have previously shown dose-response relationships for dust concentration on one hand, and asthma symptoms, coughing, BHR, and acute nasal mucosal swelling on the other (9, 10), despite a low general dust level.

The very limited number of positive persons, 14, greatly impedes our possibility of arriving at a conclusion. Though, our result point toward, that sensitization is not a strong predictor for occupational exposure to pine dust. There are some obvious limitations in our study. In a cross-sectional design it is difficult to explore selection away from the industry, but data from the first part of the present study suggest a healthy worker effect (9, 10). This could be a reason for the low frequency of sensitized persons in the furniture industry. Additionally, pine wood is widely distributed all over the society, and nonoccupational sensitization could be another explanation for sensitization among the control group. It is known from several studies, that the number of sensitized persons is positively related to increased concentrations of allergen (26, 27), making it plausible, that woodworkers have an increased risk for sensitization, despite the lack of evidence in this study.

The low protein content in pine wood dust is probably the main reason why so few individuals were specific IgE sensitized to the wood extracts.

Eight samples were positive in the pine-specific IgE immunoassay and in Western blots and all the samples came from subjects exposed to wood dust in the Danish furniture industry. Both test methods detected positive samples in the control groups not exposed to wood dust and without respiratory disease, and cannot be used alone as a definitive diagnostic tool to identify occupationally related pine allergy. Pine-specific IgE detected in Western blots were related to pollen sensitization and the same tendency was found in the pine-specific IgE immunoassay.

Occupational wood dust sensitivity from spindler tree (*Euonymus europaeus*) has been reported (28). Western blots and RAST-inhibition studies found that sera mugwort pollen-allergic subjects had specific IgE against allergens found in spindler tree. Goetz et al. (29) reported that specific IgE to mountain cedar pollen in immunoblots identifies nonpollen allergens from wood and berries of the mountain cedar tree, indicating that allergens in wood can, in some degree, be similar in structure to that of pollen allergens. Occupational asthma to wood dust from oak (4), eastern white cedar (30) and exotic woods (3, 24) has been confirmed by specific inhalation challenges generating immediate and dual asthmatic reactions. Some of the subjects were specific IgE sensitized to high molecular weight components from the wood dust.

Pine pollen allergy is very rare in Denmark as well as in other countries and is not likely to be responsible for the positive pine-specific IgE reactions in the immunoassay

Table 4. Fourteen samples positive in the pine-specific IgE immunoassay in relative light units (RLU) compared to SDS-PAGE and western blots

Wood dust exposed	Pine-specific IgE (RLU)	Number of bands in SDS-PAGE	Molecular weight in SDS-PAGE	Respiratory status (questinoires)	BHR	Clinical defined Asthma	Pollen skin prick test
F.I.	153 608	4	14, 27, 29, 43–59 kD	A		A	Pollen positive
F.I.	129 169	3	27, 29, 43–59 kD	C			
F.I.	60 313	4	25, 100 kD	A	BHR	A	Pollen positive
F.I.	65 700	4	27, 29, 36, 43–59 kD	R			
F.I.	52 629	3	27, 29, 43–59 kD	R	BHR	A	Pollen positive
F.I.	109 259	4	14, 27, 29, 43–59 kD	A			Pollen positive
F.I.	79 033	4	16, 38, 54, 100 kD	A & R			Pollen positive
F.I.	45 830	6	39, 43, 57, 71, 80, 90 kD	A		A	
F.I.	46 564	0		R		A	
F.I.	86 288	0		A & R			
F.I.	62 390	0		A	BHR	A	
C.I.	80 362	0		A	BHR	A	Pollen positive
C.I.‡	213 464‡	0		A‡		A‡	
C.I.	50 731	0		R			

Relation to wood dust exposure from the industry, clinical symptoms and skin prick test against pollen are also shown. Furniture industry (F.I.), Control industry (C.I.). One person in the control industry was previously exposed to wood dust from previous work (‡). Respiratory status from questionnaires: Asthma (A), Rhinitis (R) and Controls (C). Bronchial hyper-reactivity (BHR) and Clinical defined asthma as stated in the text.

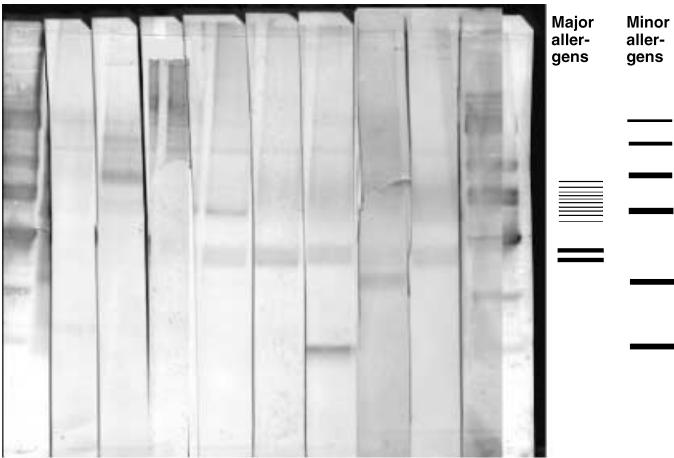


Figure 3. Western blots of eight samples classified as pine-specific immunoglobulin E-positive. We found five samples that identified two components at 27 and 29 kD, and a zone between 43 and 95 kD proposed to be major allergens. Several components in the molecular weight range of 100 – 4 kD could be identified as minor allergens. Right and left lanes Mw markers 97, 66, 45, 31, 20.1 and 14.4 kD.

or in Western blots. Specific IgE reactions against grass, birch, or mugwort may, in few cases, cross-react with pine extract. Inhibition studies need to be performed in relation to cross-reacting substances like plant profilin. Molecular relations between different IgE-binding components, e.g. potential cross-reacting carbohydrate determinants in wood dust and pollen, need to be studied in the future.

In conclusion, there seems to be a limited number of persons with sensitization to pine wood, and sensitization probably explains only a minor part of respiratory impairment among the study population. This has implications on the counseling of patients in the wood industry. Persons suffering from respiratory impairment will prob-

ably benefit from a decrease the dust level, whereas sensitized persons often have to leave the workplace. In order to prevent respiratory impairment in the wood industry, decreasing the dust level will probably be of benefit, regardless of whether it is caused by irritants or allergens.

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