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PhD THESIS

Occupational allergic and irritant skin and respiratory diseases in hairdressers

- occurrence and clinical investigations

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PREFACE

This thesis is based on scientific work performed between 2012 and 2016. It is a product of collaboration between the Research Centre for Hairdressers and Beauticians, the National Allergy Research Centre, the Allergy Clinic, and the Department of Dermatology, Copenhagen University Hospital Gentofte.

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Majken Hougaard Foss-Skiftesvik

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ABBREVIATIONS

CI	Confidence Interval
ECRHS	European Community Respiratory Health Survey
HRT	Histamine Release Test
IgE	Immunoglobulin E
IR	Incidence Rate
IRR	Incidence Rate Ratio
kDA	kilo Dalton
NOSQ	Nordic Occupational Skin Questionnaire
OR	Odds Ratio
SD	Standard Deviation
SIC	Specific Inhalation Challenge
SPSS	Statistical Package for the Social Sciences
SPT	Skin Prick Test
UK	United Kingdom

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BACKGROUND

Chemical hazards in the working environment of hairdressers and hairdressing apprentices

Hairdressing involves frequent exposure to a variety of potentially allergenic and irritant substances (1,2). Hairdressers' hands, wrists, and the mucous membranes of the respiratory tract are particularly at risk. Many hairdressing products, including hair dyes, hair bleaching products, and permanent wave solutions contain potent sensitizers. In addition to a wide range of potentially allergenic chemicals, hairdressing also involves substantial exposure to irritants such as wet work, detergents, use of gloves, and hairsprays.

Table 1 provides an overview of the most common hairdressing products that have been linked to allergic and irritant skin and respiratory diseases. In contrast to the average hair products user, hairdressers are exposed to these substances throughout their working day, 5–6 days per week, every week, all year round. As a consequence, hairdressers are at heightened risk of developing occupational skin and respiratory diseases.

There are various preventive strategies that hairdressers can adopt to reduce exposure to allergenic and irritant substances. Proper use of appropriate gloves during wet work, bleaching, dyeing, and perming, and the frequent application of moisturizing creams are essential to prevent occupational skin diseases developing (3), whilst the use of designated mixing areas and local exhaust ventilation are important in reducing airborne exposure to allergens and irritants. Unfortunately, hairdressers do not always adopt these protective measures (1,4).

Occupational contact dermatitis and contact urticaria in hairdressers

Occupational contact dermatitis predominantly affects the hands and wrists of hairdressers (5). The clinical presentation is characterized by erythema, scaling, and vesiculation during the acute phase, and lichenification and fissuring in chronic disease. Contact dermatitis can be caused by excessive exposure to sensitizers resulting in a type-4 allergic reaction (allergic contact dermatitis) or by excessive exposure to physical, mechanical, or chemical irritants resulting in a non-allergic inflammatory reaction of the skin (irritant contact dermatitis). In hairdressers, allergic contact dermatitis appears to be more common than irritant contact dermatitis (6,7).

A diagnosis of allergic contact dermatitis relies primarily on patch testing, whilst irritant contact dermatitis is diagnosed when contact allergies have been excluded by exposure analysis and patch testing using relevant allergens. When testing a hairdresser for contact dermatitis a specific hairdressers' patch test series may be applied in addition to the European baseline series. This patch test series typically contains allergens from hair dyes, hair bleaches, permanent wave solutions, and preservatives, although the allergens included can vary from country to country (8).

Table 1. Potential allergens and irritants in the hairdressing environment

Etiology	Causative agent	Disease[1]
Hair dye	p-phenylenediamine and toluene-2,5-diamine	ACD (5) CoU (9,10) Rhinitis and asthma (10,11) Anaphylaxis (10,12)
	p-methylaminophenol and p-aminophenol	ACD (5) CoU (13,14) Anaphylaxis (13,14)
	Resorcinol	ACD (5)
	Henna (lawsone)	ACD (15) CoU (16) Rhinitis and asthma (16,17)
	Senna	Rhinitis and asthma (18)
	Basic Blue 99	CoU (19,20) Rhinitis (20)
Hair bleach	Ammonium persulphate, potassium persulphate, and sodium persulphate	ACD (5) and ICD (21) CoU (22–24) Rhinitis and asthma (11,25) Anaphylaxis (26)
	Hydrogen peroxide	ICD (27) Upper respiratory tract irritation (28)
Hair spray	-	Rhinitis (29)
Permanent wave solution	Glyceryl monothioglycolate and ammonium monothioglycolate	ACD (5) CoU (30) Rhinitis and asthma (25,31)
	Cysteamine hydrochloride	ACD (5)
Shampoos	MI/MCI, formaldehyde, and other preservatives Sodium lauryl sulfate and other detergents	ACD (5,32) ICD (33)
Conditioner	Hydrolyzed wheat protein	CoU (34) Rhinitis and asthma (34)
	Panthenol	CoU (35)
Scissors, hair pins	Nickel	ACD (5)
Protective gloves	Occlusion, friction, powder	ICD (36)
	Accelerators	ACD (36)
	Latex proteins	CoU (37–39) Rhinitis and asthma (11) Anaphylaxis (36)
Glue for eyelash extensions	Acrylates	ACD (40) Rhinitis and asthma (41)
Wet work	Water	ICD (42)
Fragrances	Such as eugenol, citral, lyral, and limonene	ACD (43) and ICD (43) CoU (44) Asthma (45)

1: References are not exhaustive
ACD: allergic contact dermatitis CoU: contact urticaria ICD: irritant contact dermatitis
MI: methylisothiazolinone MCI: methylchloroisothiazolinone -: Not described in the text

The most frequent causes of sensitization in hairdressers are ammonium persulphate found in hair bleach and para-phenylenediamine and tolune-2,5-diamine found in hair dye products (5,7,46). The life-time prevalence of hand eczema in Scandinavian hairdressers is 29–44% (47–49) and the incidence is 5.6–23.8 cases/1000 person-years (6,47,50,51). It appears that hairdressing apprentices are particularly at risk with a prevalence as high as 58% (52) and an incidence of 151–328 cases/1000 person-years (53,54) during training. One of the aims of this thesis was to study the current prevalence and incidence of hand eczema in hairdressing apprentices.

Between 2008 and 2010, a clinically controlled, prospective, intervention study was carried out among hairdressing apprentices in Denmark (55). The aim was to investigate whether the risk of developing hand eczema during training could be reduced by implementing an educational programme in hairdressing schools. The evidence-based educational programme was developed in cooperation with teachers from the schools and contained information on dermal physiology, allergies and eczema, skin protection, and optimizing work procedures (56). When followed up 18 months later, the hairdressing apprentices in the intervention group had a significantly reduced incidence of hand eczema and an increased awareness of protective procedures compared with apprentice in the control group. Following these positive results, the educational programme provided to the intervention group was implemented in Danish state-approved hairdressing schools (57). One of the aims of this thesis was to examine whether the implementation of this educational program has reduced or completely eliminated the risk of hand eczema in hairdressing apprentices.

Contact urticaria is another potential problem for hairdressers (58). Here, direct contact with an offending substance produces a dermal wheal and flare reaction which is typically pruritic. It appears within 30 min and clears completely within 1–24 hours without scarring. Contact urticaria is broadly divided into two categories: immunological (IgE-mediated) and non-immunological. Immunological contact urticaria requires previous exposure of the immune system to an allergen before a reaction develops, whereas non-immunological contact urticaria does not require presensitization (58). In addition to examining a patient's medical history, the skin prick test, the determination of specific immunoglobulin E (IgE) antibodies, and the open application test are considered key diagnostic tests when diagnosing contact urticaria (58).

Although contact urticaria typically affects scalp, ears, neck, and face, the hands and wrists are most commonly affected in hairdressers (58). The main elicitors of contact urticaria in hairdressers are hair bleaches and dyes. However, hydrolysed wheat protein in conditioners (34), latex gloves (37–39), and permanent wave solutions (30) can also reportedly induce contact urticaria (see Table 1 for an overview of hairdressing products that have been linked to contact urticaria).

Epidemiologic data on contact urticaria are generally quite sparse (59), and data on occupational contact urticaria in hairdressers are limited to a few registry studies (24,60–62). In Australia, hairdressers, along with nurses and food handlers, are among the occupational groups with the highest risk of occupational contact urticaria (61), and a recent study found that hairdressers and personal care workers had the highest prevalence of occupational contact urticaria in France (24). To our knowledge, no epidemiologic data exist regarding

occupational contact urticaria in hairdressing apprentices. One of the aims of this thesis was to fill this gap in existing knowledge.

Occupational asthma and rhinitis in hairdressers

Every day, hairdressers are exposed to potentially allergenic and irritant airborne chemicals (63). Inhalation of these chemicals can result in work-related asthma and/or rhinitis (11). Work-related asthma is characterized by wheezing, chest tightness, coughing, and shortness of breath (64). In contrast, work-related rhinitis is characterized by sneezing, nasal congestion, itching, and rhinorrhea (65). Often, nasal symptoms are also present in work-related asthma (66) and the two conditions often co-exist and influence one another (67). Many hair products contain substances, for example ammonia, that can produce non-specific respiratory irritation in hairdressers (68,69). However, it is also possible for hairdressers to become sensitized to different allergens in their work environment. The hairdressing product most frequently linked to work-related rhinitis and asthma is hair bleach containing persulphates (25,70); however, hair dye ingredients, including para-phenylene-diamine (10,71,72) and henna (16,17,73), and powdered latex gloves (11) can also reportedly cause asthma and rhinitis. Hairdressers' use of hair sprays can reduce the nasal mucociliary clearance (29), and permanent wave solutions can cause nasal obstructions (74) (see Table 1 for an overview of hairdressing products that have been linked to asthma and rhinitis).

Several studies have found hairdressing to be one of the five professions most associated with occupational asthma (75–80), having an incidence rate of 0.02-0.37 cases/1000 person-years (76,77,79–83). Since hairdressers tend to underreport suspected occupational diseases (84), the actual occurrence is probably higher. Occupational rhinitis in general, has received much less attention than occupational asthma, and epidemiologic data regarding its occurrence in hairdressers are limited to only a few studies (31,85). The incidence of work-related respiratory symptoms in high-risk occupations is typically highest during the first few years (86,87). Therefore, when examining the risk of respiratory diseases in a given occupation, it is important to study apprentices. However, to date, the occurrence of asthma and rhinitis in hairdressing apprentices has received little attention (88,89).

One of the aims of this thesis was to examine the prevalence and incidence of respiratory symptoms suggestive of asthma and rhinitis in hairdressing apprentices and to determine whether hairdressing apprentices are at increased risk of developing these symptoms compared with young adults from the general population.

Healthy worker effect

The healthy worker effect is a well-known phenomenon in occupational epidemiology that presents methodological issues and can result in selection bias (90). Originally the phenomenon was used to describe the tendency of healthy individuals to be hired over unhealthy individuals (91). Today, when describing the healthy worker effect, two components are recognized: the 'healthy hire effect' describing the tendency for healthy workers to be hired and for sensitive people to avoid certain workplaces, and the 'healthy survivor effect' describing the tendency of unhealthy workers to leave certain occupations (90). Two studies have suggested that a healthy worker effect exists in hairdressing apprentices as a result of the increased risk of skin (92) and respiratory diseases (88). One of the aims of this thesis was to examine this issue further.

Diagnosing persulphate-induced rhinitis and asthma

Persulphates are low-molecular weight inorganic chemicals (<10 kDA) with strong oxidizing properties that are widely used as constituents of hair bleaches and hair dye preparations (93). In hydrogen peroxide bleaches, persulphates are added to increase oxidizing power and produce lighter shades of hair color. They exist as the salts ammonium persulphate, potassium persulphate, and sodium persulphate.

Persulphates can induce both immediate and delayed reactions that include allergic and irritant contact dermatitis, contact urticaria, asthma, rhinitis, and even anaphylaxis (5,26,94–96). Immediate reactions to persulphates predominantly occur among hairdressers, but have also been reported in workers producing persulphates (97,98) and consumers of hair bleaching products (26,99).

As with most low-molecular weight substances, the mechanism by which persulphates induce immediate reactions is not fully understood. Some studies suggest an IgE-mediated mechanism (94,98,100). Others propose the involvement of T-cells (101,102), whilst yet others have suggested that oxidative events are crucial in the development of persulphate-induced asthma and rhinitis (103). Suspected cases may be confirmed using a variety of tests, including the skin prick test (SPT), the histamine release test (HRT), and the specific inhalation challenge (SIC).

Specific inhalation challenge with persulphates

The SIC is considered the 'reference standard' for diagnosing occupational rhinitis and asthma (104,105). It is a diagnostic tool for assessing the response of an individual's airways to a suspected occupational agent in a controlled and monitored environment. In this thesis, the term SIC covers both the specific bronchial provocation tests and specific nasal provocation testing.

The 'realistic approach', which attempts to mimic working conditions, was developed in the 1970s (106). It involves the patient being exposed to the suspected occupational agent inside a specially designed provocation chamber. The occupational agents are tested in the form they occur in the workplace, e.g. liquid substances can be nebulized inside the provocation chamber and powdered substances can be administered by the 'tipping method' where powder is tipped from one tray into another by the patient. The SIC can also be performed using a 'non-realistic approach', where the patient is exposed to the substance in aerosol form using a mask or a mouth piece.

The SIC has been performed with bleaching powder since 1976 using different approaches (see Table 2). These include the 'realistic approach' where bleaching powder mixed with hydrogen peroxide (H_2O_2) (107) or persulphate powder mixed with lactose powder (108) is tipped from one tray to another inside a provocation chamber. The SIC has also been performed using the 'non-realistic approach' where aqueous solutions of persulphate are administered using a nebulizer (with a dosimeter when testing for asthma) (109), or by spraying the solution directly into the nose for a specific nasal provocation test (102). An overview of SIC methods using bleaching powder and persulphates can be found in Table 2.

Muñoz et al. validated the SIC using potassium persulphate and the 'realistic approach' by testing eight well-defined cases and a control group of similar size (108). In this validated method, the patients were exposed to a mixture of persulphate powder and lactose powder using the 'tipping method'. The exposures were performed in increments using increasing doses of persulphate over four consecutive days. On the first day, the patient was exposed for 10 min to 5 g of potassium persulphate mixed with 150 g of lactose powder. If no bronchial reaction occurred during the next 24 h, the patient was exposed to 10 g of potassium persulphate on the next day. The maximal exposure on the fourth day was 30 g of potassium persulphate for 10 min. A sensitivity of 100% and a specificity of 87.5% were reported. However, since this method takes four days to complete and the patients must be admitted to hospital during the entire procedure, the validated method is costly and time consuming for both investigator and patient. Consequently, not all centers have the facilities to perform the SIC in this manner. Therefore, with a focus on Muñoz' validated method, we attempted to establish a more rapid, but still safe, SIC using potassium persulphate to test for both asthma and rhinitis simultaneously in a single day. In addition, we were concerned that the 'tipping method' was difficult to standardize, so we tested a new 'stirring method' to examine whether this could create a reproducible level of exposure to persulphate.

Table 2. Overview of studies performing specific inhalation challenges with persulphates or bleaching powder

Year/Author	Subjects	Test substance	Exposure duration	Exposure type	Results
1976 Pepys, J. (73)	Cases: 2 hairdressers with WR asthma and rhinitis	25 g bleach powder + H_2O_2 (20%) and 30 g PP + H_2O_2	1 day 4 min and 30 min respectively	Mixing bleach powder with H_2O_2 in a mortar	SIC bleach powder: 2/2 (Immediate asthmatic reaction 1 min after exposure and non-immediate asthmatic reaction 1.5–2 hours) SIC PP: 0/2
1986 Blainey, AD. (110)	Cases: 14 hairdressers with WR asthma symptoms Controls: 4 hairdressers without WR symptoms, 4 atopic subjects	30 g bleach powder + 50 g lactose powder	1 day 10 min	Tipping method. In provocation chamber	Cases: 4/14 (All late asthmatic reactions, typically 2–4 h after exposure) Controls: 0/8
1992 Parra, FM. (111)	Case: 1 hairdresser with WR asthma symptoms Controls: 2 asthmatic subjects	Aqueous solution of PP extract	1 day 2 min at tidal volume	Aerosolized with nebulizer. Inhaled through dosimeter	Case: 1/1 (non-immediate asthmatic reaction 2 h after exposure). Controls: 0/2
1997 Schwaiblmair, M. (107)	Cases: 46 hairdressers with WR asthma and rhinitis symptoms	50 g bleach powder + H_2O_2	1 day 1 h	Tipping method. In provocation chamber	9/46 (5 immediate reactions, 4 dual reactions)
1997 Hytönen, M. (25)	Cases: 40 hairdressers with WR rhinitis symptoms	NPS with aqueous solution of AP	1 day 30–45 min	Sprayed directly into the nose	2/40
1998 Leino, T. (31)	Cases: 10 hairdressers with WR asthma symptoms	SIC with bleach powder, NPT with AP 0.1%	-	- In provocation chamber	NPT 6/10 SIC 2/10
2003 Muñoz, X. (94)	Cases: 7 patients with WR asthma and rhinitis symptoms Controls: 3 asthmatic subjects, 3 healthy subjects	PP (5, 10, 15, and 30 g) + lactose powder (150 g)	4 days 10 min per day. Incremental increase in dose	Tipping method. In provocation chamber	Cases: 7/8 (1 early asthmatic response, 5 late responses, 1 dual response) Controls: 0/6
2004 Muñoz, X. (108)	Cases: 8 patients with WR asthma symptoms Cases: 8 asthmatic controls, 10 healthy controls	PP (5, 10, 15, and 30 g) + lactose powder (150 g)	4 days 10 min per day. Incremental increase in dose	Tipping method. In provocation chamber	Cases: 8/8 Asthmatic controls: 1/8 Healthy controls: 0/10
2005 Moscato, G. (11)	Cases: 47 hairdressers with WR asthma and rhinits	Aqueous solution of AP	1 day 30 min	Aerosolized with nebulizer in provocation chamber	SIC: 21/47 NPT: 11/47
2006 Harth, V. (112)	Case: 1 hairdresser with WR asthma symptoms	AP dissolved in PBS. (0.0004 mg, 0.0045 mg, 0.045 mg and 0.45 mg = cumulative dose of 0.5 mg)	-	Aerosolized with nebulizer. Inhaled through dosimeter	1/1 (isolated late asthmatic reaction after 210 min)
2011 Pala, G. (113)	Case: 1 hairdresser with WR asthma and rhinitis symptoms	Aqueous solution of AP (8 mg in 3 ml distilled water)	1 day cumulative exposure of 240 min.	Aerosolized with nebulizer in provocation chamber	0/1 The patient was diagnosed with non-asthmatic eosinophilic bronchitis
2012 Herin, F. (114)	Case: 1 hairdresser	Bleaching substances	1 day. Consecutive exposure (2, 3, 5 and 10 min) with 10 min pauses in between.	Preparing of bleaching substance 30 cm away from face inside provocation chamber	0/1 Upper airway endoscopy revealed irritant-associated vocal cord dysfunction syndrome
2015 Hagemeyer, O. (109)	Cases: 8 hairdressers	AP dissolved in PBS. Either as 4-step protocol (0.01, 0.10, 1.00, 10 mg/mL) or 6-step protocol (0.010, 0.039, 0.156, 0.625, 2.5, 10 mg/mL)	1 day. Exposures to increasing doses.	Aerosolized with nebulizer. Inhaled through dosimeter	4/8 (2 from 4-step protocol and 2 to 6-step protocol). All show isolated late reactions (onset ≥ 2 h after end of exposure).

AP: ammonium persulphate NPT: nasal provocation test PBS: phosphate buffered saline PP: potassium persulphate SIC: specific inhalation challenge WR: work-related
-: Not described in the text

Skin prick tests and scratch tests with persulphates

The scratch test with persulphates was first described in 1963 (115). Since then, several studies have performed scratch tests and later SPTs with persulphates (see Table 3). It is not clear, why the scratch test was abandoned and replaced by the SPT, but this could be because the SPT is easier to standardize. There is no general consensus on the best choice of allergen (ammonium persulphate, potassium persulphate, or sodium persulphate), concentration, or solvent to use for the SPT with persulphates. The test has often been performed with one or two persulphates and concentrations have ranged from 0.05% to 23%. At present, the value of the test is unknown because some studies detect positive reactions, whilst others fail to do so. One of the aims of this thesis was to evaluate the value of SPT with persulphates to diagnose persulphate-induced asthma and rhinitis.

Table 3. Overview of studies performing skin prick tests with persulphates

Year/ author	Subjects	Test and substance	Conc. of persulphate	Solvent	Results (positive SPT/number tested)
1963 Calnan, S. (115)	Cases: 5 hairdressers with hand dermatitis and scalp irritation Controls: 57 healthy subjects	Scratch test AP	8.5%	Aqueous solution	Cases: 5/5 (after 15-30 min) Controls: 4/57
1976 Pepys, J. (73)	Case: 1 hairdresser with WR respiratory symptoms and urticaria	SPT Hair bleach	0.1—5%	-	1/1 to a component identified as persulphate
1976 Fisher, AA. (99)	Case: 1 hairdresser with WR respiratory symptoms	Scratch test AP	1% (freshly prepared)	Aqueous solution	1/1
1977 Widstrom, L. (116)	Case: 1 hairdresser with WR symptoms of rhinitis and hand eczema	Scratch test AP	1%	Aqueous solution	1/1 (after 5 min.)
1986 Blainey, AD. (110)	Cases: 14 hairdressers exposed to persulphates Controls: 6 healthy atopic subjects	SPT AP, PP	2.3 x10^{-5} –23% (AP) 2.7x10^{-5} –2.7% (PP)	Sterile water	Cases: 1/14 AP (conc. 23%) 1/14 PP (conc. 2.7%) Controls: 0/6 AP and PP
1992 Parra, FM. (111)	Case: 1 hairdresser with WR asthma and dermatitis Controls: 5 non-exposed atopic subjects and 5 healthy subjects	SPT SP, PP	20%	PBS (dialysed and non-dialysed)	Case: 1/1 PP and SP (dialysed and non-dialysed) Controls: 0/10 SP and PP
1995 Wrbitzky, R. (98)	Cases: 52 exposed industrial workers Controls: 13 non-exposed subjects	SPT AP, PP	1% and 5% (freshly prepared)	0.9% Saline	Cases: 8/52 (2/52 PP only) (3/52 AP only) (3/52 both PP and AP) Controls: 0/13 AP and PP
1996 Merget, R. (97)	Cases: 25 exposed industrial workers Controls: 18 non-exposed subjects	SPT AP, SP	8% (max. 3 days old in 4°C)	Distilled water and PBS	Cases: 0/25 AP and SP (both distilled water and PBS) Controls: 0/18
1997 Hytonen, M. (25)	Cases: 40 hairdressers with WR rhinitis symptoms	SPT AP	0.1% (freshly prepared weekly)	Aqueous solution	2/40
1997 Schwaiblmair, M. (107)	Cases: 54 hairdressers with asthma or rhinitis symptoms	SPT Hair bleach	1%	PBS	13/54
1998 Leino, T. (31)	Cases: 107 hairdressers with suspected WR skin and respiratory disease	SPT AP, PP	2%	-	4/107 PP 3/107 AP
1999 Yawalkar, N. (101)	Case: 1 hairdresser with WR respiratory symptoms and dermatitis Controls: 15 healthy subjects	SPT AP	1% and 5%	-	Case: 1/1 (after 24 hours) Controls: 0/15

Table 3 (continued). Overview of studies performing skin prick tests with persulphates

Year/ author	Subjects	Test and substance	Conc. of persulphate	Solvent	Results (positive SPT/number tested)
1999 Borelli, S. (117)	Case: 1 hairdresser with WR respiratory symptoms and dermatitis Controls: 10 atopic and 10 non-atopic subjects	SPT AP	2.5%	-	Case: 1/1 Controls: 0/20
2001 Rodriguez, JLE. (22)	Case: 1 consumer with CoU to hair dye product Controls: 4 healthy subjects	SPT PP	3.3% and 10%	PBS	Case: 0/1 (conc. 3.3%) 1/1 (conc. 10%) Controls: 0/4
2003 Aalto-Korte, K. (100)	Cases: 138 patients with suspected persulphate allergy Controls: 20 healthy subjects	SPT AP, PP	2% (freshly prepared)	Sterile water	Cases: 7/138 (1/138 PP only) (3/138 AP only) (3/138 both) Controls. 0/20 AP and PP
2005 Moscato, G. (11)	Cases: 14 hairdressers with OA verified by SIC	SPT AP	1% and 5% (freshly prepared)	Saline	0/14 (read at 20 min, 2 h, 4 h and 24 h)
2006 Harth, V. (112)	Case: 1 hairdresser with late asthmatic reaction in SIC to AP	SPT AP	0.1%	Saline and phenol	0/1
2008 Diab, KK. (102)	Cases: 15 hairdressers with WR rhinitis symptoms Controls: 14 asymptomatic hairdressers, 12 atopic subjects	SPT PP	0.05%, 0.1% and 0.5%	Sterile water	Cases: 0/15 Controls: 0/26
2008 Munoz, X. (118)	Cases: 10 patients with OA to persulphate diagnosed 3 years previously	SPT AP, PP	5%	PBS-buffered saline	5/10 at baseline (after 15 min) 2/10 at follow-up
2008 Figueiredo, JP. (119)	Case: 1 hairdresser with OR and OA to persulphate diagnosed by SIC	SPT AP, PP	2% and 5%	-	0/1
2009 Bregnhoj, A. (120)	Case: 1 hairdresser with WR hand eczema and asthma	SPT AP, PP	0.1%	Sterile water	0/1 PP 1/1 AP
2010 Moscato, G. (121)	Cases: 26 patients with OA and/or OR to persulphate diagnosed by SIC	SPT AP	1% and 5% (freshly prepared)	Saline	0/26
2011 Pala, G. (113)	Case: 1 hairdresser with non-asthmatic eosinophilic bronchitis to AP	SPT AP	-	-	0/1
2012 Hoekstra, M. (26)	2 cases a) with anaphylaxis b) with asthma, rhinitis and urticaria	SPT AP, PP	0.1%, 1% and 2%	Aqueous solution	a) 0/1 AP and PP b) 1/1 to AP and PP (conc. 1%)
2012 Diab, KK. (122)	Cases: 17 hairdressers with WR rhinitis symptoms Controls: 19 asymptomatic hairdressers,10 atopic subjects	SPT PP	0.05%, 0.1% and 0.5%	Sterile water	Cases: 0/17 Controls: 0/29
2013 Polychronakis, I. (123)	Case: 1 patient with late asthmatic reaction in SIC to AP	SPT AP	10%	-	0/1
2015 Hagemeyer O. (109)	Cases: 8 patients with suspected OA	SPT AP	2%	-	3/8
2016 Kleniewska M. (124)	Case: 1 ex-hairdresser persulphate-induced asthma, rhinitis, urticaria, and anaphylaxis	SPT AP, PP	0.001%	-	1/1 AP 1/1 PP

AP: ammonium persulphate CoU: contact urticaria OA: occupational asthma OR: occupational rhinitis PBS: phosphate buffered saline PP: potassium persulphate SIC: specific inhalation challenge SP: sodium persulphate SPT: skin prick test Conc.: concentration WR: work-related -: Not described in the text

Histamine release test with persulphates

The HRT is another potential diagnostic tool for examining patients with suspected persulphate-induced asthma and rhinitis. The HRT uses glass microfibers with high affinity for histamine to measures basophil histamine release in whole blood samples from patients with an IgE-mediated allergy (125). The HRT with persulphates has only been described for a single case (111), and the results were inconclusive. One of the aims of this thesis was to assess the value of the HRT with persulphates to diagnose persulphate-induced asthma and rhinitis.

OBJECTIVES

The overall aims of this PhD thesis were: 1) to investigate the epidemiology of occupational allergenic and irritant skin and respiratory diseases in Danish hairdressing apprentices and 2) to improve the current diagnostic tests for diagnosing patients with persulphate-induced asthma and rhinitis.

The specific aims of the individual parts of the study were as follows:

Study part I – Epidemiology of skin and respiratory diseases in hairdressing apprentices

- To determine the current prevalence and incidence of skin and respiratory diseases in Danish hairdressing apprentices.
- To examine whether hairdressing apprentices are at increased risk of skin and respiratory diseases compared with other young adults from the general population.
- To examine whether a healthy worker effect with respect to skin and respiratory diseases exists in hairdressing apprentices.

Study part II – Specific inhalation challenge with persulphate

- To establish a new rapid SIC to diagnose persulphate-induced asthma and rhinitis.

Study part III – Skin prick tests and histamine release test with persulphates

- To examine the value of the SPT and HRT with persulphates in well-defined cases of patients with persulphate-induced asthma and rhinitis confirmed by the SIC.

MANUSCRIPTS

Manuscript I

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Occupational skin diseases in hairdressing apprentices – has anything changed?

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Summary

Background. Hairdressers are at risk for occupational skin diseases. Since 2008, an educational programme has been conducted in Danish hairdressing schools to prevent occupational skin diseases. Its effect is unknown.

Objective. To examine the current frequency of self-reported hand eczema and contact urticaria in Danish hairdressing apprentices as compared with controls, and to determine the occurrence of hand eczema and contact urticaria in hairdressing apprentices with different durations of exposure to the trade.

Methods. This was a cross-sectional, web-based questionnaire study conducted among 504 hairdressing apprentices and a control group of 1400 adolescents from the general population.

Results. Hand eczema was significantly more prevalent in the hairdressing apprentices than in controls (34.5% versus 18.8%, $p < 0.001$). The incidence rate of hand eczema among hairdressing apprentices was 98 cases/1000 person-years. Contact urticaria was also more prevalent in the hairdressing apprentices (7.3% versus 4.2%, $p = 0.006$). Both diseases increased with increasing duration of exposure to the trade.

Conclusion. Despite educational efforts to prevent occupational skin diseases in the hairdressing schools, Danish apprentices are still at increased risk for hand eczema and contact urticaria. Both diseases develop after only a few years of work in hairdressing. Further preventive strategies are warranted.

Key words: contact urticaria; hairdressing apprentices; hand eczema; web-based questionnaire.

Hairdressers have daily exposure to various chemicals and allergens, and are consequently at risk for occupational skin diseases. In Danish hairdressers, a lifetime prevalence of hand eczema of 42–44% has been reported (1, 2). Hairdressing apprentices seem to have a particularly high (3) risk, with a reported lifetime prevalence of 27–58% (4–6).

Common hairdressing allergens in Denmark include *p*-phenylenediamine and toluene-2,5-diamine in hair dyes, and persulfates in bleaching products. Additionally, glyceryl monothioglycolate (GMTG) in permanent wave solutions (7, 8) is a frequent cause of contact dermatitis in hairdressers. Although the withdrawal of GMTG in some European countries has resulted in a reduction in GMTG allergy in these countries (9), GMTG is still used in permanent wave solutions in Denmark. In addition to the heavy allergen exposure, hairdressing apprentices frequently perform wet work, which strongly predisposes them to the development of irritant contact dermatitis.

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Since 2008, an education programme has been conducted in Danish hairdressing schools in order to prevent hand eczema. Whether it has had an effect is unknown.

In addition to hand eczema, hairdressers are at risk of developing contact urticaria (10–14). The causal agents may be persulfates, natural rubber latex proteins, hydrolysed wheat protein (15), or hair dye ingredients (16). According to Australian findings, hairdressers have one of the highest frequencies of occupational contact urticaria (12). However, few studies on contact urticaria in hairdressers have been published, and studies on contact urticaria in hairdressing apprentices have never been performed.

The objectives of this study were to examine the current prevalence and incidence of hand eczema in Danish hairdressing apprentices, and thereby evaluate whether hairdressing apprentices are still at significant risk of developing hand eczema after the introduction of the educational programme in 2008. We also investigated whether self-reported contact urticaria was more prevalent in hairdressing apprentices than in adolescents from the general population. Finally, we determined the occurrence of hand eczema and contact urticaria in hairdressing apprentices with different durations of exposure to the trade.

Materials and Methods

A cross-sectional, case–control questionnaire study was conducted among all hairdressing apprentices in Denmark and a control group comprising adolescents from the general population.

The study was approved by the Danish Data Protection Agency.

The Danish training programme for hairdressing apprentices

Hairdressing training in Denmark is provided by 10 governmentally supported vocational schools distributed evenly throughout the country. Apprenticeship takes 4 years: 60 weeks in the schools, and 148 weeks working in different hairdressing salons. Exposure to hair dyes, hair bleaches, permanent wave solutions and styling products starts within the first weeks of apprenticeship. Since 2008, several lessons have been given during the first 20 weeks of apprenticeship about chemistry and protective measures against occupational skin diseases.

Study population

All 10 vocational schools in Denmark were invited to participate in the questionnaire study; eight participated. Contact information on 1128 hairdressing apprentices was collected. We included apprentices at all levels of apprenticeship, including apprentices who had recently graduated (within 1 year). Both hairdressing apprentices training in schools and hairdressing apprentices currently training in salons were included.

For the control group, contact information on 2701 persons of similar age, sex and post code was obtained from the Danish Research Services (Statens Serum Institut, Copenhagen, Denmark).

In April–July 2013, 3829 invitation letters and two reminders for a web-based questionnaire (Enalyzer Survey Solutions) were sent by post to cases and controls. To increase the participation rate, participants were automatically enrolled in a prize draw.

Questionnaire

The web-based questionnaire contained 99 questions, of which approximately half concerned atopic dermatitis, hand eczema, contact urticaria, smoking status, education, and work. Questions on atopic dermatitis were based on the UK Working Party's criteria (17). Questions from the Nordic Occupational Skin Questionnaire (NOSQ) (18) were used to identify hand eczema and urticaria.

Definitions of outcome variables

Hand eczema was defined by an affirmative answer to the question: 'Have you ever had hand eczema?'

Incident hand eczema was defined as hand eczema with onset at the same time as starting current education or work or later.

Urticaria on the hands, wrists or forearms was defined as an affirmative answer to the question: 'Have you ever had itchy wheals appearing and disappearing rapidly (within hours) on your hands, wrists or forearms (urticaria or nettle rash)?'

Contact urticaria was defined as an affirmative answer to the question: 'Have these itchy wheals (urticaria) on your hands, wrists or forearms been caused by skin contact with rubber gloves, hair dyes, cosmetics or the like?'

Covariates

Atopic dermatitis was defined, according to the UK Working Party's diagnostic criteria (17), as the presence of one major criterion and two or more minor criteria.

Personal smoking status was recorded according to ECRHS III (19), and defined as never smoker, ex-smoker, and current smoker. Cigarettes/day were calculated for current smokers.

Hairdressing school takes 4 years in Denmark. For level of apprenticeship, the apprentices were grouped according to their current year of apprenticeship.

Statistical analysis

The statistical program IBM SPSS™ version 19 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. A chi-squared test was used for binary data, and a *t*-test was used for normally distributed continuous data. A two-sided *p*-value of ≤ 0.05 was considered to be statistically significant.

Binary logistic regression analyses were performed to study the development of hand eczema and contact urticaria in hairdressing apprentices at different levels of apprenticeship. Adjustment was made for age, sex, atopic dermatitis, and smoking status. Associations were expressed as odds ratios (ORs) and their accompanying 95% confidence interval (CIs). Hairdressing apprentices who had dropped out of hairdressing school were not included in regression analyses.

Results

Of the 3829 invited persons (1128 hairdressing apprentices and 2701 controls), 1908 successfully completed the questionnaire (505 hairdressing apprentices and 1403 controls). Controls with previous exposure to hairdressing work were excluded from the study. One respondent from the case group was excluded because of a language barrier. This left 1904 respondents for analyses.

The overall participation rate was 49.8% (1904/3825). There were no major differences in age, sex or geographical distribution between responders and non-responders.

The participation rate was significantly lower for cases than for controls (Table 1). Both groups consisted mainly of females. The median age was 22 years in both groups. The geographical distribution of cases and control was similar (results not given). Current smoking was significantly more common in hairdressing apprentices than in controls.

Of the hairdressing apprentices, 18.1% were first-year apprentices, 20.0% were second-year apprentices, 20.2% were third-year apprentices, 21.6% were fourth-year apprentices, 19.0% had recently graduated, and 5 (1%) had dropped out of school since their information was provided by the schools.

Occurrence of hand eczema

The prevalence of hand eczema was significantly higher in hairdressing apprentices across all groups (34.5%) than in controls (18.8%) ($p < 0.001$) (Table 2). Hairdressing apprentices were older than controls at the onset of hand eczema (18 versus 15 years, $p < 0.001$). Among the participants reporting hand eczema, hairdressing apprentices had a significantly higher proportion of incident hand eczema (Table 2). The incidence of hand eczema was 98 cases/1000 person-years.

Table 1. Main characteristics of participants

	Hairdressing apprentices (n = 504)	Controls (n = 1400)	OR (95% CI)*	*p*-value
Participation rate, % (no.)	44.7 (504/1127)	51.9 (1400/2698)	**0.75 (0.65–0.86)**	**< 0.001**
Female, % (no.)	94.6 (477/504)	95.7 (1340/1400)	0.79 (0.50–1.26)	0.323
Age (years), mean (SD)	22 years (3.8)	22 years (4.1)	–	0.545
Never-smoker, % (no.)	56.5 (285/504)	71.6 (1002/1400)	**0.52 (0.42–0.64)**	**< 0.001**
Current smoker, % (no.)	28.4 (143/504)	17.2 (241/1400)	**1.91 (1.50–2.42)**	**< 0.001**
Ex-smoker, % (no.)	15.1 (76/504)	11.2 (157/1400)	**1.41 (1.05–1.89)**	**0.023**
Cigarettes/day, mean (SD)	10.04 (5.6)	9.45 (6.3)	–	0.353
Level of apprenticeship				
None, no. (%)	–	1400 (100)		
First year, % (no.)	18.1 (91)	0		
Second year, % (no.)	20.0 (101)	0		
Third year, % (no.)	20.2 (102)	0		
Fourth year, % (no.)	21.6 (109)	0		
Recently graduated, % (no.)	19.0 (96)	0		
Dropped out, % (no.)	1.0 (5)	0		

CI, confidence interval; OR, odds ratio; SD, standard deviation.
Bold type indicates statistical significance.
*Crude OR for cases as compared with controls.

Table 2. Hand eczema and urticaria in hairdressing apprentices as compared with controls

	Hairdressing apprentices (n = 504)	Controls (n = 1400)	Crude OR (95% CI)	*p*-value
Hand eczema	34.5 (174/504)	18.8 (263/1400)	**2.3 (1.8–2.9)**	**< 0.001**
Incident hand eczema	78.2 (136/174)	24.0 (63/263)	**11.4 (7.2–18.0)**	**< 0.001**
Age at onset of hand eczema (years), mean (SD)	18 (4.7)	15 (6.3)	–	**< 0.001**
Urticaria on hands, wrists, or forearms	23.2 (117/504)	24.3 (340/1400)	0.9 (0.7–1.2)	0.63
Contact urticaria	7.3 (37/504)	4.2 (59/1400)	**1.8 (1.2–2.8)**	**0.006**

CI, confidence interval; OR, odds ratio; SD, standard deviation.
Values are % (n/N) unless otherwise stated.
Bold type indicates statistical significance.

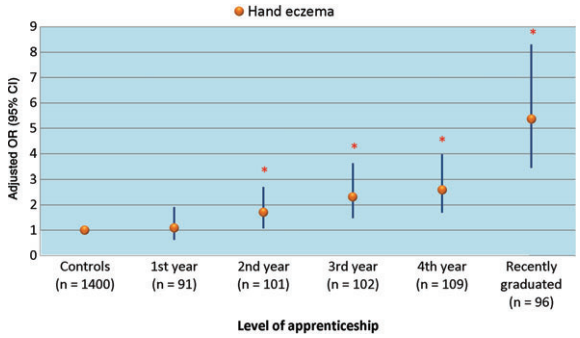

Fig. 1. The association between hand eczema and level of apprenticeship was studied with a binary regression analysis. Adjustment was performed for sex, age, level of apprenticeship, atopic dermatitis, and smoking status. Results are expressed as adjusted odds ratios (ORs) with accompanying 95% confidence intervals (CIs). ORs marked with an asterisk are statistically significantly different from controls.

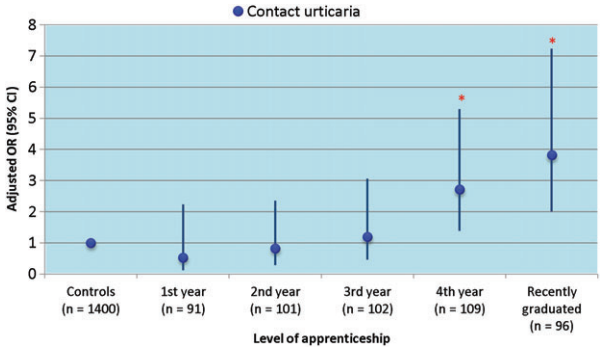

Fig. 2. The association between contact urticaria and level of apprenticeship was studied with a binary regression analysis. Adjustment was performed for sex, age, level of apprenticeship, atopic dermatitis, and smoking status. Results are expressed as adjusted odds ratios (ORs) with accompanying 95% confidence intervals (CIs). ORs marked with an asterisk are statistically significantly different from controls.

Occurrence of urticaria

The prevalence of urticaria on the hands, wrists or forearms was similar in hairdressing apprentices and controls (Table 2). However, self-reported contact urticaria caused by skin contact with rubber chemicals, hair dyes, cosmetics or the like was significantly more prevalent in hairdressing apprentices, with a frequency of 7.3% as compared with 4.2% in controls ($p = 0.006$).

Effect of level of apprenticeship on hand eczema and contact urticaria

The prevalence of hand eczema increased with increasing level of apprenticeship: 18.7% of first-year apprentices, 29.7% of second-year apprentices, 33.3% of third-year apprentices, 36.7% of fourth-year apprentices, and 53.1% of recently graduated hairdressing apprentices ($p < 0.001$).

The risk of hand eczema increased with increasing level of apprenticeship (Fig. 1).

Apprentices in the second year of hairdressing school had almost twice as high an OR for hand eczema as adolescents from the general population (OR 1.7, 95% CI 1.07–2.70). Recently graduated hairdressers had a more than five times higher OR than controls (OR 5.34, 95% CI 3.44–8.30).

Neither age nor smoking was associated with an increased risk of hand eczema. Atopic dermatitis was a risk factor for hand eczema (OR 2.75, 95% CI 2.19–3.45).

The prevalence of contact urticaria also increased with increasing level of apprenticeship: 2.2% of first-year apprentices, 4.0% of second-year apprentices, 4.9% of third-year apprentices, 11.0% of fourth-year apprentices, and 14.6% of recently graduated hairdressing apprentices ($p < 0.001$).

The risk of contact urticaria increased with increasing level of apprenticeship (Fig. 2).

Apprentices in the fourth year of hairdressing school had an almost three times higher OR for contact urticaria than adolescents from the general population (OR 2.71, 95% CI 1.39–5.30). Recently graduated hairdressers had an almost four times higher OR than controls (OR 3.81, 95% CI 2.01–7.23).

Atopic dermatitis was a risk factor for contact urticaria (OR 2.69, 95% CI 1.77–4.11). Current smoking tended to increase the risk of contact urticaria (OR 1.57, 95% CI 0.97–2.53). Age was not associated with an increased risk of contact urticaria.

Discussion

Hand eczema is a well-recognized problem in hairdressing, and there have been several efforts to reduce its incidence. During 2008–2010, a clinically controlled prospective intervention study was conducted (20), and showed that the implementation of a training programme in Danish hairdressing schools resulted in a significantly reduced incidence of hand eczema in the intervention group as compared with controls. As a result, hairdressing schools in Denmark have, since then, provided several lessons on chemistry and protective measures in hairdressing during the first 20 weeks of apprenticeship. In spite of these efforts, we show that hand eczema is still a substantial problem for hairdressing apprentices in Denmark. In the first year of apprenticeship, the risk of hand eczema has increased, and in the second year of apprenticeship the OR has significantly increased to almost double that of adolescents from the general population. In 2009, 49.4% of hairdressing apprentices in Denmark had experienced hand eczema (1). This prevalence is comparable to our data on hairdressing apprentices in the third and fourth years of apprenticeship.

Our findings underline the need for further efforts to reduce hand eczema in hairdressing apprentices. Educating apprentices in the schools is a welcome effort; however, two-thirds of the apprenticeship is spent training in the salons, and this needs to be considered in any effective prevention strategy.

We report here an incidence of 98 cases/1000 person-years for hand eczema in Danish hairdressing apprentices. This incidence is much higher than that reported for trained Swedish hairdressers younger than 25 years (37.1 cases/1000 person-years) (21). However, it is much lower than that reported by Uter et al. in 1999 (343 cases/1000 person-years) among German hairdressing apprentices (6) and that reported by Smit et al. in 1994 (22) (328 cases/1000 person-years). Unfortunately, study methods vary between the four studies, which makes comparison of results difficult.

The questions used to examine self-reported hand eczema in this study have previously proven to be in good agreement with clinical examination, and are considered to constitute a valid method for estimating the prevalence of hand eczema, although they might underestimate the true prevalence (1, 23).

This is the first study to investigate contact urticaria in hairdressing apprentices. A previous study investigating contact urticaria in trained hairdressers reported a prevalence of 16% (24). This is comparable to our findings among apprentices in the fourth year of apprenticeship and recently graduated hairdressers. Contact urticaria in hairdressing apprentices has not been identified before, and further studies should be performed to substantiate the findings and investigate the consequences.

The association between hairdressers' occupational skin diseases and smoking remains controversial. In line with previous investigations (25, 26), we found that smoking is more common in hairdressing apprentices than in controls. Smoking seems to play a role in the development of contact urticaria, but was not associated with hand eczema.

The defective skin barrier seen in atopic dermatitis is hypothesized to predispose to allergic reactions of both type 1 (27) and type 4 (28). As expected, we found that atopic dermatitis was a strong risk factor for the development of hand eczema and contact urticaria in both hairdressing apprentices and controls. More precisely, the risk of hand eczema and contact urticaria increased almost threefold in participants with a history of atopic dermatitis. Knowledge of this strong association is important, as it enables career guidance for young people with concurrent atopic dermatitis and a desire to become hairdressers.

A majority of the questions used in this study were adopted from previously validated questionnaires. In order to make the question concerning contact urticaria more relevant for the hairdressing apprentices, it was slightly changed from the original version by the NOSQ: 'Have these itchy wheals (urticaria) on your hands, wrists or forearms been caused by skin contact with fruits, vegetables, rubber gloves, animals, etc.?' to 'Have these itchy wheals (urticaria) on your hands, wrists or forearms been caused by skin contact with rubber gloves, hair dyes, cosmetics or the like?' This modification of the question has potentially introduced a bias, as hairdressing apprentices inevitably have more exposure to rubber gloves, hair dyes, cosmetics or the like than adolescents from the general population. They might therefore have been more prone to answer 'yes' to this question.

In Denmark, internet access has been available to > 80% of the population since 2009 (29). Therefore, we believed that distribution of questionnaires via the

internet was suitable for the study. However, achieving a high response rate can be challenging in web-based questionnaire studies (29), so we tried to overcome this obstacle by enrolling respondents in a prize draw. This may also have limited the potential bias resulting from the fact that sick individuals are more prone to answer questionnaires on health. The response rate may be a limitation of the study. However, as the available demographic data do not differ between respondents and non-respondents, we see no immediate reason to believe that the two groups differ from each other.

Conclusion

In spite of efforts to prevent hand eczema by means of education in the hairdressing schools, hairdressing apprentices are still at increased risk for hand eczema. Hairdressing also seems to increase the risk for contact urticaria. Both hand eczema and contact urticaria develop after only few years of work in the hairdressing trade. Further preventive strategies to reduce occupational skin diseases in hairdressing apprentices are warranted, especially in the hairdressing salons.

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Manuscript II

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High occurrence of rhinitis symptoms in hairdressing apprentices

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Background: **Little is known about the occurrence of respiratory symptoms among hairdressing apprentices during their training. Therefore, in this study, we examined whether hairdressing apprentices are at increased risk of rhinitis and asthma symptoms when compared with other young adults from the general population.**

Methods: **A questionnaire was completed by 504 hairdressing apprentices and 1400 control participants from the general population with a similar age, gender, and geographic distribution.**

Results: **The 1-year prevalence of rhinitis symptoms was higher in hairdressing apprentices than in controls (58.1% vs 46.6%; odds ratio, 1.59; 95% confidence interval, 1.30-1.98), and the prevalence was higher among hairdressing apprentices in the last years of training compared with apprentices in the first year of training (62.4% vs 41.8%, $p = 0.003$). Current smoking was more common in hairdressing apprentices (28.4% vs 17.2%, $p < 0.001$). Asthma symptoms were equally common in the 2 groups; however, hairdressing apprentices had a later age of onset of wheezing than did the controls (18 years vs 14 years, $p < 0.00001$) and a decreased risk of wheezing (odds ratio, 0.72; 95% confidence interval, 0.54 to 0.95) after adjusting for smoking, education level, and degree of rurality. Bleaching products were the most frequently reported cause of rhinitis and asthma symptoms in hairdressing apprentices.**

Conclusions: **Hairdressing apprentices seem to have an increased risk of occupational rhinitis, and bleaching products are the main cause of respiratory symptoms. In addition, our findings suggest that a healthy worker effect exists in relation to asthma among hairdressing apprentices.** © 2016 ARS-AAOA, LLC.

Key Words:
hairdressers; hairdressers apprentices; wheeze; occupational asthma; occupational rhinitis; rhinitis symptoms; self-reported respiratory symptoms

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Airborne exposure to irritant and allergenic chemicals from hairdressing increases the risk of occupational asthma and rhinitis among hairdressers. Surveillance studies based on occupational disease reporting from physicians[1–6] showed that hairdressers were among the top 5 occupational groups with the highest risk of occupational asthma, with incidence rates of 0.2 to 3.7 cases per 10,000 person-years. Because hairdressers tend to underreport suspected occupational diseases,[7] the actual occurrence is probably higher.

Conversely, in contrast to occupational asthma, occupational rhinitis has received little attention, with the exception of 2 Finnish studies.[8,9] One of these reported a prevalence of occupational rhinitis in trained hairdressers of 1.7%,[8] and the other noted that hairdressers were among the top 13 occupational groups with the highest risk of occupational rhinitis.[9]

The incidence of work-related respiratory symptoms in high-risk occupations is greatest during the first years

after starting exposure.[10,11] Thus, it is important to identify respiratory symptoms in apprentices. Unfortunately, little is known about respiratory symptoms among hairdressing apprentices during training, and the only study conducted[12] did not address rhinitis.

Therefore, we performed a study to investigate whether hairdressing apprentices have an increased risk of symptoms suggestive of rhinitis and/or asthma compared with a control group comprising young adults from the general population.

Patients and methods

A cross-sectional, web-based questionnaire was used to survey participants in Denmark. The study was approved by the Danish Data Protection Agency.

Study population

Hairdressing training in Denmark is provided by 10 governmentally supported vocational schools distributed evenly throughout the country. Apprenticeship takes 4 years: 60 weeks of training in a hairdressing school and 148 weeks of training in different hairdressing salons. Exposure to all hairdressing chemicals starts within the first weeks of apprenticeship.[13]

All 10 schools in Denmark were invited to participate in the study, and 8 schools comprising 1128 hairdressing apprentices agreed to participate. Apprentices of all levels (first to fourth year), including those recently graduated (within 1 year), were invited to participate.

A comparable control group of more than twice the size was obtained via the Danish Central Personal Registry (Ministry of Internal Affairs). Civil registration numbers were used to identify 2701 people with similar characteristics (age, gender, and postcodes).

Questionnaire and outcome variables

The questions concerning respiratory health were selected from the European Community Respiratory Health Survey (ECRHS) questionnaire.[14] A Danish translation was provided by the Danish ECRHS group. Results regarding skin diseases in this cohort are presented elsewhere.[15]

Invitation letters (n = 3829) and reminders (n = 2) were sent by post between April and July 2013. To increase participation, responders were enrolled into a prize draw ($730/670€).

Questionnaire items considered relevant for our outcomes are as follows: (1) Have you had a problem with sneezing or a runny or blocked nose when you did not have a cold or the flu in the last 12 months? (2) Has this nose problem been accompanied by itchy or watery eyes? (3) Have you had wheezing or whistling in your chest at any time in the last 12 months? (4) Have you been at all breathless when the wheezing noise was present? (5) How old were you when you first experienced wheezing or whistling in your chest? (6) Have you been woken by an attack of coughing at any time in the last 12 months? (7) Have allergies or respiratory problems affected your choice of education/work? (8) Do these nasal problems/respiratory problems improve or stop during the weekend or holidays? (9) When you are near (hair bleaching/hair coloring/hairspray/permanent wave solutions) do you ever: Start to cough? Start to wheeze? Get a feeling of tightness in your chest? Start to feel short of breath? Get a runny or stuffy nose or start to sneeze? Get itchy or watering eyes?

Smoking status was divided into 3 categories: never smoker; former smoker; and current smoker.

The current education level of participants was classified according to the Danish International Standard Classification of Education (DISCED 15). A lower DISCED number indicated a lower education level. A DISCED 90 refers to "Not given or no education."

Participants were classified as living in "urban municipalities," "intermediate municipalities," "rural municipalities," or "peripheral municipalities."[16]

Statistical analyses

The statistical program IBM SPSS version 19 (SPSS, Inc., Chicago, IL) was used for statistical analyses. A chi-square test was used for binary data, and independent samples *t* tests were used for normally distributed, continuous variables. A 2-sided $p \leq 0.05$ was considered statistically significant.

A binary logistic regression was performed to compare the risk of respiratory symptoms in never smokers, former smokers, and current smokers. Never smokers were used as the comparison group. Binary logistic regressions were performed to assess for interaction between smoking status and hairdresser status for the presence of rhinitis and asthma symptoms. As shown in Table 1, adjusted binary logistic regression was performed to compare the risk of respiratory symptoms between hairdressing apprentices and controls. Participants having an education level of DISCED 90, never having smoked, and living in a peripheral municipality comprised the comparison group.

Results

Respondents and nonrespondents

Of the 3829 potential participants (1128 hairdressing apprentices and 2701 controls), 1904 successfully completed the questionnaire (504 hairdressing apprentices [44.7%] vs 1400 controls [51.9%], $p < 0.001$), giving an overall response rate of 49.7%. The characteristics of respondents and nonrespondents are summarized in Table 2.

Main characteristics of participants

The mean age and the degree of rurality were similar between the 2 groups. The demographic characteristics of participants are presented in Table 1.

TABLE 1. Main characteristics of study participants

	Hairdressing apprentices (n = 504)	Controls (n = 1400)	*p* value
Participation rate, % (n/N)	44.7 (504/1128)	51.9 (1400/2698)	<0.001[a]
Female, % (n)	94.4 (476)	95.7 (1340)	0.24
Mean age in years (SD)	22.0 (3.8)	22.0 (4.1)	0.55
Smoking status, % (n)			
Never smoker	56.5 (285)	71.6 (1002)	<0.001[a]
Current smoker	28.4 (143)	17.2 (241)	<0.001[a]
Ex-smoker	15.1 (76)	11.2 (157)	0.02
Cigarettes/day, mean (SD)	10.04 (5.6)	9.45 (6.3)	0.35
Level of apprenticeship, % (n)			
First year	18.1 (91)		
Second year	20.0 (101)		
Third year	20.2 (102)		
Fourth year	21.6 (109)		
Recently graduated	19.1 (96)		
Dropped out	1.0 (5)		
Current educational level, % (n)[b]			
DISCED 20		3.1 (43)	
DISCED 30	100 (504)	46.4 (650)	
DISCED 50		4.1 (58)	
DISCED 60		24.2 (339)	
DISCED 70		4.6 (64)	
DISCED 80		0.4 (5)	
DISCED 90[c]		12.7 (241)	
Degree of rurality, % (n)			
Urban municipality	49.6 (250)	55.1 (772)	0.12
Intermediate municipality	12.5 (63)	10.1 (141)	
Rural municipality	27.2 (137)	25.6 (359)	
Peripheral municipality	10.7 (54)	9.1 (128)	

[a]Statistically significant ($p < 0.05$).
[b]A lower DISCED number indicates a lower educational level.
[c]DISCED 90 refers to "Not given or no education."
DISCED = Danish International Standard Classification of Education; SD = standard deviation.

Smoking

Current smoking was more common among hairdressing apprentices than controls (28.4% vs 17.2%, $p < 0.001$; Table 1), and hairdressing apprentices smoked more than controls with a similar education level (28.4% vs 17.4%, $p < 0.001$; data not shown). In all participants, former smoking was associated with "sneezing or runny or blocked nose" (odds ratio [OR], 1.64, 95% confidence interval [CI], 1.24 to 2.18), and both current smoking and former smoking were associated with "wheezing or whistling in your chest" (current smoker: OR, 3.19; 95% CI, 2.50 to 4.10; former smoker: OR, 1.47; 95% CI, 1.07 to 2.03). There was no interaction between smoking status and being a hairdressing apprentice on the prevalence of rhinitis or asthma symptoms (data not shown).

Rhinoconjunctival symptoms

The 1-year prevalence of respiratory symptoms is shown in Table 3. The prevalence of sneezing or a runny or blocked nose was significantly higher in hairdressing apprentices

TABLE 2. Main characteristics of respondents and nonrespondents

	Respondents (n = 1904)	Nonrespondents (n = 2701)	*p* value
Mean age (SD)	22.0 (4.0)	22.4 (4.4)	0.002
Females, %	95.4	93.1	0.003
Degree of rurality, %			
Urban municipality	53.7	55.3	
Intermediate municipality	10.7	10.5	0.07
Rural municipality	26.1	23.2	
Peripheral municipality	9.6	11.0	

SD = standard deviation.

(58.1%) than in controls (46.6%; OR, 1.6; 95% CI, 1.3 to 1.9). The difference persisted after adjusting for smoking status, education level, and degree of rurality (OR, 1.6; 95% CI, 1.3 to 2.1). The prevalence of rhinitis symptoms was also higher in hairdressing apprentices than in controls with a similar educational level (DISCED 30; 58.1% vs 45.7%, $p < 0.001$). Accompanying conjunctival symptoms were also more prevalent among hairdressing apprentices, but this did not reach statistical significance (OR, 1.2; 95% CI, 0.9 to 1.4). Of the participants with rhinitis symptoms, more hairdressing apprentices than controls had an improvement during weekends and holidays (43.4% vs 18.5%, $p < 0.001$).

Rhinitis symptoms were significantly more prevalent in apprentices with >2 years of training compared with apprentices in the first year of training (Figure 1). Although not significant, hairdressing apprentices in the first year of training reported fewer rhinitis symptoms than controls (41.8% vs 46.6%, $p = 0.37$; data not shown).

Asthma symptoms

Hairdressing apprentices and controls reported a similar prevalence of wheezing (25.3% vs 26.2%; Table 3). Nevertheless, the adjusted regression analysis showed a negative association between wheezing and being a hairdressing apprentice (OR, 0.8; 95% CI, 0.6-1.0). Among participants reporting wheezing, hairdressing apprentices had a significantly later onset than controls (18 years vs 14 years, $p < 0.00001$). Hairdressing apprentices attending the first year of training had a lower prevalence of wheezing compared with controls (22.0% vs 26.2%, $p = 0.37$), but the numbers did not reach significance (data not shown). Improvement of wheezing during weekends and holidays was significantly more common among hairdressing apprentices (8.8% vs 5.1%, $p = 0.003$).

TABLE 3. The 1-year prevalence of respiratory symptoms among participants*

	Hairdressing apprentices (n = 499[a])	Controls (n = 1400)	*p* value	Crude OR (95% CI)	Adjusted OR[b] (95% CI)	Adjusted OR[c] (95% CI)
Sneezing, or runny, or blocked nose without cold or the flu during last 12 months, %	58.1	46.6		**1.6 (1.3–1.9)**	**1.6 (1.3–1.9)**	**1.6 (1.3–2.1)**
Above plus itchy or watery eyes, %	31.9	28.8		1.2 (0.9–1.4)	1.1 (0.9–1.4)	1.1 (0.9–1.5)
Wheezing or whistling in the chest in the last 12 months, %	25.3	26.2		1.0 (0.8–1.2)	**0.8 (0.6–1.0)**	**0.8 (0.6–1.0)**
Wheezing with breathlessness, %	8.6	10.9		0.8 (0.5–1.1)	**0.7 (0.5–1.0)**	0.9 (0.6–1.3)
Age of onset of wheezing in years, mean (SD)	18.1 (4.8) (n = 108[d])	14.4 (6.5) (n = 283[d])	**<0.00001**	—	—	—
Woken by attack of coughing during last 12 months, %	38.7	39.9		1.0 (0.8–1.2)	0.9 (0.7–1.1)	**0.8 (0.6–1.0)**
Choice of education/work affected by consideration of allergies or respiratory problems, %	7.8	3.6		**2.3 (1.5–3.5)**	—	**1.8 (1.1–3.1)**

*Bold values indicate statistically significant differences.
[a]The 5 hairdressing apprentices who had dropped out of school are not included.
[b]Adjusted for smoking status.
[c]Adjusted for smoking status, education level, and degree of rurality.
[d]The count is lower than expected because some participants answered "Don't know."
CI = confidence interval; OR = odds ratio; SD = standard deviation.

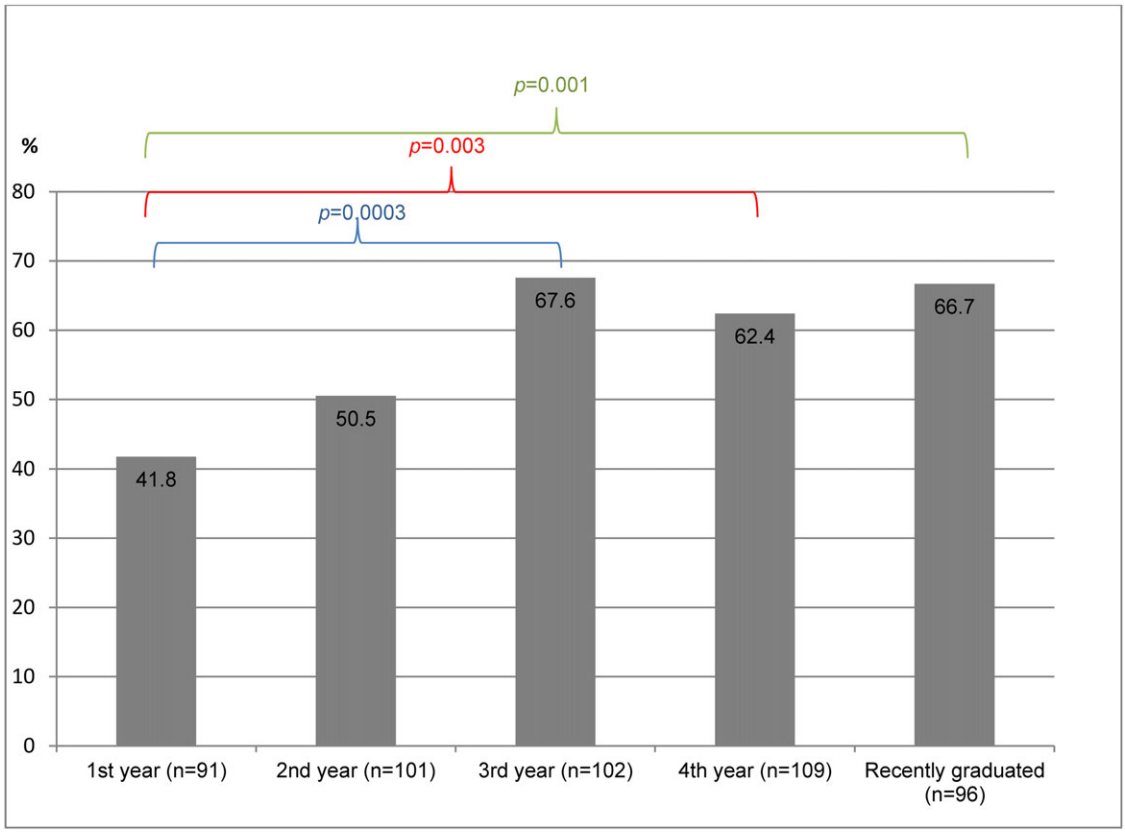

FIGURE 1. Comparison of the 1-year prevalence of rhinitis symptoms between 499 hairdressing apprentices by stage of training. Individual bars represent the prevalence of rhinitis symptoms among hairdressing apprentices by level of training. A binary regression analysis was performed with rhinitis symptoms as the dependent variable and level of apprenticeship as the independent variable. The model was adjusted for age and hairdressing apprentices in their first year of training were used as the comparison group. The *p* values indicate a significant increase in the prevalence of rhinitis symptoms between hairdressing apprentices in the first year of training and hairdressing apprentices in the third or fourth year of training and recent graduates.

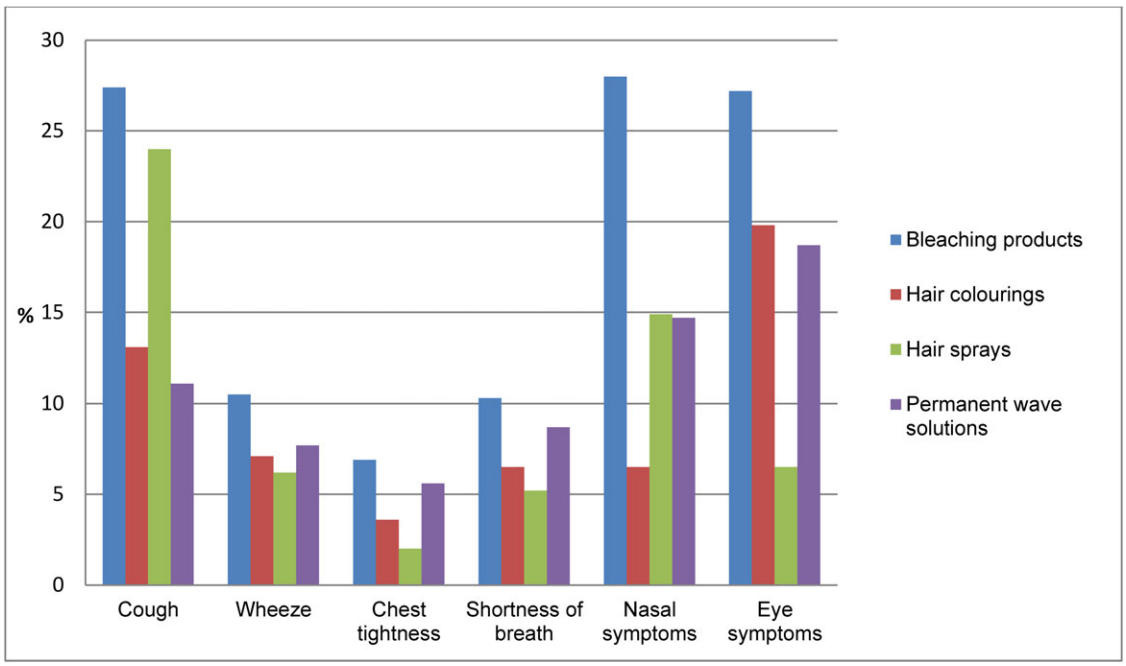

FIGURE 2. The frequency of respiratory symptoms caused by different hairdressing chemicals in 504 hairdressing apprentices.

Effect of hairdressing on respiratory health

Hairdressing apprentices were more likely than controls to have considered allergies and respiratory problems when choosing their career (7.8% vs 3.6%, $p < 0.001$; Table 3). When apprentices were asked about respiratory symptoms at work, they responded that exposure to bleaching products most frequently provoked both rhinitis and asthma symptoms (see Figure 2). In addition, hair spray induced cough in >20%, and 15% reported nasal symptoms from working with hair spray (Figure 2).

Discussion

This is the first study to examine the risk of rhinitis symptoms in hairdressing apprentices during training and the largest study concerning asthma symptoms in this group. Rhinitis symptoms were significantly more prevalent in hairdressing apprentices than in controls, and the prevalence increased with duration of exposure to the hairdressing trade (41.8% among first-year apprentices compared with 67.6% among third-year apprentices). In addition, >40% of hairdressing apprentices had an improvement in rhinitis symptoms during weekends and holidays, indicating a relation to exposure at work.

Our findings support those of previous studies reporting a higher risk of rhinitis symptoms in trained hairdressers when compared with saleswomen and office workers.[17,18] In contrast, a study conducted among 91 hairdressers and 80 office workers indicated no difference between the 2 groups.[19]

Our adjusted analyses showed a decreased risk of wheezing and waking from coughing among hairdressing apprentices. These results are in accordance with a previous study[12] reporting fewer asthma symptoms in hairdressing apprentices compared with office apprentices. Also, we found that hairdressing apprentices, more often than controls, had considered allergies and respiratory problems when choosing their career, and their age of onset of wheezing was significantly later than that of controls. Finally, although not significant, apprentices in their first year of training reported less wheezing than controls. Taken together, it seems likely that a healthy worker effect exists among hairdressing apprentices in relation to asthma.

Smoking was significantly more common in hairdressing apprentices than in controls and our data show an association between respiratory symptoms and smoking. Hence, an important step in improving respiratory health in hairdressers is to reduce smoking in this group.

We found hair-bleaching products to be the major causal agent for respiratory problems in hairdressing apprentices. Bleaching substances are both irritating and sensitizing and known to cause respiratory diseases.[20] It is concerning that these substances, widely used in hairdressing, begin causing symptoms as early as the apprenticeship years. In addition to bleaching products, permanent wave solutions, hair colorings, and hair sprays frequently induced rhinoconjunctivitis symptoms. Yet, hairdressing apprentices work with these substances every day, and the frequency of reported symptoms is unacceptably high.

Occupational rhinitis alone affects the quality of life and the ability to work and therefore it should be properly diagnosed and treated. Moreover, because nasal symptoms appear before the onset of occupational asthma in 21% of workers exposed to low-molecular-weight allergens,[21] the high prevalence of rhinitis in apprentices may be interpreted as a sign of respiratory impairment, and therefore a potential predictor of occupational asthma.[22] For a full preventive effect, intervention in the work environment should be undertaken during apprenticeship.

Although this study has several strengths, such as including a carefully selected control group and using the ECRHS questionnaire, which has been used in several nationwide studies to assess the prevalence of rhinitis and asthma,[23,24] there are also some limitations. For example, the response rate for this study was 49.7%, thus a potential for selection bias must be acknowledged. After examining the available data for nonrespondents (age, gender, and geography), we found no geographic differences between respondents and nonrespondents, but respondents were marginally younger and tended to be women. However, these differences are not expected to have introduced a bias regarding the occurrence of asthma and rhinitis. For example, the similar geographic distribution between the controls and hairdressing apprentices implies that the urban-rural gradient of allergic diseases, which was recently documented in Denmark,[25] is not a cause of selection bias in our study. Furthermore, one could speculate that persons with respiratory symptoms are more inclined to participate; however, this incentive is probably similar in both groups, precluding a selection bias. In addition, enrolling respondents into the prize draw may have strengthened the incentive for healthy persons to complete the questionnaire.

Conclusions

Hairdressing apprentices seem to have an increased risk of rhinitis caused by exposure to hairdressing chemicals, and the occurrence of rhinitis symptoms shows an increasing trend with duration of training. Our study suggests that a healthy worker effect exists in relation to asthma as apprentices have a later onset of wheeze and also a decreased risk of wheeze when compared with controls. More focus is needed on preventing rhinitis among hairdressing apprentices. Furthermore, hairdressing schools should focus on reducing exposure to hair-bleaching products and increasing awareness of the negative effects of smoking.

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Manuscript III

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Incidence of skin and respiratory diseases among Danish hairdressing apprentices. Manuscript submitted to Contact Dermatitis, 2016.

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Running head: Allergy in hairdressing apprentices

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Summary

Background: Hairdressing belongs to the professions with the highest risk of occupational skin and respiratory diseases. The incidence of these diseases in hairdressing apprentices has only been studied sparsely.

Objective: To determine the incidence of skin and respiratory disease in hairdressing apprentices, and to explore whether hairdressing apprentices leave the trade during training due to these diseases.

Materials/Methods: A prospective questionnaire study was conducted among 248 hairdressing apprentices and a control group comprising 816 young adults from the general population.

Results: The incidence rate ratios for contact urticaria (IRR 4.74, 95% CI 2.6–8.6), hand eczema (IRR 1.68, 95% CI 1.1–2.6), and rhinitis symptoms (IRR 1.61, 95% CI 1.2–2.2) were significantly increased in the hairdressing apprentices, whereas wheezing was similar between groups. During follow-up, 21.8% of the hairdressing apprentices had left the trade, and 70.3% of these left due to healthy complaints. The reasons most frequently reported as cause for leaving were musculoskeletal pain (47.4%) and skin diseases (47.4%) followed by respiratory symptoms (23.7%).

Conclusions: Hairdressing apprentices are at increased risk of contact urticaria, hand eczema, and rhinitis symptoms compared with the general population, and a substantial proportion leave the trade due to these diseases, indicating a 'healthy survivor effect'.

Key words: hairdressing apprentices, contact urticaria, hand eczema, wheezing, rhinitis symptoms, incidence, occupational

Background

Hairdressers are exposed daily to various allergenic and irritant substances (1,2). Especially the hands and respiratory tract are exposed, and consequently hairdressers are at risk of occupational skin and respiratory diseases such as hand eczema, contact urticaria, asthma, and rhinitis. Various types of epidemiologic studies have assessed the occurrence of these diseases in hairdressers. Registry studies have identified hairdressing as being among the occupations with the highest risk of occupational hand eczema (3–5), contact urticaria (6,7), rhinitis (8), and asthma (9–12). In these registry studies, the primary focus is on diagnosed cases of disease and thereby conservative risk estimates of the actual occurrence are given. Several cross-sectional studies investigating the occurrence of self-reported disease have also been conducted among hairdressers (13–18). These studies give higher estimates of the disease frequency, but the cross-sectional design risks being subject to a healthy worker effect if sensitive individuals have already left the trade and therefore are not included in the data. Prospective questionnaire studies examining the incidence of new cases of diseases are usually the preferred approach when studying the cause of disease, because they use all of the available information on the studied population. Such studies are, however, sparse among hairdressing apprentices (19–21).

A healthy worker effect has been suggested to exist in hairdressers in relation to both skin and respiratory diseases as a result of a 'healthy hire effect'(22) since newly started hairdressing apprentice have less hand eczema and atopic dermatitis (23) and less respiratory symptoms and a better lung function (21) compared with control groups. Whether sensitive individuals discontinue training because of skin and respiratory diseases, resulting in a 'healthy survivor effect' (22), has not been examined in hairdressing apprentices.

The aims of this study were to examine the incidence of skin and respiratory disease in hairdressing apprentices, and to explore whether hairdressing apprentices leave the trade during training due to these diseases.

Material and methods

The study was conducted as a 3-year follow-up questionnaire study among hairdressing apprentices and a comparable reference group comprising young adults from the general population (referred to as the population controls). The study population is described in detail in previous studies (24,25). The baseline study was conducted between April and July 2013 as a web-based questionnaire study among 504 hairdressing apprentices and 1400 population controls in Denmark. For further details,

see previous studies (24,25). In the baseline study, personal registration numbers and e-mail addresses of individual participants were acquired. For the follow-up study in 2016, the current addresses of participants were obtained through the Danish Research Services (Forsker Service, Statens Serum Institute, Copenhagen) using their personal registration numbers.
The follow-up study was conducted during April and June 2016. First participants received an invitation e-mail, plus two reminders, with a link to the web-based questionnaire. Non-responders subsequently received a paper questionnaire and a pre-paid return envelope by post. One postal reminder was given.

Questionnaire

The questionnaire contained questions concerning respiratory symptoms, asthma, rhinitis, urticaria on the hands, contact urticaria, hand eczema, smoking habits, occupational exposures, and education level. Questions were adopted from the Nordic Occupational Skin Questionnaire 2002 (NOSQ-2002) (26) and the European Community Respiratory Health Survey (ECRHS) (27). For details concerning the definition of wheezing, rhinitis symptoms, hand eczema, urticaria on the hands, and contact urticaria see previous studies (24,25).
To determine incident cases of disease since baseline, the following questions were used: 1) Have you had wheezing or whistling in your chest at any time since the last survey? 2) Have you, since the last survey, had a problem with sneezing or a runny or blocked nose when you did not have a cold or the flu? 3) Have you had hand eczema since the last survey? 4) Have you, since the last survey, had itchy wheals appearing and disappearing rapidly (within hours) on your hands, wrists, or forearms (urticaria or nettle rash)? 5) Have these itchy wheals (urticaria) on your hands, wrists, or forearms been caused by skin contact with rubber gloves, hair dyes, cosmetics, or the like?
To examine the degree of discontinuation from the trade, the hairdressing apprentices were asked the following questions: 1) Are you still a hairdressing apprentice? 2) Did you leave training because of, or partly because, of disease? 3) Which disease? (You can tick off one or more of the following): asthma, allergy symptoms related to the nose and/or eyes, hand eczema, nettle rash, depression/stress, pain in muscles or joints, other.
Ever smokers were defined as participants giving an affirmative answer to the following question: Have you ever smoked for as long as a year? (Yes means at least 20 cigarettes in a life-time or at least one cigarette per day for one year).
Atopic dermatitis was defined according to the U.K. Working Party's diagnostic criteria (28), as the presence of one major criterion and at least two of four minor criteria.

The highest completed education level of participants was classified according to the Danish International Standard Classification of Education 2015 (DISCED-15)(29). A higher DISCED number indicated a higher education level, except for DISCED 90 which refers to "Not given or no education". DISCED 20 refers to a lower secondary education, whilst DISCED 80 refers to a doctoral degree or equivalent education.

Data handling and statistics

The distribution of the web-based questionnaire by e-mail and the subsequent entry of responses were performed using the online questionnaire tool Enalyzer Survey Solutions (30). The questionnaire was answered electronically by 741 respondents, and 323 answered the paper questionnaire. The answers in the paper questionnaires were subsequently entered into Enalyzer, and the full dataset was finally drawn from Enalyzer as an excel file.

The statistical program IBM® SPSS™ version 19 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. A chi-square test was used for binary data and independent samples t-tests were used for normally distributed continuous variables. A two-sided *p*-value ≤ 0.05 was considered statistically significant.

The incidence rates (IRs) were calculated as the number of new cases of wheezing, rhinitis symptoms, hand eczema, urticaria on the hands, and contact urticaria, respectively, since the baseline study, per person-year of observation. The population at risk of hand eczema and urticaria were defined as participants who had never had hand eczema or urticaria at baseline. The population at risk of rhinitis symptoms was defined as participants who had never had nasal allergies, including hay fever, at baseline, and the population at risk of wheezing was defined as participants who had never had asthma at baseline. Since the follow-up study was conducted exactly three years after baseline, all participants were considered having been observed for three years. Hairdressing apprentices who had left the trade were not included in calculations of IRs. Incidence rate ratios (IRRs) with 95% confidence intervals (95% CI) were calculated with the online calculator OpenEpi. Meaningful adjustment for smoking and atopic dermatitis could not be performed due to small sample sizes.

Results

Results from the baseline study in 2013 are presented elsewhere (24,25). In the follow-up study, an overall response rate of 55.9% (1064/1904) was obtained after two e-mail reminders and one postal reminder. The response rate was significantly lower in hairdressing apprentices compared with population controls (49.2% (248/504) vs. 58.2% (816/1400), $p<0.001$). Main characteristics of responders are summarized in Table 1. Both hairdressing apprentices and population controls consisted mainly of females with a mean age of 25 years. Atopic dermatitis was equally present in the two groups (36.3% vs. 35.5%, $p<0.82$), but smoking was significantly more prevalent among hairdressing apprentices (35.9% vs. 25.4%, $p<0.001$). The distribution of highest completed education level in the two groups is presented in Table 1. The majority (85.5%) of the participants that were hairdressing apprentices at baseline were currently in the category DISCED 30, meaning they had graduated from hairdressing schools, which correspond to an upper secondary education. More than half (56.1%) of the population controls also belonged to the category DISCED 30, but many (39.3%) had attained higher education levels.

Occupational exposure for more than two hours a day to wet work, rubber gloves, cleaning agents, and chemicals fumes was significantly more common among the hairdressing apprentices ($p<0.001$) (see Table 1).

Non-responders

Since the invitation e-mail and letter contained information concerning the purpose of the study, we wanted to investigate if a selection bias had been introduced in the sense that persons with pre-existing skin or respiratory diseases at baseline would be more inclined to complete the follow-up questionnaire. In Table 2, we compared the baseline life-time prevalence of diseases between responders and non-responders to the follow-up study. No significant selection bias could be demonstrated, but the hairdressing apprentices that responded to the follow-up questionnaire tended to have a higher baseline prevalence of hand eczema (38.3% vs. 30.9%, $p=0.08$).

Reasons for leaving the trade

Of the 248 cases that were hairdressing apprentices at baseline, 78.2% (194) were still active in the hairdressing trade at follow-up; 53 were still training and 141 were working as trained hairdressers. Hence, 54 (21.8%) had left the trade (see Table 1), of whom 38 (70.4%) reported leaving because of, or partly because of, disease. Six left before finishing apprenticeship and 32 left after completing

apprenticeship. The reasons most frequently reported as cause for leaving were musculoskeletal pain and skin diseases (hand eczema or urticaria), which were both reported by 47.4% (18/38). The second most common reason for leaving was respiratory symptoms (rhinoconjunctivitis symptoms or asthma), which was reported by 23.7 (9/38) % (see Table 3).

Incidence of skin and respiratory diseases in hairdressing apprentices

Amongst the 1467 participants who had never had hand eczema at baseline (330 hairdressing apprentices and 1137 population controls), 813 (153 hairdressing apprentices and 660 population controls) completed the follow-up questionnaire three years later. However, 25 of the hairdressing apprentices had left the trade during follow-up, leaving 128 hairdressing apprentices for analysis of incidence rates. At follow-up, 106 new cases of hand eczema were reported (26 hairdressing apprentices and 80 population controls), giving calculated IRs of 68 cases/1000 person-years among hairdressing apprentices and 40 cases/1000 person-years among controls. The IRs for wheezing, rhinitis symptoms, urticaria on the hands, and contact urticaria were calculated in the same manner. Results are presented in Table 4.

The IRRs for rhinitis symptoms (1.61, 95% CI 1.2–2.2), hand eczema (1.68, 95% CI 1.1–2.6), urticaria on the hands (1.85, 95% CI 1.2–2.8), and contact urticaria (4.74, 95% CI 2.6–8.6) were significantly increased in the hairdressing apprentices (see Table 4), whereas the incidence of wheezing was similar in the two groups (0.95, 95% CI 0.6–1.5), with a cumulative incidence of 15.4% vs. 16.3%, respectively (p=0.79).

Discussion

To the best of our knowledge, this is the first study to investigate the incidence of urticaria and rhinitis symptoms in hairdressing apprentices. Interestingly, we found significantly increased IRRs in the hairdressing apprentices for rhinitis symptoms, hand eczema, urticaria on the hands, and contact urticaria caused by rubber gloves, hair dyes, cosmetics or the like, whilst an increased risk of wheezing could not be detected.

Since the incidence of rhinitis symptoms has not been investigated in hairdressing apprentices before, comparison to similar studies is not possible. However, a large-scale retrospective questionnaire study (31) conducted among trained Swedish hairdressers and women from the general population, also found a significantly increased incidence of nasal blockage in the hairdressers (17.3 vs. 11.4 cases/1000 person-years, IRR 1.5, 95% CI 1.3-1.8). In addition, several

cross-sectional studies have reported a higher prevalence of rhinitis symptoms in trained hairdressers compared to different control groups (32–34).

In contrast to our discovery of an increased incidence of rhinitis symptoms, we could not detect an increased risk of wheezing in the hairdressing apprentices. Our findings are in agreement with a previous prospective study in hairdressing apprentices (21) which reported a similar cumulative incidence of wheezing in hairdressing apprentices and office apprentices (10.0% vs. 11.5%, p=0.66). In conclusion, although hairdressing is among the professions with the highest risk of occupational asthma, an increased incidence of wheezing cannot be detected during training. We did, however, detect an increased risk of rhinitis symptoms, which is generally considered a risk factor for asthma. It appears that respiratory symptoms can be induced by excessive exposure to hairdressing products, and that upper respiratory tract symptoms develop faster than lower respiratory tract symptoms.

Epidemiologic data on contact urticaria in hairdressers are sparse (35,36) and the only existing data on its occurrence in hairdressing apprentices derive from our baseline study where a prevalence of 7.3% was found (24). Interestingly, the incidence of urticaria on the hands was as high as that of hand eczema in both hairdressing apprentices and population controls. The fact that many of the symptoms of urticaria have a substantial degree of overlap with symptoms of hand eczema might have given rise to misclassification partly explaining this high incidence. Nevertheless, urticaria on the hands and contact urticaria caused by exposure to hairdressing products seem to be a problem for a substantial fraction of hairdressing apprentices and further attention should be paid to this disease. Symptoms of contact urticaria are usually mild, limited to small areas of the skin, and of short duration. The diagnostic test, the open application test, is rarely performed (37), and thus contact urticaria may probably be underdiagnosed. Hopefully, the results of this study will lead to more focus on the disease in hairdressers and the performance of sufficient diagnostic tests by clinicians.

We report an IR for hand eczema of 68 cases/1000 person-years among the hairdressing apprentices and an increased IRR compared to young adults from the general population. In the 1990s, two prospective studies also examined the incidence of hand eczema in hairdressing apprentices (19,20). The apprentices were included soon after they began training and were followed with questionnaire and clinical examinations until the end of training. Both studies found high IRs of hand eczema (151 and 328 cases/1000 person-years), and a peak incidence was reported during the second quarter of training (572 cases/1000 person-years) (20). The higher IRs in these studies are probably partly due to the fact that they followed hairdressing apprentices from the beginning of training,

whilst we followed hairdressing apprentices across all levels. However, the lower incidence in our study could also suggest an improvement in work habits and use of protective measures during the last 20-30 years.

In this 3-year prospective questionnaire study, we found that more than one in five of the hairdressing apprentices leave the trade and that the majority reported leaving because of, or partly because of, health complaint. The reasons most frequently reported as causes for leaving the trade were musculoskeletal pain and skin diseases, followed by respiratory symptoms. A large-scale study conducted among trained hairdressers reported an even higher discontinuation rate of 44.3% among trained hairdressers after finishing hairdressing school (13). In this group, musculoskeletal pain was the main cause for leaving (41.9%), followed by hand eczema (23.1%). In a Finnish study, hand eczema and asthma were the top reasons for leaving the trade, followed by musculoskeletal pain and rhinitis (38). In conclusion, the existence of a 'healthy survivor effect' in regards to skin and respiratory diseases is supported both by the findings in our study and by studies among trained hairdressers. Although it was not the main focus of this study, musculoskeletal pain proved to be a substantial health complaint in hairdressing apprentices and the hairdressing schools should increase the focus on prevention of musculoskeletal pain by teaching the apprentices about healthy working postures and habits.

A relatively low response rate of 55.9% in our study gives rise to concern about selection bias. A particular concern is if individuals with diseases were more prone to complete the follow-up questionnaire which would result in overestimation of IRs. However, comparison of the baseline prevalence of disease between responders and non-responders revealed similar occurrences of disease in the two groups.

Another concern was that ex-hairdressers would be less inclined to complete the questionnaire thereby resulting in false low discontinuation rates and wrong estimates of reasons for leaving the trade. However, our dropout rate was similar to data available from Statistics Denmark (39), showing discontinuation rates of 22-28% among hairdressing apprentices in the years 2013, 2014, and 2015 (39).

In conclusion, the novel finding of a significantly increased incidence of self-reported rhinitis symptoms, urticaria on the hands, and contact urticaria among hairdressing apprentices compared with young adults from the general population should encourage greater attention to these diseases in the hairdressing trade. Reducing the occurrence of these diseases along with hand eczema and musculoskeletal pain are important steps in improving working conditions for hairdressers and hairdressing apprentices.

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Table 1. Main characteristics of participants

	Hairdressing apprentices (n=248)	Population controls (n=816)	*p*-value
Mean age, years (SD)	25 (4.0)	25 (4.3)	0.88
Female, % (n)	96.4 (239)	96.3 (786)	0.97
Ever smoked, % (n)	35.9 (89)	25.4 (207)	0.001
Atopic dermatitis, % (n)	36.3 (90)	35.5 (289)	0.82
Highest completed education, % (n)			
- DISCED 90	0	0.9 (7)	
- DISCED 20	13.7 (34)	3.7 (30)	
- DISCED 30	85.5 (212)	56.1 (458)	
- DISCED 50	0.4 (1)	5.9 (48)	
- DISCED 60	0.4 (1)	21.4 (175)	
- DISCED 70	0	11.5 (94)	
- DISCED 80	0	0.5 (4)	
Exposures > 2 hr/day, % (n)			
- Wet work	63.7 (158)	15.2 (124)	<0.001
- Rubber gloves	56.5 (140)	13.6 (111)	<0.001
- Cleaning agents	8.5 (21)	4.0 (33)	<0.001
- Dusts	6.5 (16)	5.3 (43)	0.70
- Chemical fumes	66.5 (165)	1.1 (9)	<0.001
Completed training, % (n)	75.8 (188)		
- Active hairdressers	56.8 (141)		
- Ex-hairdresser	19.0 (47)		
Still training, % (n)	21.4 (53)		
- Maternity leave	0.4 (1)		
- 4th year of training	19.8 (49)		
- 3rd year of training	0.8 (2)		
- 2nd year of training	0.4 (1)		
Dropped out during training, % (n)	2.8 (7)		

DISCED: Danish International Standard Classification of Education. A lower DISCED number indicates a lower education level. DISCED 90 refers to “Not given or no education”.

Table 2. Comparison of the reported life-time prevalence of diseases at baseline between responders and non-responders to the 3-year follow-up questionnaire

	Hairdressing apprentices (n=504)			Population controls (n=1400)		
	Responders (n=248)	Non-responders (n=256)	*p*-value	Responders (n=816)	Non-responders (n=584)	*p*-value
Asthma, % (n)	16.9 (42)	17.6 (45)	0.85	17.9 (146)	16.1 (95)	0.38
Allergic rhinitis, % (n)	36.3 (90)	35.2 (90)	0.79	34.1 (278)	32.9 (192)	0.64
Hand eczema, % (n)	38.3 (95)	30.9 (79)	0.08	19.1 (156)	18.3 (107)	0.71
Urticaria on hands, % (n)	21.0 (52)	25.4 (65)	0.24	25.2 (206)	22.9 (134)	0.32
CoU to cosmetics, % (n)	6.5 (16)	8.2 (21)	0.45	4.0 (33)	4.5 (26)	0.71
Atopic dermatitis, % (n)	36.3 (90)	30.9 (79)	0.20	35.4 (289)	31.7 (185)	0.15

CoU: contact urticaria

Table 3. Reported reasons for leaving the hairdressing trade

	Hairdressing apprentices (n=6)	Trained hairdressers (n = 32)	Total (n=38)
Asthma, % (n)	0 (0)	3.1 (1)	2.6 (1)
Allergy symptoms from nose/eyes, % (n)	0 (0)	25.0 (8)	21.1 (8)
Hand eczema, % (n)	66.7 (4)	37.5 (12)	42.1 (16)
Urticaria, % (n)	0 (0)	6.3 (2)	5.3 (2)
Depression/stress, % (n)	16.7 (1)	15.6 (5)	15.8 (6)
Musculoskeletal pain, % (n)	16.7 (1)	53.1 (17)	47.4 (18)

Table 4. Crude incidence rates and incidence rate ratios (IRRs) with 95% CIs of disease in hairdressing apprentices and population controls

	Hairdressing apprentices			Population controls			
	Cases	Person-years	Incidence (/1000 py)	Cases	Person-years	Incidence (/1000 py)	**IRR (95% CI)**
Wheezing	25	486	51	109	2007	54	0.95 (0.6–1.5)
Rhinitis symptoms	59	381	154	155	1611	96	**1.61 (1.2–2.2)**
Hand eczema	26	384	68	80	1974	40	**1.68 (1.1–2.6)**
Urticaria on hands	31	459	68	66	1821	37	**1.85 (1.2–2.8)**
CoU to cosmetics	23	543	42	21	2349	9	**4.74 (2.6–8.6)**

CoU: contact urticaria CI: confidence interval py: person-years

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Optimizing diagnostic tests for persulphate-induced respiratory diseases

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Abstract

Background: Persulphates from hair bleaching products are considered the major cause of occupational-rhinitis and asthma in hairdressers. The specific inhalation challenge (SIC) is considered 'reference standard' for diagnosing persulphate-induced asthma and rhinitis; however, the currently validated method of performing SIC with persulphate powder is time consuming with a duration of up to 4 days. The value of skin prick tests (SPTs) and histamine release tests (HRTs) with persulphates is unknown. The aim of this study was to establish a novel rapid SIC with persulphate powder to test for both rhinitis and asthma simultaneously in 1 day. In addition, we assessed the suitability of SPTs and HRTs for detecting persulphate-induced respiratory diseases.

Methods: The study population included 19 hairdressers with a history of work-related rhinitis and/or asthma symptoms, 12 symptomatic controls (10 with concurrent allergic asthma and rhinitis and two with non-allergic asthma), and 40 healthy controls. A previous severe asthmatic reaction and/or anaphylactic reaction to persulphates was considered an exclusion criterion for hairdressers. The 19 hairdressers and 12 symptomatic controls had SIC performed with 3 × 5 min exposures to potassium persulphate powder in a provocation chamber. All participants, including the 40 healthy controls, were subjected also to SPTs and HRTs with three persulphate salts at concentrations of 2–20 % and 0.03–1 %, respectively.

Results: None of the symptomatic controls had a nasal or bronchial response to SIC with potassium persulphate. Six hairdressers presented a nasal and two a bronchial response. No severe reactions occurred. No positive SPTs were recorded, neither among hairdressers, symptomatic controls, nor healthy controls. All three groups showed nonspecific non-IgE mediated histamine release to persulphates in HRT.

Conclusions: The proposed method for performing SIC showed a high specificity for detecting persulphate-induced asthma and rhinitis. The rapid SIC was able to produce positive nasal and bronchial responses in symptomatic hairdressers without any severe reactions occurring. SPTs and HRTs cannot predict asthma or rhinitis caused by persulphates.

Keywords: Specific inhalation challenge, Persulphates, Persulphate salts, Histamine release test, Skin prick tests, Occupational asthma, Occupational rhinitis

Background

Persulphates are low-molecular weight chemicals (<10 kDA) with strong oxidizing properties and wide application in hair bleaching products. They are also found in dental prosthesis cleaners, food starch, paper and cellophane, as a reducing agent in photography, and as etching solution for printed circuit boards [1]. Persulphates can induce immediate and delayed reactions, such as contact dermatitis, contact urticaria, asthma, rhinitis, and anaphylaxis [2–6]. Reported cases of immediate type reaction caused by persulphates are predominantly among hairdressers, but also workers producing persulphates [7, 8] and consumers of hair bleaching products [3, 9] have been reported to react.

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As with most low-molecular weight agents, the mechanism by which persulphates induce immediate reactions is not fully understood. Immunoglobulin E (IgE) [4, 8, 10], T-cells [11, 12], and oxidative events have been proposed to contribute to the development of persulphate-induced asthma and rhinitis [13].

When assessing a patient with possible persulphate-induced rhinitis or asthma, various tests can be considered. Several studies describe the use of skin prick tests (SPTs) [10, 14, 15]; however, validation and standardization are lacking. Only one study addressed the use of histamine release test (HRT) [16] and results were inconclusive.

The specific inhalation challenge (SIC) is held as 'reference standard' for diagnosing occupational-rhinitis and asthma [17]. SIC with persulphate has been performed with a realistic approach attempting to reproduce conditions in the hairdressing salon [18–20]. Typically, mixtures of persulphate powder and lactose powder [20], or bleaching powder and hydrogen peroxide [21] are tipped from one tray to another inside a specially designed provocation chamber. The test has also been performed by administering an aqueous persulphate solution with a nebulizer and by spraying the solution directly into the nose when examining asthma [22, 23] and rhinitis [12], respectively. The SIC performed with persulphate in the realistic approach has previously been validated [20]. In this validated approach, the patient is exposed to a mixture of persulphate powder and lactose powder. The exposure is performed step-wise with increasing doses of persulphate during four consecutive days. The maximal exposure on the fourth day is 30 g of potassium persulphate for 10 min. A sensitivity of 100 % and a specificity of 87.5 % for diagnosing persulphate-induced asthma were reported. A disadvantage of this approach is, that it is very time consuming for both investigator and patient.

The aim of our study was, with a focus on Munoz' validated method, to establish a new realistic approach rapid SIC performed with potassium persulphate to test for both rhinitis and asthma simultaneously in 1 day. Instead of using the step-wise approach over several days, we exposed the patients to 30 g of potassium persulphate on the first day for 3×5 min. Instead of the typical tipping method, we used a new stirring method in order to obtain a more reproducible exposure. In addition, we assessed the potential for diagnosing persulphate-induced asthma and rhinitis by SPTs and HRTs using three different persulphates (ammonium persulphate, potassium persulphate and sodium persulphate) in concentrations from 2–20 and 0.03–1 %, respectively.

Methods

The study was performed as a clinical single-blinded case–control study between February 2014 and May 2016.

Hairdressers

Hairdressers with work-related respiratory symptoms who had either contacted the hot-line of the Research Center for Hairdressers and Beauticians or were refereed to our unit for suspected occupational asthma and/or rhinitis were eligible for inclusion in this study. Hairdressers with a history of severe asthmatic reactions and/or anaphylactic reactions to hair bleaching products were excluded. Standardized interviews were employed to obtain a detailed medical and occupational history, as well as records of atopic diseases and smoking. Respiratory symptoms suggestive of asthma and rhinitis were assessed and their association with exposure to persulphates and other hairdressing chemicals was explored. A positive stop/resume test was defined as respiratory symptoms improving after periods away from work and worsening at the workplace [24]. A physical examination that included rhinoscopy was performed to exclude nasal conditions mimicking rhinitis.

Symptomatic controls

Individuals with a history of asthma and rhinitis without known sensitization or exposure to persulphates were recruited among patients in our unit and through an advertisement on a website for research subjects.

Healthy controls

For the SPT and HRT with persulphates we recruited a group of healthy controls without known asthma, rhinitis, or urticaria.

Prior to any clinical tests, inhaled corticosteroids were discontinued for 2 weeks, oral antihistamine and nasal corticosteroids for 72 h, long-acting beta$_2$-agonist and leukotriene receptor antagonists for 48 h, and short-acting beta$_2$-agonist for 8 h. The following were considered exclusion criteria: unstable asthma during the last 3 months before inclusion, regular use of oral corticosteroids, baseline forced expiratory volume in 1 s (FEV_1) $\leq$70 % of predicted normal value, recent (<4 weeks) respiratory tract infection, chronic obstructive pulmonary disease, severe hypertension, immunological diseases, pregnancy or unstable cardiovascular diseases.

Immunologic tests

SPTs were performed in duplicate with 10 common aeroallergens (Soluprick SQ®; ALK-Abelló, Hørsholm, Denmark), latex, and chlorhexidine digluconate (5 mg/mL). Negative (diluent) and positive (histamine 10 mg/mL) controls were also included. A positive reaction was defined by a wheal with a diameter $\geq$3 mm. The SPT was only considered to be valid when the positive control was positive and the negative control was negative. Atopy was

defined as a positive SPT reaction to one or more of the common allergens.

In addition, SPTs were performed with freshly prepared solutions of ammonium persulphate (ACS reagent ≥98.0 %, CAS 7727-54.0), potassium persulphate (ACS reagent, ≥99.0 %, CAS 7727-21-1), and sodium persulphate (purum p.a., ≥99.0 %, CAS 7775-27-1); all Sigma-Aldrich, St. Louis, MO, USA. The persulphates were dissolved in physiologic saline solution. Ammonium and sodium persulphate were prepared at 2, 5, 7.5, 10 and 20 (wt/vol). Potassium persulphate was used at 2, 5, and 7.5 (wt/vol), as it was insoluble at higher concentrations. The solutions' pH ranged from 1.45 to 5. First, the lowest three concentrations of the persulphates solutions were applied. If no reaction occurred within 15 min, 10 % solution was applied. Finally, if no reaction occurred again, the test was performed with the 20 % solution. Reactions were recorded after 15 and 30 min.

Heparinized blood (5 mL) for HRT was collected at and sent to RefLab ApS (Copenhagen, Denmark) according to standard procedures. Blood samples were stored at room temperature for a maximum of 6 h prior to analysis. Persulphate solutions were prepared daily and tested at concentrations of 0.03, 0.06, 0.125, 0.25, 0.5 and 1.0 (wt/vol) in duplicates. Briefly, 25 µL aliquots were incubated with 25 µL persulphate dilutions at 37 °C for 1 h. During incubation, the released histamine bound to a glass fiber coated microtitre plate and was detected fluorometrically after coupling to *o*-phthaldialdehyde [25]. Positive reactions were categorized according to the lowest concentration producing significant histamine release (10 ng histamine/mL blood). If no histamine was released, the result was categorized as negative.

Finally, whole blood was collected, serum was separated and stored at −20 °C until total IgE was measured by the ImmunoCap® assay (Thermo Fisher Scientific, Waltham, MA, USA).

Lung function tests

Hairdressers and symptomatic controls had relevant asthma medication discontinued prior to the performance of any lung function tests. Spirometry, including reversibility test and methacholine challenge was performed for each hairdresser and control 2–3 days before SIC.

Forced expiratory flow in the first second (FEV_1) and forced vital capacity (FVC) within 2 standard deviations (SD) of predicted normal values were considered normal. The reversibility test was deemed positive if FEV_1 increased by ≥12 % or >200 ml upon inhalation of β_2-agonist. Bronchial hyperresponsiveness (BHR) was assessed by the bronchial provocation test with methacholine. The provocative dose of methacholine producing a 20 % fall in FEV_1 (PD20) was expressed in micrograms.

After spirometry, fractional exhaled nitric oxide (FeNO) was measured with a DENOX 88 analyzer (ECO MEDICS AG, Duernten Switzerland) and was considered elevated at ≥25 ppb [26].

SIC with persulphate

SIC was performed on an outpatient basis. On a separate control day, SIC was performed with 50 g D-lactose monohydrate (Sigma-Aldrich). In the absence of a bronchial- and nasal response during the following 24 h, subjects were exposed to a mixture of 30 g potassium persulphate and 20 g lactose powder. The participants, but not the investigator, were blind to the nature of the challenges.

During exposure, participant sat at a table inside a provocation chamber (2.1 m × 2.2 m × 2.3 m) at ambient temperature and humidity. Fresh air was supplied at 0.5/h through a high efficiency particulate air and carbon filters. Test substances were contained in a 1-L Erlenmeyer flask (Schott, Mainz, Germany), placed 30 cm from the subjects' face on a magnetic stirrer (IKAMAG® RCT basic; IKA, Staufen, Germany), and swirled in the air by stirring the magnet (length: 7 cm) at 810 rpm. Maximal exposure consisted of 3 × 5 min, with 20-min intervals in between. During pauses and after maximal exposure was reached, participants were removed from the provocation chamber. Exposure was discontinued if the patient developed a significant bronchial response before maximal exposure was reached. Monitoring for a bronchial and nasal response was performed at baseline; in between each exposure; 15, 30, and 60 min after exposure; and hourly thereafter until sleep. Participants were monitored in the hospital during the first 8 h; thereafter, they performed self-measurements of FEV_1 and nasal symptoms at home until sleep and again the following morning when waking up.

Quantification of potassium persulphate during SIC

To assess the reproducibility of the stirring method, the amount of potassium persulphate in the provocation chamber was quantified during three challenges on three separate days. Particles sized 10–300 nm and 0.1–10 µm were counted using a NanoTracer PNT800 (Philips Electronics, Eindhoven, The Netherlands) and a Dust Trak™ Aerosol Monitor Model 8520 (TSI, Shoreview, MN, USA), respectively, placed 30 cm away from the Erlenmeyer flask.

Evaluation of bronchial response

Airway obstruction was assessed by FEV_1 using a portable asthma monitor (AM1; Jaeger, Hoechberg, Germany). A sustained ≥15 % decrease in FEV_1 from baseline was considered a positive result for asthma, provided that fluctuations in FEV_1 were ≤10 % on the control day [27].

Evaluation of nasal response

Rhinitis was measured using three tests: Linder's symptoms score scale, changes in nasal cavity volume, and anterior rhinoscopy. SIC with persulphate was considered positive for rhinitis if ≥2 tests were positive and the participant had <2 positive tests on the control day.

Linder's symptoms score scale

Subjective symptoms of rhinoconjunctivitis were scored according to Linder's symptoms score scale [28, 29]. Participants rated sneezing, rhinorrhea, and nasal congestion from 0 to 3. Ocular symptoms scored 1 point, and itchiness of the nose, ears or palate scored 1 point for each location with itch. An increase of ≥3 points from baseline was considered a positive result.

Changes in nasal cavity volume

Swelling of the nasal mucosa was assessed by means of acoustic rhinometry using a Rhinoscan® SRE 2000 (RhinoMetrics A/S, Lynge, Denmark) as previously described [30]. Participant had acclimatized for 20 min before baseline measurements were performed. Total nasal volume (TNV) was measured at 2–6 cm from the nares. A ≥25 % fall in TNV after exposure was considered a positive result [28].

Scoring by anterior rhinoscopy

Anterior rhinoscopy was performed and rhinorrhea and nasal congestion were scored separately according to the method proposed by Hytonen [31]. A change in nasal status score of ≥4 points between baseline and exposure was considered a positive response [31].

Statistical analysis

Data were analyzed using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA). Results for categorical variables are presented as numbers and frequencies, and are compared by the Fischer's exact test. *P* values ≤0.05 were considered statistically significant (two-tailed tests). Continuous variables were compared with the Mann–Whitney U test and expressed as means ± SDs.

Results

Hairdressers

A total of 20 hairdressers were considered eligible for inclusion; one was excluded because of unstable asthma. All were female and the mean age was 31 years (Table 1). Six hairdressers were atopic and three had atopic dermatitis. FeNo was elevated in three, FEV1/FVC was reduced in three, and five showed bronchial hyperresponsiveness in the methacholine challenge. Seven hairdressers used asthma medication and six used rhinoconjunctivitis medication (Table 1). When asked about work-related symptoms, one hairdressers reported asthmatic symptoms (≥2 of the following: wheeze, cough, shortness of breath or hoarseness), one reported rhinitis symptoms (≥1 of the following: nasal itching, runny nose, blocked nose, itchy and watery eyes), and 17 reported both asthmatic and rhinitis symptoms. All 19 hairdressers reported symptoms in relation to hair bleaching and 11 (58 %) admitted that their symptoms could also be provoked by other hairdressing products such as hair dyes, hairsprays, permanent wave solutions, and perfume (Table 1).

Symptomatic controls

A total of 14 symptomatic controls were eligible for inclusion in the study; two had to be excluded due to unstable asthma leaving ten with concomitant allergic asthma and rhinitis and 2 with non-allergic asthma. The mean age was 21 years and 58 % were female (Table 1). Half had atopic dermatitis. Elevated FeNO was detected in 42 %, FEV1/FVC was reduced in three, and the methacholine challenge was positive in seven. All used asthma medication, whilst only the ten with concomitant allergic rhinitis used rhinitis medication (Table 1).

Healthy controls

A total of 40 healthy participants had SPT and HRT with persulphates performed.

Results of SIC

None of the participants reacted to placebo. None of the symptomatic controls developed a nasal or bronchial response when exposed to potassium persulphate in SIC. A total of six (32 %) hairdressers showed a positive reaction to SIC with persulphate; four had a nasal response, and two had a combined bronchial and nasal response (Table 2).

All hairdressers with a positive SIC, reported a positive stop/resume test, whereby their symptoms subsided in periods away from work and deteriorated again when returning to work. They had all been exposed to hairdressing for ≥6 months before developing work-related respiratory symptoms. The typical time interval between initiating work with bleaching products and the appearance of symptoms, was minutes (n = 3), hours (n = 2), or it could not be defined (n = 1). Half of the hairdressers had discontinued their work, and hence were no longer exposed to persulphates on a

Table 1 Main characteristics of participants

	Hairdressers (n = 19)	Symptomatic controls (n = 12)	Healthy controls (n = 40)	*P* value[α]
Mean age, years (SD)	31 (10.5)	21 (2.6)	35 (12.9)	0.002
BMI, mean (SD)	22.5 (3.8)	22.4 (3.4)	24.7 (4.3)	1.000
Sex (% female)	19 (100)	7 (58)	43	0.02
Smoking status, n (%)				
Smoker	7 (37)	5 (42)	–	0.79
Never smoker	12 (63)	7 (58)	–	
Atopic dermatitis, n (%)	3 (16)	6 (50)	0 (0)	0.06
Total IgE, mean (SD)	58.3 (76)	156.5 (202)		0.22
Atopy[a] (%)	6 (32)	10 (83)		0.009
FeNO ≥ 25 ppb, n (%)	3 (16)	5 (42)		0.20
FeNO ≥ 50 ppb, n (%)	1 (5.2)	3 (25)		
Lung function, mean (SD)				
% FEV1	101.7 (9.7)	106.6 (14.8)		0.48
% FVC	105.3 (8.7)	116.8 (14.1)		0.025
FEV1/FVC	84.8 (7.6)	78.9 (6.6)		0.43
Methacholine test				
BHR, n (%)	5 (26)	7 (58)		0.13
Asthma medication, n (%)				
None	12 (63)	0 (0)		
SABA	3 (16)	7 (58)		
SABA + low dose ICS	1 (5)	3 (25)		
SABA + medium dose ICS	1 (5)	2 (17)		
SABA + LABA/ICS	1 (5)	0 (0)		
SABA + LTRA	1 (5)	0 (0)		
Rhinitis medication, n (%)				
None	13 (68)	2 (17)		
OA	2 (11)	8 (67)		
INS	1 (5)	1 (8)		
OA + INS	2 (11)	1 (8)		
OA + antihistamine eye drops	1 (5)	0 (0)		
Work-related symptoms, n (%)				
Rhinitis symptoms	1 (5)	–		
Asthma symptoms	1 (5)	–		
Both	17 (90)	–		
Trigger of symptoms, n (%)				
Bleaching products	19 (100)	–		
Hair dye	9 (49)	–		
Hair spray	4 (21)	–		
Permanent solution	3 (16)	–		
Perfume	3 (16)	–		
Positive stop/resume test, n (%)	16 (84)	–		

SD standard deviation, *BMI* body mass index, *SABA* short-acting beta$_2$-agonists, *LABA* long-acting beta$_2$-agonists, *ICS* inhaled corticosteroids, *LTRA* leukotriene receptor antagonists, *INS* intra-nasal steroid

[a] Defined as 1 ≥positive SPT or 1 ≥positive specific IgE to common inhalant allergens

[α] Comparing hairdressers with controls

daily basis. The nasal responses to SIC began within minutes (n = 2), after 1 h (n = 3), and after 3 h (n = 1). The two hairdressers reacting with bronchoconstriction did so after 3 h and 8 h, respectively. The characteristics of hairdressers with negative SICs are presented in Table 3.

Table 2 Characteristics of hairdressers with a positive specific inhalation challenge

ID	Age (y)	WRAS	WRRS	Stop/resume test	Duration of exposure before symptoms (y)	Time from exposure to symptom	Time since last exposure to persulphates (y)	Baseline FEV1/FVC (% of pred.)	MCh PD20 (μg)
2	21	+	+	P	5	Within hours	CE	87 (103 %)	330
5	29	+	+	P	11	Not definable	CE	86.7 (104 %)	N
8	32	+	+	P	1.5	Within minutes	8	85.5 (103 %)	346
10	22	+	+	P	4–5	Within hours	CE	80.6 (96 %)	N
16	23	+	+	P	0.5–1	Within minutes	2	85.4 (101 %) Rever: 12 %	N
19	23	+	+	P	0.5–1	Within minutes	1/3	94.9 (113 %)	N

ID	Age (y)	FeNO (ppb)	T-IgE (kU/L)	Atopy[a]	HRT PP (mg/mL)	HRT SP (mg/mL)	HRT AP (mg/mL)	SIC response	Classification of SIC response
2	21	6.3	73.2	No	N	N	2.5	R A	4 and 8 after 3rd exposure (late reaction)
5	29	6.2	8.7	Yes	N	10.0	10.0	R	1 h after 3rd exposure (immediate reaction)
8	32	*31.2*	46.4	Yes	N	N	5.0	R	1 h after 3rd exposure (immediate reaction)
10	22	7.4	26.6	No	N	N	10.0	R A	1 and 3 h after 3rd exposure (immediate reaction/late reaction)
16	23	13.0	39.8	Yes	10.0	2.5	1.25	R	After 2nd exposure (immediate reaction)
19	23	16.9	63.6	Yes	0.63	–	0.63	R	After 3rd exposure (immediate reaction)

y years, *WRAS* work-related asthma symptoms, *WRRS* work-related rhinitis symptoms, *MCh* methacholine challenge, *FeNO* fractional exhaled nitrogen oxide (increased values in italics), *T-IgE* total immunoglobulin E, *HRT* histamine release test, *PP* potassium persulphate, *AP* ammonium persulphate, *SP* sodium persulphate, *SIC* specific inhalation challenge, *N* negative, *P* positive, *CE* currently exposed, *R* rhinitis, *A* asthma

[a] Defined as ≥1 positive SPT to common inhalant allergens

Quantification of potassium persulphate

Before exposure, the amount of particles sized 0.1–10 μm inside the provocation chamber ranged from 7 to 18 μg/m^3 and the number of ultra-fine particles was 347–1260/cm^3. No additional ultrafine particles were detected during a 3 × 5 min exposure to 50 g pure potassium persulphate in the Erlenmeyer flask.

The mean amount of particles sized 0.1–10 μm measured during a 5-min exposure to a mixture of 30 g potassium persulphate and 20 g lactose ranged from 0.25–0.57 mg/m^3 and the proportion of potassium persulphate to lactose powder in the flask was 3:2. Thus, the estimated concentration of potassium persulphate in the air during a 5-min exposure was 150–340 μg/m^3 with a mean of 240 μg/m^3 and a standard deviation of 0.6 μg/m^3. During the 20-min pause in between exposures, the amount of particles in the air returned to baseline values.

SPT results

In two hairdressers, the negative control was positive due to dermographism and therefore their SPTs could not be evaluated (Table 4). All participants reacted to the positive control (histamine), whilst none were positive to latex, chlorhexidine, or any of the three tested persulphates (Table 4).

Results of HRT with persulphates

Of the six hairdressers with a positive SIC, four (66.7 %) did not react to HRT with potassium persulphate or sodium persulphate at any of the tested concentrations. In contrast, all six hairdressers with positive SICs released histamine in response to ammonium persulphate at concentrations ranging from 0.063 to 1 %. So did also 96.2 % of symptomatic controls and healthy controls. For all three persulphates, the lowest concentration producing histamine release in the controls and healthy controls was 0.125 %, whilst some of the hairdressers reacted to concentrations of 0.06 %. None of the participants showed histamine release to any of the persulphates in concentration of 0.031 %.

Discussion

SIC

In this study, we aimed at improving the currently validated SIC with persulphate. The improvements consisted

Table 3 Characteristics of hairdressers with a negative specific inhalation challenge

ID	Age (y)	WRAS	WRRS	Stop/resume test	Duration of exposure before symptoms (y)	Time from exposure to symptoms	Time since last exposure to persulphates (y)	Baseline FEV1/FVC (% of pred.)
1	52	+	+	N	3	Within hours	5	86 (109 %)
3	45	+	+	P	20	Within hours	CE	83 (104 %)
4	49	+	+	P	28	Within minutes	CE	65 (81 %) Rever: 2.9 %
6	30	–	+	P	10	Within minutes	3	81 (97 %)
7	27	+	+	P	1	Not definable	1	84.9 (103.3 %)
9	20	+	+	P	1	Within minutes	CE	98 (116 %)
11	43	+	+	P	20	Within hours	CE	83 (103 %)
12	23	+	+	N	3	Within hours	CE	74 (88 %) Rever: 12 %
13	46	+	–	N	2	Not definable	3	83 (103 %)
14	27	+	+	P	7	Within minutes	CE	83 (99 %)
15	31	+	+	P	4	Within minutes	1/2	75 (90 %) Rever: 7.8 %
17	20	+	+	P	4	Not definable	1/6	91 (108 %)
18	29	+	+	P	8	Within hours	CE	91 (109 %)

ID	Age (y)	MCh PD20 (µg)	FeNO (ppb)	T-IgE (kU/L)	Atopy[a]	HRT PP (mg/mL)	HRT SP (mg/mL)	HRT AP (mg/mL)
1	52	N	*25*	3.6	No	1.25	0.63	1.25
3	45	N	9.9	6.7	No	N	5.0	2.5
4	49	N	16.0	2.2	No	10.0	10.0	5.0
6	30	400	*64.1*	155	No	N	10.0	5.0
7	27	N	19.3	127	D	N	5.0	2.5
9	20	N	6.6	<2	No	N	10.0	5.0
11	43	N	7.1	22.5	No	N	10.0	5.0
12	23	N	21.8	122	No	10.0	5.0	2.5
13	46	626	12.1	35.4	No	N	5.0	1.25
14	27	N	11.8	3.7	No	N	10.0	5.0
15	31	720	9.0	308	D	5.0	2.5	1.25
17	20	N	9.0	4.3	No	10.0	2.5	2.5
18	29	N	19.7	58.3	No	N	N	N

y years, *WRAS* work-related asthma symptoms, *WRRS* work-related rhinitis symptoms, *MCh* methacholine challenge, *FeNO* fractional exhaled nitrogen oxide (increased values in italics), *T-IgE* total immunoglobulin E, *HRT* histamine release test, *PP* potassium persulphate, *AP* ammonium persulphate, *SP* sodium persulphate, *SIC* specific inhalation challenge, *N* negative, *P* positive, *CE* currently exposed, *D* dermographism, *R* rhinitis, *A* asthma

[a] Defined as ≥1 positive SPT to common inhalant allergens

of: a more rapid approach; using the "stirring method" instead of the "tipping method"; and assessing not only asthma but also rhinitis.

When Munoz et al. validated the realistic method [20], repeated exposures on consecutive days were performed with a mixture of potassium persulphate and 150 g lactose using the tipping method. The duration of the exposure was 10 min each day, and the dose of potassium persulphate was increased from 5 to 30 g over 4 days until a positive reaction occurred. The patient was hospitalized during the entire procedure. The method proved safe and a sensitivity of 100 % and a specificity of 87.5 % for diagnosing occupational asthma were reported.

In our method, we skipped the first 3 days with low exposure, and went straight to exposing the patient to 30 g of potassium persulphate. Instead of 10 min exposure we performed 15 min exposure. To reduce the risk of adverse reaction, exposure was performed step-wise; 5 min at a time with 20 min pauses in between, and severe asthmatic reactions and/or anaphylactic reactions to bleaching products were considered exclusion criteria.

Table 4 Results from skin prick tests

	Hairdressers (n = 19)	Symptomatic controls (n = 12)	Healthy controls (n = 40)
Positive control (p/n)	19/0	12/0	40/0
Negative control (p/n)	2/17	0/12	0/40
Potassium persulphate (p/n) Conc. (%)	0/17	0/12	0/40
2	–	–	–
5	–	–	–
7.5	–	–	–
Ammonium persulphate (p/n) Conc. (%)	0/17	0/12	0/40
2	–	–	–
5	–	–	–
7.5	–	–	–
10	–	–	–
20	–	–	–
Sodium persulphate (p/n) Conc. (%)	0/17	0/12	0/40
2	–	–	–
5	–	–	–
7.5	–	–	–
10	–	–	–
20	–	–	–

p positive, *n* negative

Given that none of the symptomatic controls with allergic asthma and rhinitis reacted to SIC, it seems that the proposed method has a high specificity for persulphate-induced asthma and rhinitis. In this group of hairdressers, SIC produced a nasal response in 33 % (6/18 with work-related rhinitis symptoms) and a bronchial response in 11 % (2/18 with work-related asthma symptoms).

We registered no adverse events or severe asthmatic reactions although our exposure was higher than Munoz' on the fourth day. Hence, it seems that the rapid method is safe when tested in patients without a history of severe asthmatic reactions or anaphylactic reactions to bleaching products.

We have several reasons for using the level of exposure we did. Firstly, we chose 3 × 5 min exposure to better mimic the hairdressers' exposure during a typical working day. Since hairdressers are mainly exposed to persulphates when they mix bleaching powder with hydrogen peroxide [32], we wanted to mimic this process. We estimated that a typical hairdressers performs this process three times a day. Secondly, the ratio of persulphate to lactose powder was changed as to better mimic the level hairdressers are exposed to in their daily practice. During mixing of the paste that is applied to the clients hair, 20–80 g bleaching powder [33], containing up to 60 % persulphate (12–48 g) [1], is typically used. We therefore used a ratio of persulphate to lactose powder of 3:2 (30 g persulphate:20 g lactose powder). To obtain a more uniform and reproducible exposure, we used a magnetic stirrer. In our study, the participants were exposed to levels of up to 0.34 mg/m^3 for 3 × 5 min during SIC. The permissible threshold limit value of exposure to potassium persulphate, as defined by the Occupational Safety and Health Administration in the United States, is a time weighted average (TWA) of 0.1 mg/m^3 during a typical working day of 8 h. According to the excursion limit of potassium persulphate, the TWA should not be exceeded more than 3 times for no longer than 30 min during a working day. Hence, the TWA was exceeded during our exposure, but the excursion limit was respected.

A limitation of our approach is that the patients were sent home after 8 h of observation in the clinic. This is convenient for the patient, but it introduces a potential bias. If the patient develops a positive nasal or bronchial response during this period at home, it is difficult to interpret whether the response was caused by exposure to persulphates or by exposure to other allergens encountered outside the hospital. However, in our study, all hairdressers reacted whilst being monitored in our department, so it is unlikely that this is a problem in our results.

Another limitation of our study is that the included hairdressers were merely under suspicion of having occupational asthma and rhinitis, but they were not clear-cut cases, which explains why only some hairdressers had a positive reaction to SIC. Firstly, they did not have serial peak flow measurement at and away from work performed prior to inclusion. If we had included only patient with a peak flow pattern suggestive of occupational asthma it might have improved the sensitivity of the test for detecting persulphate-induced asthma. Secondly, many had normal findings in spirometry, FeNO, and the methacholine challenge suggesting that they did not in fact have asthma although they reported asthmatic symptoms. Third, although persulphates are considered the major cause of occupational asthma and rhinitis in hairdressers [34] more than half reported that their work-related respiratory symptoms could also be provoked by other hairdressing products suggesting that their respiratory symptoms were not merely caused by persulphates. Also, some of the hairdressers had not been active hairdressers for several years and therefore were not still exposed to persulphates meaning that they could have lost airway responsiveness. Taken together, several factors exist that could explain why not all hairdressers reacted to the SIC and consequently the sensitivity of our approach cannot be determined.

HRT and SPTs with persulphate salts

This is the first study, to the best of our knowledge, to report results of HRT with persulphates. We found that persulphates, especially ammonium persulphate, induced non-IgE-mediated histamine release in both hairdressers and controls. Additionally, most of the SIC-positive hairdressers did not show histamine release. Ammonium persulphate has recently shown to have oxidative activity capable of promoting degranulation of human mast cells and basophils [13]. Thus, persulphates stimulate nonspecific non-IgE-mediated histamine release even in individuals without symptoms of persulphate-induced respiratory diseases, voiding the use of HRT to document asthma or rhinitis caused by persulphates.

We performed SPTs in duplicate with all three persulphates simultaneously, at concentrations as high as 20 %. To our knowledge, this has not been done before. We did not register any positive SPTs with persulphates in any of the participants, although all responded positively to the histamine control. Given the high persulphate concentrations applied, lack of positive reactions does not seem to be caused by using excessively low dosage. In addition, by testing all three persulphates, we ensured that we would not miss any patient sensitized to only one of the three persulphates [35].

Although several reports of positive SPTs with persulphates exist [8, 19, 21, 23], an equal amount of studies have failed to produce positive reactions [7, 14, 30, 31]. Moreover, in some patients, positive reactions are not reproducible over time [36].

The fact that specific IgEs to persulphates have been detected in only three [10, 37], out of more than 40 reported positive SPT cases, indicates that positive SPT reactions are caused by nonspecific non-IgE mediated histamine release. Indeed, when researchers with a method capable of detecting specific IgE to persulphates tested five patients with positive SPT reactions, they found that only two had demonstrable specific IgE [10], suggesting that the remaining positive SPT reactions were not mediated by IgE.

All in all, the majority of positive SPT reactions appear to be caused by direct histamine release rather than IgE-mediated mechanisms. Moreover, they have been reported by only a fraction of investigators, and are not always reproducible. Taken together, this indicates that SPTs cannot be applied to testing for persulphate-induced asthma and rhinitis.

Conclusions

The new rapid SIC with potassium persulphate proved safe when tested in hairdressers without a history of previous serious asthmatic reactions and had a high specificity for diagnosing persulphate-induced asthma and rhinitis. Based on our results, neither histamine release nor SPTs with persulphates appear adequate in predicting asthma and rhinitis caused by persulphates.

Abbreviations

ACS: American Chemical Society; BHR: bronchial hyperresponsiveness; CAS: Chemical Abstract Service; FeNO: fractional exhaled nitric oxide; FEV_1: forced expiratory volume in 1 s; HRT: histamine release test; IgE: immunoglobulin E; rpm: revolutions per minute; SIC: specific inhalation challenge; SPT: skin prick test; TNV: total nasal volume.

Authors' contributions

MHF-S, LW, HFM, PSS, MSO, HS, CZ, JDJ, CRJ participated in the study design. Drs. MHF-S and CRJ were responsible for conduction of the clinical investigations. Dr. PSS was responsible for performing the HRTs. Dr. MHF-S conceived the manuscript, drafted the initial version, and revised the final edition, whilst the remaining co-authors revised the initial and final manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The clinical data supporting the conclusion of this article contains direct and indirect identifiers. We do not have informed consent of participants for publication of dataset. Also, publication of the dataset would breach our local data protection law. For these reasons, data will not be shared.

Ethical approval and consent to participate

Approval was obtained by the Danish Data Protection Agency and the local ethics committee (H-2-2013-124). All participants gave informed consent and received financial compensation.

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CONSIDERATIONS AND COMMENTS ON METHODOLOGY

In this section, additional information and comments on methodology are included that have either not already been covered or are only discussed briefly in the manuscripts.

Study part I

Study design

A cross-sectional questionnaire study was performed at baseline to determine the prevalence of self-reported skin and respiratory diseases in hairdressing apprentices and a reference group comprising young adults from the general population. An alternative way of studying the epidemiology of a diseases is to perform a registry study (e.g. combining data from the Danish Register of Occupational Diseases with data from Statistics Denmark); however, as hairdressers tend to underreport suspected occupational diseases (84,126), we were concerned that this design would underestimate the disease occurrence in the cohort.

To determine the incidence of diseases we performed a 3-year prospective follow-up study among all respondents from the baseline study. The relatively short follow-up time decreases the risk of recall bias. The prospective study design also has the advantage of enabling us to also determine the rate of dropout from the trade and thereby examine the 'healthy survivor effect', while at the same time determining the incidence rates of the diseases.

We decided to use a web-based questionnaire because our study population consisted primarily of young people who generally have excellent computer skills and easy Internet access. Web-based questionnaires have several advantages over paper questionnaires. For example, data quality is generally better because validating checks can be incorporated and data entry errors are reduced because data are entered electronically and can be automatically transformed into analysable formats. In addition, the response times are faster and the costs are lower (127). One disadvantage of using web-based, compared with paper, questionnaires is that response rates are often lower (127). In the follow-up study, we combined web-based and postal questionnaires to increase the participation rate.

Study population

To assess the early effects of the hairdressing environment on the disease occurrence, we compared hairdressing apprentices to a non-exposed control group comprising young adults from the general population. This enabled us to examine whether hairdressing apprentices had an increased overall risk of disease compared with the general population, rather than examining if hairdressing apprentices had an increased risk compared to other occupational groups without exposure to the particular hazards in hairdressing. By selecting the

general population as the reference group, we demonstrate conservative differences in occurrence, because some individuals in the general population might be exposed to some of the same hazards as in hairdressing, e.g. wet work.

We chose to include hairdressing apprentices of all stages of their training in order to maximize the size of the group and to be able to compare the prevalence of diseases between apprentices with different durations of exposure to the trade. In the follow-up study we also included hairdressing apprentices of all stages of training. An alternative approach would have been to follow only newly started hairdressing apprentices. By examining apprentices of all levels of training, we probably report conservative incidence rates for the investigated diseases, because sensitive hairdressing apprentices may have already left the trade.

Execution of the study

In spring 2013, all ten public hairdressing vocational schools in Denmark were contacted and invited to participate in the baseline questionnaire study; eight participated and provided contact information, including civil registration numbers, on 1356 registered hairdressing apprentices. Data from Statistics Denmark indicated that 1473 hairdressing apprentices were registered as training in Danish hairdressing schools in 2013; hence 117 apprentices from the two non-participating schools were not included in the study.

A list with the civil registration numbers of the 1356 hairdressing apprentices was sent to the Danish Research Services (Forskerservice, Statens Serum Institut, Copenhagen, Denmark) to generate a control group that was twice the size and matched for age, sex, and postal code. The Danish Research Services returned a list of 2712 suitable controls subjects, and at the same time informed us that 242 of the hairdressing apprentices were covered by 'researcher protection', meaning that they had exercised their right to restrict the Danish Research Services from providing their current address to researchers. These individuals were therefore ineligible and the remaining 1128 were included in the baseline study. Eleven control individuals were excluded due to unknown address, leaving a total control group of 2701 subjects.

Between April and July 2013, the study population received a letter by post inviting them to complete a web-based questionnaire. The invitation letter included a link to the web-based questionnaire and a personal ID and password. Two reminders were given. All responders were enrolled in a prize draw ($ 730/670 €/5000 dkk) to provide an incentive to participate.

In 2016, the follow-up study was conducted among all participants from the baseline study. E-mail addresses of participants had been obtained in the baseline study, which enabled us to distribute the follow-up questionnaire by e-mail. As we also wanted to distribute the questionnaire by post, we contacted the Danish Research Services to obtain the current address of the participants. Between April and June 2016, all participants from the baseline study received three e-mails and two letters by post containing the follow-up questionnaire.

Questionnaire and definitions of outcome variables in baseline study

Hand eczema and contact urticaria

We used questions from the previously validated Nordic Occupational Skin Questionnaire 2002 (NOSQ-2002)(128). The validity of the questions on hand eczema has previously been compared with the gold standard of clinical examination in a group of hairdressing apprentices. A sensitivity of 70.3% and a specificity of 99.8% were reported (129), making the questionnaire a good tool for investigating hand eczema, although it tends to underestimate disease prevalence.

The original question on contact urticaria in the NOSQ lists a few typical urticariants (e.g. fruits, vegetables, rubber gloves, animals etc.), but not those associated with hairdressing. Therefore, we were concerned that using the original wording of the question in our questionnaire would result in a false reduction in the occurrence of contact urticaria among our hairdressing apprentices. For this reason, the question was changed to list more relevant urticariants (e.g. rubber gloves, hair dyes, cosmetics or the like). In both the original and adapted versions of the question, bias should be considered, because individuals with contact urticaria to an unlisted allergen might mistakenly answer "no" to the question. Therefore, on the basis of the response to this question, we cannot determine whether the risk of contact urticaria in general is higher in hairdressing apprentices compared with the general population. We can however, examine whether hairdressing apprentices have a higher risk of contact urticaria that is caused by the listed allergens (e.g. rubber gloves, hair dyes, cosmetics, and the like).

Respiratory symptoms

Questions from the European Community Respiratory Health Survey (ECRHS) (130) were used to examine respiratory symptoms and smoking. This questionnaire has previously been used in several nationwide studies, both as an interviewer administered questionnaire, and in a shorter screening version as a self-administered questionnaire (131,132). The questions on asthma and wheezing have been validated against bronchial hyperresponsiveness, spirometry, and by clinical evaluation (133,134). The validity of the Danish version of the questionnaire was guaranteed by the Danish ECRHS centre, who translated and back-translated the questionnaire to minimize linguistic bias.

There is a lack of consensus on the standard operational definitions of asthma (135) and rhinitis (136,137) for epidemiologic studies. Some studies use self-reported disease whilst others use self-reported symptoms as outcome. We decided to focus on self-reported symptoms because this approach is less likely to be biased by health care practices and diagnostic activities. We decided to focus on wheezing because this is the symptom most commonly used and it also has the highest sensitivity for asthma (133,135). The rhinitis questions in the ECRHS questionnaire asks whether the classical symptoms of sneezing, or a runny or blocked nose (in the absence of a cold or the flu) are present. This wording is the same as is used in the International Survey of Asthma and Allergies in Childhood (ISAAC), and the same as recommended in a position paper on the epidemiologic identification of rhinitis (137). Because responses may vary with seasonal changes (137), it is important that all participants receive the questionnaire at the same time of year.

Questionnaire and definitions of outcome variables in follow-up study

We used the same questions from the ECRHS and NOSQ that were asked in the baseline study, and the definitions of wheezing, rhinitis symptoms, hand eczema, urticaria on the hands, and contact urticaria were the same. The questions were altered slightly and now asked about the occurrence of diseases and symptoms within the last three years. The individuals at risk of disease during the follow-up period were defined as those reporting at baseline that they had never had the disease. This approach gives more conservative incidence rates than defining individuals at risk as those without current disease or without having had the disease within the last 12 months.

The participants were asked if they were still hairdressing apprentices and how many years of training they had completed. If they reported having completed their training entirely, they were asked whether they were still active as hairdressers. Participants who had left the trade during training or after completing their training, were asked whether they had left due to, or partly due to, health problems. Those participants who did attribute their leaving the trade, at least in part, to ill health, were asked to identify one or more of the following as the reason: asthma, allergy symptoms related to the nose and/or eyes, hand eczema, nettle rash, depression/stress, pain in the muscles or joints, or other disease.

Study part II

Study design

In order to establish a new rapid SIC with potassium persulphate, we performed a single-blinded observational clinical case-control study. The study was conducted at the Allergy Clinic, Copenhagen University Hospital Gentofte between February 2014 and May 2016. Ideally, a longer study period would have been preferable to optimize the size of the study group. Previous similar studies have had a duration of 5-12 years (108,121). A possible alternative design would have been a retrospective review of a sufficient number of patients with a positive SIC response, all of whom had been tested in exactly the same manner. These results could subsequently be compared with those from identical SIC tests on a control group. Since we had only recently acquired a provocation chamber, we obviously did not have such records.

Determining the sensitivity of a test requires a reference standard to compare the new test to. Since the SIC is considered the reference standard for diagnosing occupational asthma and rhinitis, the new SIC must be compared with a previously validated standardized SIC. In this case, Munoz' method was the only option. So to determine the sensitivity of the test, we would first have to perform Munoz' method and then our rapid method on every patient and compare the results. For practical and logistical reasons, this design was abandoned and the sensitivity of the new rapid SIC was not determined. However, we did examine whether the test was able to produce positive reactions in patients reporting asthmatic and nasal symptoms when exposed to hair bleaching products, and also determined the specificity of the test by examining a group of asymptomatic subjects without known exposure to persulphates.

Study population

To generate a study population we recruited hairdressers with occupational respiratory symptoms referred to our department, those with respiratory complaint who had contacted our hotline at the Research centre for Hairdressers and Beauticians, and those who answered an advert in the hairdressers' trade magazine 'Spejlet'. Hairdressers and hairdressing apprentices, both active and inactive in the hairdressing trade, who were between the ages of 18 and 60 years old and reported work-related respiratory symptoms were included in the study. A history of excessive asthmatic or anaphylactic reactions to bleaching powder was used as exclusion criteria; however, none of the hairdressers reported these reactions. Narrower inclusion criteria might have improved the strength of the study, e.g. including only hairdressers currently active in the hairdressing trade or only those where the suspicion of occupational asthma and rhinitis was supported by an objective test (such as peak flow measurements both in and away from the workplace). However, because we wanted to optimize the size of the case group, we maintained broad inclusion criteria.

The symptomatic control group comprised both men and women between the ages of 18 and 60 who had been diagnosed with asthma. The exclusion criteria for the control group included previous or current work as a hairdresser, or persulphate-related respiratory symptoms.

Additional exclusion criteria for both groups included pregnancy, unstable asthma within the last three months, baseline forced expiratory volume in 1 s ≤ 70% of predicted normal value, chronic obstructive pulmonary disease, regular use of systemic corticosteroids, severe hypertension, immunological disease, or unstable cardiovascular disease. A recent upper respiratory tract infection resulted in postponement of any clinical investigations for four weeks.

The specific inhalation challenge

The SIC is described in detail in manuscript IV page 2.

Assessment and definitions of positive responses

Bronchial response

Bronchoconstriction was measured using a portable asthma monitor. At each assessment point, three consecutive measurements were made and the best value was noted. If a large decrease in FEV1 was noted, the participant was instructed to contact the investigator and the measurement was reassessed to make sure that the decrease was not attributable to improper technique.

Asthma was defined according to the consensus statement from 2014, as a sustained fall in FEV ≥15% from baseline, provided that fluctuations in FEV_1 were ≤10% on the control day (105).

Nasal response

In the absence of a consensus on how to monitor the nasal response during the SIC, we chose to use a combination of three tests: one subjective, one semi-objective, and one objective. The SIC was considered positive for a nasal response if ≥ 2 tests were positive on the test day and the participant had < 2 positive tests on the control day. Including additional objective test, for example measuring nasal peak inspiratory flow, might improve the study method because both the Linder symptom score scale and scoring by anterior rhinoscopy are highly subjective and rely on the observer (138). The study might also be improved if it was double blind.

For the subjective test, we used Linder's symptom score scale (138,139)(see Table 4). Originally this score was defined as positive if a participant scored more than 5 points. Instead of using this approach, we defined a positive test as a minimum increase of 3 points between baseline and post-exposure. We made this change because we wanted to monitor the change in nasal status and not merely the presence or absence of rhinitis. Validation of this approach should be investigated in future studies.

For the semi-objective scoring of nasal congestion and rhinorrhea using anterior rhinoscopy, we adopted the scoring system proposed by Hytonen et al. (140) (see Table 5). For the objective test we used acoustic rhinometry as proposed by Hilberg et al. (141). For a detailed description, see manuscript IV page 4.

Table 4. Linder Symptom Score Scale

Symptoms	Severity	Score (points)
Sneezes	0−2	0
	3−4	1
	≥ 5	3
Itchiness	Nose, palate, ear 1 point for each	
Rhinorrhea		0-3
Nasal obstruction		0-3
Ocular symptoms		1

Positive if ≥ 5 (maximum possible score 13)

Table 5. Scoring of nasal blockage and rhinorrhoea with anterior rhinoscopy

Rhinorrhea

0: dry mucous membrane
1: slightly moist mucous membrane
2: some mucus collecting at the bottom of the nasal cavity
3: mucus dripping out of the nose

Nasal obstruction

0: no swelling (the bony configuration of the anterior turbinate is seen)
1: slightly mucous membrane swelling of the inferior turbinate
2: moderate mucous membrane swelling (if there is no septal deviation, the inferior turbinate is close to the septum)
3: the nasal cavity is (almost) completely blocked

Each nostril is scored separately (maximum possible score 12 points). The test is considered positive if a change in score of ≥4 point is observed between baseline and post-exposure.

Exposure

We focused on the level of exposure used by Muñoz et al. on the fourth day of their protocol, where 30 g of potassium persulphate is mixed with 150 g of lactose powder for 10 min using the 'tipping method'. Potassium persulphate was chosen because, in contrast to the other persulphates, it is odorless, and this facilitates blinding. A total of 30 g of persulphate was used because this is a typical amount a hairdresser may use for bleaching (142). However, instead of mixing this with 150 g of lactose powder we used 20 g of lactose powder, because persulphates can constitute up to 60% of a hair bleaching product (93). Several studies have suggested that the main exposure to persulphates occurs during the mixing of bleaching powder with hydrogen peroxide (2,143). Because mixing typically takes 2–5 min (143), we exposed for 5 min at a time for a total of three exposures. In accordance with the consensus statement from the ERS task force (105), we gradually increased the duration of exposure. Instead of the 'tipping method', we used a magnetic stirrer for mixing in an attempt to obtain a more uniform and reproducible exposure. To assess the reproducibility of our method, we quantified the powder inside the provocation chamber on three separate days, during three distinct exposures, each 5 min in duration (30 g potassium persulphate and 20 g lactose powder). We chose to measure particles with a maximum size of 10 µm during sampling because larger particles are deposited in the upper respiratory tract (144).

Study part III

Study design

To assess the value of SPT and HRT using persulphates we conducted a clinical case-control study.

Study population

The study population consisted of hairdressers from part II of the study who had a positive nasal or bronchial response to the SIC. The control groups comprised hairdressers with a negative response to the SIC and also symptomatic controls. The inclusion and exclusion criteria for these groups were the same as in part II of the study, but dermographism was also added to the exclusion criteria.
In order to increase the statistical power of the study, we included an additional control group comprising 40 healthy control subjects. The exclusion criteria for this group were a history of asthma, rhinitis, urticaria, or dermographism.

Skin prick tests with persulphates

To properly assess the SPT using persulphates we wanted to ensure that negative reactions could not be ascribed to testing persulphate concentrations that were too low or to testing only particular persulphates. Due to a lack of recommendations regarding the use of solvents, we chose to use saline and the test solutions were made up in concentrations ranging from 2% to 20% (w/v). Solutions containing high concentrations of persulphates had pH-values < 2, which we predicted could potentially induce false positive reactions. Since the importance of testing with freshly prepared solutions has been stressed (100), we performed tests within 30 min of preparing solutions. To ensure accuracy, we carried out the SPT in duplicate. Only one previous study has done this when testing with persulphates (97). The test was regarded as positive if both pricks had produced a wheal with a diameter of $\geq$3 mm (145) providing the positive control was positive and the negative control was negative.

Histamine release tests with persulphates

For the HRT, we also decided to test all three persulphates. We used concentrations of 0.03%, 0.06%, 0.125%, 0.25%, 0.5% and 1.0% (w/v). Blood samples were analysed on the day of collection and tested for histamine release using freshly prepared solutions of persulphates in saline. Positive reactions were categorized according to the lowest concentration producing significant histamine release from the basophils (10 ng histamine/mL blood). Therefore, if a patient's cells released histamine when exposed to a solution containing the lowest concentration of persulphate, this was categorized as class 6, and if cells responded only to the highest concentration of persulphate, this was categorized as class 1. If no reactions occurred to any of the tested concentrations, the test was class 0 (negative).

Scratch test with persulphates (not in manuscript)

After conducting parts II and III of the study, we thought it possible that scratch testing with persulphate powder mixed with saline directly on the skin might be more sensitive than the SPT, because the persulphate solution would be 100% freshly made and with a higher concentration, so more persulphate would get in contact with the mast cells of the skin.

Therefore, we contacted all participants from the clinical study and invited them to take the scratch test. Because not all patients could be contacted and some were now pregnant, we studied a subsample of three hairdressers with a positive response to the SIC, nine hairdressers who had a negative SIC, and eight symptomatic control subjects. The scratch test was carried out by scratching the skin of the forearm slightly with a lancet at three different locations. Concentrated powder containing one of the three persulphates was applied to each scratch and a drop of saline was added. The solution was gently rubbed into the scratch for a few seconds using a sterile cotton swab. A positive control test containing histamine (10 mg/ml) and a negative control test containing saline only were also applied. The reactions were assessed after 15 and 30 min. A wheal > 3 mm in diameter was regarded as positive, if the negative control was negative and the positive control was positive.

Results are presented in the discussion of results.

DISCUSSION OF RESULTS

This section includes additional discussion of the results presented in the manuscripts as well as a more general discussion.

Study part I

Response rate

In total, 1908 of the 3829 eligible subjects completed the baseline questionnaire (505 hairdressing apprentices and 1403 controls). One hairdressing apprentice was excluded due to lack of Danish language skills, and three control subjects were excluded due to current or previous exposure to the hairdressing trade. The resulting overall response rate was 49.8% (1904/3825), and the response rate was significantly lower for the hairdressing apprentices compared with the control subjects (44.7% vs. 51.9%, $p< 0.001$).
Taking into account the two hairdressing schools that did not participate (117 apprentices) and the 242 apprentices that were covered by researcher protection, the participation rate to the baseline study from hairdressing apprentices was 34% (504/1473). Unfortunately, no data are available on the 117 hairdressing apprentices from the two non-participating schools. The hairdressing apprentices covered by researcher protection did not differ significantly from the participating hairdressing apprentices with respect to age or sex (mean age = 22.8 years, 95.1% female), but another selection bias could potentially have been introduced. Whenever a low response rate is obtained in an epidemiologic study, one has to consider the introduction of selection bias, e.g., individuals with hand eczema or respiratory symptoms might be more inclined to answer a questionnaire on allergic diseases. However, the fact that all responders were enrolled in a prize draw may have increased non-diseased individuals' incentive to participate. In addition, the fact that our control group had similar frequencies of hand eczema (146), wheezing (147), and rhinitis symptoms (148) to those previously observed in the general Danish population, suggests that selection bias has not been introduced in the baseline study. We compared the available demographic data between responders and non-responders and found no significant differences in age, sex, or geographical distribution between the two groups. An alternative strategy to investigate a potential selection bias between responders and non-responders would have been to telephone a subsample of the non-responders to provide them with a short version of the questionnaire to compare disease prevalence between the two groups. This would have strengthened the validity of our results, but unfortunately we did not have the necessary approval.

In the follow-up study, a total of 1064 (248 hairdressing apprentices and 816 control subjects) of 1904 participants from the baseline study responded to the follow-up questionnaire resulting in a relative low response rate of 55.9%. As previously, the response rate from hairdressing apprentices was significantly lower than that from control subjects (49.2% vs. 58.3%, $p<0.001$).

We compared the prevalence of disease at baseline between responders and non-responders to assess if diseased individuals were more inclined to participate in the follow-up questionnaire. The results did not indicate the introduction of selection bias. However, since further information regarding non-responders are not available, one could still speculate that individuals who had developed the disease during the follow-up period were more inclined to participate, resulting in overestimation of IRs in both hairdressing apprentices and population controls. The relatively high IR of hand eczema and wheezing in the population controls compared to findings from similar studies in the general population (146,149) might be interpreted as a sign of selection bias in the control group. However, differences in study methods between our study and the mentioned studies, such as longer follow-up periods giving rise to recall bias, and an older mean age of the study population, might also partly explain the differences in results.

The healthy hire effect

Hairdressing apprentices have been found to have a lower prevalence of respiratory symptoms and skin diseases when they start training (88,92) compared with office workers and the general population, suggesting a 'healthy hire effect'. In our baseline study, we made several observations that support this suggestion. First, the 91 hairdressing apprentices in the first year of their training had a lower prevalence of wheezing, rhinitis symptoms, urticaria on the hands, and contact urticaria when compared with the general population (wheezing: 22% vs. 26.2%, $p = 0.37$; rhinitis symptoms: 41.8% vs. 46.6%, $p = 0.37$; urticaria on hands: 17.6% vs. 24.3%, $p = 0.15$; contact urticaria: 2.2% vs. 4.2%, $p = 0.34$). Although these findings were not significant, atopic dermatitis was significantly less prevalent in the first year apprentices (22.0% vs. 33.9%, $p = 0.02$). In addition, more hairdressing apprentices than control subjects had considered allergies and respiratory problems when choosing their career and had significantly later onsets of wheezing and hand eczema. Overall, it seems plausible that there is a 'healthy hire effect' in hairdressing apprentices with respect to respiratory symptoms and skin diseases. To fully investigate the extent of the 'healthy hire effect' however, hairdressing apprentices should ideally be examined on the first day of their training rather than during the first year, as in our study.

The healthy survivor effect

During the 3-year follow-up period, 21.8% of the hairdressing apprentices left the trade and most of them had left due to disease (70.4%). This finding indicates that a 'healthy survivor effect' exists in hairdressing apprentices during their early years in the trade. The largest contribution to this 'healthy survivor effect' is attributable to musculoskeletal pain and skin disease (hand eczema and urticaria), which were both reported as a reason for leaving the trade by 47.4% of ex-hairdressers, whilst 23.7% reported respiratory symptoms

(asthma or rhinoconjunctivitis) as a reason for leaving. As discussed in detail in manuscript III, our findings agree with studies conducted among trained hairdressers in Denmark (49) and Finland (150) and support the existence of a 'healthy survivor effect' in hairdressers and hairdressing apprentices which is strongly attributable to musculoskeletal pain and skin diseases, but also to respiratory symptoms.

Occurrence of hand eczema

An overall increased prevalence of hand eczema was reported among the hairdressing apprentices (34.5% vs. 18.8%, $p<0.001$) and a dose response effect of training duration on hand eczema was observed as the prevalence increased from 18.7% among first year apprentices, to 36% among fourth year apprentices, to more than 50% among recently graduated hairdressers. A significantly increased OR in apprentices attending second year of training compared with the controls indicates that hand eczema develops quickly during the early years of training.

In the follow-up study, the hairdressing apprentices also had an increased incidence rate ratio (IRR) for hand eczema (IRR: 1.68, 95% CI 1.1–2.6) compared with the population controls. As discussed in detail in manuscript III, the IR of hand eczema of 68 cases/1000 person-years is low compared with previous findings among apprentices in the 1990s (53,54), but almost twice as high as the 37 cases/1000 person-years reported among trained Swedish hairdressers who were < 25 years (47). These differences are probably partly due to differences in study methods, but they also suggest that hairdressing apprentices are at an increased risk compared with qualified hairdressers; however, improvements in working practices and the implementation of protective measures over the last 20–30 years may have resulted in a decreased incidence of hand eczema. Nevertheless, it is clear that despite receiving education in hairdressing schools on the prevention of occupational skin diseases, hairdressing apprentices in Denmark are still at increased risk of developing hand eczema during their training.

Recently, a 6-year follow-up study was conducted on Danish hairdressing apprentices who were originally recruited as part of an intervention study by Bregnhøj et al. in 2008. The aim of the follow-up study was to examine the long-term effects of the initial intervention (57). In agreement with our findings, no sustained reduced risk of hand eczema was detected in hairdressing apprentices who had received training in preventing the condition. Therefore, both the hairdressing apprentices participating in the initial study on prevention and the hairdressing apprentices subsequently attending Danish hairdressing schools remained at high risk of developing occupational hand eczema.

So why has the implementation of the education program not had a full preventive effect? Hairdressing apprentices spend around 2/3 of their apprenticeship training in salons, and results from a previous study showed that hairdressing apprentices use gloves less frequently in the salons than they do in the schools (55) and in some salons the apprentices turned the gloves inside out and reused them. The reason for this difference in the use of protective work habits between the schools and the salons is unknown, but one could speculate, that some salons owners are less supportive of protective work habits than the hairdressing schools are.

Occurrence of urticaria

Urticaria on the hands was equally common among hairdressing apprentices and control subjects with a prevalence of 23.2% vs. 24.3%. However, following a similar trend to hand eczema, the prevalence of urticaria on the hands increased with training duration from 17.6% in first year apprentices to 33% in fourth year apprentices (results not in manuscript), indicating that exposure to the hairdressing trade increases the risk of urticaria on the hands. The prevalence of contact urticaria caused by rubber chemicals, hair dyes, cosmetics or the like was 7.3% in the hairdressing apprentices and also showed a tendency to increase with increasing duration of exposure, with a prevalence of 14.6% in recently graduated hairdressers. The results from the follow-up study further supported our findings of an increased risk of both urticaria on the hands and contact urticaria in the hairdressing apprentices compared with the population controls (IRR urticaria on the hands: 1.85, 95% CI 1.2–2.8, IRR contact urticaria: 4.74, 95% CI 2.6–8.6). Urticaria has not been previously studied in hairdressing apprentices and epidemiologic data on contact urticaria among hairdressers is sparse (7,60) and mostly confined to case reports (10,23,34). Our results in the baseline study are consistent with the frequency of 16% observed in qualified Greek hairdressers (60).

In the hairdressing apprentices, the IR for urticaria on the hands was as high as the IR of hand eczema (68 cases/1000 person-years for both diseases). This is a surprising finding that also raises concerns about the possibility of misclassification. The substantial overlap in symptoms between hand eczema, atopic dermatitis, and urticaria might partly explain this surprisingly high incidence of urticaria on the hands. For future research, the use of clinical pictures in the questionnaire should be considered to improve clarity of questions on skin diseases. Nevertheless, our findings indicate that hairdressing apprentices are at risk of contact urticaria and the profile of a disease, which has previously received little attention, should be raised.

In more than 40% of occupational contact urticaria cases, patients have a co-diagnosis of irritant contact dermatitis (ICD) or allergic contact dermatitis (ACD) (151). A defective skin barrier might facilitate skin penetration by allergens and therefore the high frequency of hand eczema in hairdressing apprentices might lead to an increased risk of occupational contact urticaria.

Occurrence of rhinitis symptoms

Rhinitis symptoms were significantly more prevalent in the hairdressing apprentices across all levels of training compared with the controls (58.1% vs. 46.6%, OR 1.6, 95% CI 1.3–2.1) and increased significantly from 41.8% among apprentices in their first year of training to more than 60% in apprentices with three or more years of training (see Figure 1 in manuscript II). In the follow-up study, we also observed a significantly increased risk of rhinitis symptoms in the hairdressing apprentices compared with the population control (IR rhinitis symptoms: 154 vs. 96 cases/1000 person-years, IRR 1.61, 95% CI 1.2–2.2.). As our study is the first to examine the prevalence and incidence of rhinitis in hairdressing apprentices, comparison with similar studies is not possible. However, our results from the baseline study are consistent with studies among trained hairdressers reporting a higher prevalence of rhinitis symptoms compared with saleswomen (152) and office

workers (153,154), whilst studies that compared the prevalence of hay fever between hairdressers and the general population (143) and office and shop workers (152,155,156) did not detect differences in prevalence. Only one study has examined the incidence of rhinitis symptoms in trained hairdressers and they also reported an increased incidence of nasal blockage in hairdressers compared with women from the general population (17.3 vs. 11.4 cases/1000 person-years, IRR 1.5, 95% CI 1.3–1.8) (157).

In conclusion, exposure to the hairdressing environment during training increases the risk of developing rhinitis symptoms. Our study demonstrates that rhinitis symptoms develop after only a few years of exposure. Since rhinitis is generally considered a risk factor for asthma, and nasal symptoms have been found to develop before bronchial symptoms in 21% of workers exposed to low-molecular weight chemicals (158), the increased risk of rhinitis symptoms should be taken seriously and attempts made to prevent the disease developing during hairdressing apprenticeship.

Occurrence of wheezing

Wheezing was similar in prevalence in both hairdressing apprentices and young adults from the general population (25.3% vs. 26.2%). This finding differs from those of a study that reported a higher prevalence of wheezing in hairdressing apprentices attending their last year of training compared with pupils from low-risk occupations (26.7% in hairdressers vs. 16.5% in butchers and 9.5% in sales workers) (89). In contrast, Iwatsubo et al. (88) examined hairdressing apprentices with 2–3 years of training and uncovered a significantly lower prevalence of wheezing in the hairdressing apprentices compared with office apprentices (10% vs. 18.8%, $p = 0.004$). The inconsistencies between the results of the three studies might be due to differences in the study methods – e.g. comparison with a low-risk occupational group versus comparison with the general population, or by examining hairdressing apprentices without taking into account the stage of their training versus examining apprentices in the last year of their training. When we performed analyses adjusted for smoking, we also found a decreased OR for wheezing in the hairdressing apprentices compared with the controls (OR 0.8, 95% CI 0.6–0.95).

In the follow-up study, a similar incidence of wheezing was observed in the two groups (51 vs. 54 cases/1000 person-years, IRR: 0.95, 95% CI 0.6–1.5), despite smoking being significantly more common among hairdressing apprentices (ever smoker: 35.9% vs. 25.4%, $p=0.001$) and a greater proportion of apprentices reporting exposure to chemical fumes for more than two hours daily (66.5% vs. 1.1%, $p<0.001$).

Our finding of a similar incidence of wheezing in both hairdressing apprentices and control subjects is supported by a previous prospective study conducted among French hairdressing apprentices (88). Interestingly, a study conducted in trained hairdressers reported a higher IR of wheezing in the hairdressers compared with the general population (157). The significantly higher incidence was, however, only present in non-smoking individuals without atopy.

In conclusion, although hairdressing has one of the highest risks of occupational asthma, an increased risk of wheezing is not consistently detected in apprentices. However, an increased risk of rhinitis symptoms can be

detected, and this is generally considered a risk factor for asthma. Therefore, it appears that excessive exposure to hairdressing can induce respiratory tract symptoms, but seemingly upper respiratory tract symptoms are more common and develop faster than lower respiratory tract symptoms.

Causes of respiratory symptoms in hairdressing apprentices

The hairdressing product most frequently associated with both upper and lower respiratory problems was hair bleach (see Figure 2 in manuscript II). Therefore, an important step in reducing the risk of respiratory problems is reduction of exposure to hair bleaching products containing persulphates. To the best of our knowledge, no hair bleaching products exist that do not contain at least one of the three persulphates. If product manufacturers were able to substitute the persulphate component of hair bleach with a less problematic alternative, this would be most welcome. The Danish "Branchearbejdsmiljøråd" (159) recommends local exhaust ventilation (with an air extraction capacity of 100 m^3 per hour) at every work station where bleaching, perming, or dyeing is being performed. They also recommend using designated mixing stations provided with local exhaust ventilation to avoid spreading chemicals to the rest of the hairdressing salon. Local exhaust ventilation is accessible in 90% of hairdressing salons in Denmark (84), but only 63% of hairdressers report using it while performing permanent waving, hair dyeing, and bleaching, and only 41% use local exhaust ventilation during mixing. Hence, there is potential for improvement in the use of ventilation in hairdressing salons and this is one aspect that preventive strategies should focus on.

Study part II

The new rapid SIC produced a nasal response in 6 of 18 hairdressers with rhinitis symptoms, and a bronchial response in 2 of 18 hairdressers with asthmatic symptoms. None of the symptomatic controls subjects reacted to the test. Hence the test is able to produce positive reactions in hairdressers reporting respiratory symptoms to bleaching powder, and the specificity of the test is high.

Usability of the new rapid SIC

Because the sensitivity of the new rapid SIC was not determined, we do not know the rate of false negative responses. Perhaps the short duration of exposure to persulphate has caused us to underdiagnose some actual cases. This would be unlikely if persulphate-induced asthma and rhinitis are IgE-mediated diseases, but if they are caused by irritant mechanisms then more than one day of exposure may be required to provoke a reaction. However, to date, only Muñoz et al. have implemented gradual exposures to persulphate over successive days. All other studies have performed the test in a single day. As discussed previously, many centres may lack the facilities and resources to admit patients for SIC testing over several days. To determine whether the four-day protocol is superior to the one-day protocol, the two tests should be compared using the same group of patients. Until the better method has been identified, we suggest conducting the new rapid SIC along with pre- and post-challenge methacholine provocation as recommended by the ERS task force (105) to identify participants that would benefit from additional challenge testing.

Safety

We did not register any severe adverse reactions during of the new rapid SIC procedure, and the proposed method appears to be safe for patients with no history of excessive asthmatic or anaphylactic reactions in response to bleaching products. When reading the literature on work-related asthma in hairdressers it appears that severe reactions are rare (73,94,111,112). Although a previous severe asthmatic reaction was an exclusion criterion in our study, none of the hairdressers were excluded on this basis. Therefore, the method proposed is probably suitable for the majority of hairdressers.

Exposure

We quantified the powder inside the provocation chamber to assess the reproducibility of exposure (see Tabel 6). The mean quantity of particles 0.1–10 µm in size inside the provocation chamber was 0.4 mg/m^3 (range 0.25–0.57 mg/m^3). Because the ratio of persulphate to lactose powder was 3:2, we estimated that the concentration of persulphate ranged between 0.15 and 0.34 mg/m^3 (mean 0.24 mg/m^3). Therefore, the ‘stirring method’ apparently produces reproducible levels of exposure to persulphates; however, further research would be required to determine whether it is more reproducible than the ‘tipping method’.

The level of exposure to potassium persulphate produced using the 'stirring method' is lower than the 1–6 mg/m^3 produced using Muñoz' 'tipping method'. The level of persulphate hairdressers are exposed to in Swedish hairdressing salons has previously been studied (143). Here, personal samplings revealed a level of 35–150 µg/m^3 of airborne persulphates during a 5 min mixing of persulphate with hydrogen peroxide (143). Another study measured airborne levels of persulphates in French hairdressing salons and found a mean conc. of 20 µg/m^3 during an entire shift in both personal exposure measurement and at the mixing stations (63). It should be noted, that the measurements performed in the Swedish salons stem from short periods, focused on mixing of hairdressing products, while the French data correspond to shift averages over all daily activities. By comparing the exposure levels used in our method and that of Muñoz to the measurements made in the hairdressing salons, we can conclude that our 'stirring method' produced more realistic persulphate exposure levels (similar to those in the salon).

Table 6. Quantification of particles between 0.1-10µm in size during exposure

		Mean quantity of particles (mg/m^3)	Estimated quantity of PP (mg/m^3)
Day 1	1. exposure	0.37	0.22
	2. exposure	0.32	0.19
	3. exposure	0.36	0.22
Day 2	1. exposure	0.37	0.22
	2. exposure	0.49	0.29
	3. exposure	0.57	0.34
Day 3	1. exposure	0.37	0.22
	2. exposure	0.48	0.29
	3. exposure	0.25	0.15
		Mean (SD): 0.40 (0.09)	Mean (SD): 0.24 (0.06)

PP: potassium persulphate

A more precise estimate of the persulphate concentration inside the provocation chamber can be made by sampling dust on filters in air samplers and subsequently analysing of their persulphate content using mobile phase ion chromatography (11,63,143). We collaborated with scientists at the Danish Technical University, who made several attempts to establish such an analysis, unfortunately without success.

Study part III

Skin prick tests with persulphates

None of the patients with persulphate-induced asthma or rhinitis had a positive SPT with persulphates, and none of the participants in the control group has a false positive reaction when tested with high concentrations of persulphates.

A weakness of our study is that SPTs using different solvents were not performed, and therefore our negative findings could potentially be due to using the wrong solvent. When reviewing results from previous studies, choice of solvent does not appear to influence the rate of positive and negative reactions; hence it is unlikely that this has influenced our results. However, future studies should consider the optimal solvent for persulphate and the stability of persulphates in different solvents. Some low-molecular weight substances can bind to human serum albumin and create a hapten which then triggers the immune response. However, it was recently demonstrated that ammonium persulphate cannot conjugate with human serum albumin to form a hapten (103), making human serum albumin an unsuitable solvent for persulphates.

When comparing studies that have performed SPTs using persulphates, it becomes clear that results are diverging; some detect positive reactions (25,31,100,107,109,118), whilst others do not (11,97,112,121,122). As discussed in detail in manuscript IV, true IgE-mediated persulphate sensitivity appears to be very rare as it has only been demonstrated in three cases to date (100,160), and therefore the majority of the reported positive SPT reactions were probably caused by non-IgE mediated histamine release.

Scratch tests with persulphates

The results of the scratch test are summarized in Table 7. All positive reactions occurred after 30 min. One of the hairdressers who had a positive response to SIC reacted to the scratch test with all three persulphates. In total, four hairdressers with a negative SIC response reacted to the scratch test; two reacted to only one persulphate, whilst two showed positive responses to all three persulphates. Two symptomatic control subjects also responded positively to the scratch test; one reacted to all three persulphates, whilst one reacted only to sodium persulphate.

Table 7. Results of scratch test with persulphates

	SIC positive hairdressers (n=3)	SIC negative hairdressers (n=9)	Symptomatic controls (n=8)
Potassium persulphate	33.3% (1)	33.3% (3)	12.5% (1)
Sodium persulphate	33.3% (1)	33.3% (3)	25.0% (2)
Ammonium persulphate	33.3% (1)	22.2% (2)	12.5% (1)

The conclusion to this small study was that the scratch test with persulphates was not suitable for diagnosing persulphate-induced asthma and rhinitis, because it produced positive reactions in both SIC-positive and controls subjects, and not all patients with persulphate-induced asthma and rhinitis reacted to the test.
The findings further support the hypothesis that persulphates can induce non-IgE mediated histamine release from mast cells and basophils. The difference between the scratch test and the SPT is that higher concentrations of persulphate are used in the scratch test. Here the solutions are mixed with saline directly on the skin and we can be relatively sure that persulphate has not disappeared from the solution. In addition, perhaps scratching the skin better ensures that persulphate comes into contact with mast cells.
In 1963, Calnan and Shuster (115) scratch tested five hairdressers with immediate reactions to hair bleaching products using saturated solutions of ammonium persulphate and found all had a positive reaction after 15 to 30 min. These reactions were inhibited by intramuscular injection of antihistamine and could not be elicited in skin depleted of histamine with prior injection of compound 48/80 suggesting a histamine response. In this study, a passive transfer test involving intradermal injection of serum of the hairdressers into six normal subjects did not confer the ability to respond to ammonium persulphate, thus suggesting that ammonium persulphate did not react via IgE.

Histamine release tests with persulphates

This is the first study to test the HRT with persulphates in cases with persulphate-induced asthma and rhinitis confirmed by SIC. The results are summarized in Table 8. The HRT with potassium persulphate and sodium persulphate produced histamine release in only two in six cases with a positive response to SIC, whilst ammonium persulphate induced histamine release in all six cases. However, ammonium persulphate also induced histamine release in the majority of controls. These findings further suggest that persulphates, in particular ammonium persulphate, have the ability to induce histamine release by non-IgE mediated mechanisms. The fact that not all participants reacted to persulphates by releasing histamine suggest that persulphates do not merely cause histamine release by chemical initiation and therefore some type of immunological mechanism may account for the selective histamine release. Oddly, there was no connecting between the participants with a positive scratch test and patients with a positive histamine release tests.
One interesting finding of the HRT is that potassium persulphate was apparently better tolerated by all participants. This should be noted by hairdressers choosing hair bleaching products, because those containing only potassium persulphate will probably be less problematic to work with.

Table 8. Results of HRT with potassium persulphate, sodium persulphate, and ammonium persulphate in cases and controls

	SIC positive hairdressers (n = 6)	All symptomatic hairdressers (n = 19)	Symptomatic controls (n = 12)	Healthy controls (n = 40)	Symptomatic + healthy controls (n = 52)
HRT response to PP					
Class 6	-	-	-	-	-
Class 5	1 (16.7%)	1 (5.3%)	-	-	-
Class 4	-	1 (5.3%)	-	1 (2.5%)	1 (1.9%)
Class 3	-	-	-	-	-
Class 2	-	1 (5.3%)	1 (8.3%)	8 (20%)	9 (17.3)
Class 1	1 (16.7%)	4 (21.1%)	5 (41.7%)	6 (15%)	11 (21.2%)
Class 0	4 (66.7%)	12 (63.2%)	6 (50%)	25 (62.5%)	31 (59.6%)
HRT response to SP					
Class 6	-	-	-	-	-
Class 5	-	1 (5.3%)	-	-	-
Class 4	-	-	-	1 (2.5%)	1 (1.9%)
Class 3	1 (16.7%)	3 (15.7%)	2 (16.7%)	4 (10%)	6 (11.5%)
Class 2	-	4 (21.1%)	4 (33.3%)	11 (27.5%)	15 (28.8%)
Class 1	1 (16.7%)	6 (31.6%)	5 (41.7%)	10 (40%)	15 (28.8%)
Class 0	4 (66.7%)	5 (26.3%)	1 (8.3%)	14 (35%)	15 (28.8%)
HRT response to AP					
Class 6	-	-	-	-	-
Class 5	1 (16.7%)	1 (5.3%)	-	-	-
Class 4	1 (16.7%)	4 (21.1%)	2 (16.7%)	8 (22%)	10 (19.2%)
Class 3	1 (16.7%)	5 (26.3%)	3 (25.0%)	12 (30%)	15 (28.8%)
Class 2	1 (16.7%)	6 (31.6%)	5 (41.7%)	11 (27.5%)	16 (30.8%)
Class 1	2 (33.3%)	2 (10.5%)	2 (16.7%)	7 (17.5%)	9 (17.3%)
Class 0	0%	1 (5.3%)	0%	2 (5%)	2 (3.8%)

AP: Ammonium persulphate HRT: histamine release test PP: Potassium persulphate SIC: specific inhalation challenge
SP: Sodium persulphate
Definition of classes: A Class 6 response defines a release of histamine in response to the lowest dose of persulphate (0.03%.). A Class 1 response defines a release of histamine only to the highest dose of persulphate (1.0%.) A Class 0 response defines no response

CONCLUSION AND PERSPECTIVES FOR FUTURE RESEARCH

The epidemiology of allergic and irritant skin and respiratory diseases in hairdressing apprentices was studied using both a cross-sectional and prospective questionnaire study comparing prevalence and incidence of diseases in the apprentices with a reference group of young adults from the general population.

Hairdressing apprentices in the first year of their training had a lower prevalence of the diseases investigated and a significantly later disease onset compared with controls subjects indicating a 'healthy hire effect'. The prospective study revealed that almost 22% of hairdressing apprentices had left hairdressing during the 3-year follow-up period, and more than 70% of those leaving reported disease as a reason for this decision, supporting the existence of a 'healthy survivor effect'. Musculoskeletal pain and skin diseases were the dominant reasons for leaving, followed by respiratory symptoms. In conclusion, a healthy worker effect with regards to skin and respiratory diseases apparently exists in hairdressing apprentices both as a 'healthy hire effect' and a 'healthy survivor effect', and this should be taken into account when interpreting epidemiologic studies in this group. Although musculoskeletal pain was not the primary focus of this thesis, the condition contributed substantially to discontinuation of training by hairdressing apprentices, and future studies should focus on preventive strategies against this condition.

Despite the efforts of hairdressing schools to increase awareness and prevention, hairdressing apprentices in Denmark are still at high risk of hand eczema. The hairdressing schools have taken important steps in adopting preventive strategies against occupational skin diseases, nevertheless, to achieve a full preventative effect, it is important that the salons also support the appropriate use of gloves and moisturizers and participate in the dissemination of knowledge on how to prevent occupational skin diseases among hairdressers.

One novel finding from our study is that hairdressing apprentices have an increased risk of self-reported urticaria on the hands and contact urticaria caused by rubber gloves, hair dyes, cosmetics or the like. The substantial overlap in symptomology between urticaria and hand eczema might have given rise to some misclassification and thereby overestimation of occurrence; however, conversely, the high frequency of hand eczema might also be a predisposing factor for allergen penetration and thereby the development of disease. Contact urticaria is a hitherto overlooked disease in hairdressers and our finding should encourage greater attention to this condition in clinical settings and further research into its occurrence.

Although hairdressing is considered a high-risk profession for the development of occupational asthma, we did not detect an increased prevalence or incidence of wheezing among hairdressing apprentices. We did, however, find an increased risk of rhinitis symptoms, which is considered a risk factor for occupational asthma. In conclusion, it seems that exposure to the hairdressing work environment can induce upper respiratory tract symptoms within a few years, whilst the development of lower respiratory tract symptoms require prolonged exposure and occur less frequently. Occupational rhinitis is often overlooked in hairdressing apprentices and our findings suggest it should receive greater attention. The examination of apprentices for rhinitis might enable individuals at risk of occupational asthma to be identified early and enhance the potential for intervention before asthma develops.

The hairdressing products that most often induce respiratory symptoms are hair bleaching products containing persulphates and an important step in preventing respiratory diseases in hairdressers would be to reduce exposure to these, either by sufficient use of ventilation or by removing persulphates from hair bleaching products.

The technique presented here for performing a rapid 'realistic approach' SIC using the 'stirring method' demonstrated high specificity for persulphate-induced asthma and rhinitis and was safe when tested in this study population. The 'stirring method' was reproducible and generated exposure levels similar to those in the hairdressing salon. The sensitivity of the test was not determined however, and future studies should compare different methods of performing the SIC to determine the optimal technique.

In our study, the SPT with persulphate could not detect patients with persulphate-induced asthma or rhinitis. It appears that persulphate-induced asthma and rhinitis are rarely caused by IgE-mediated mechanisms and results from studies that have performed SPT with persulphates have been diverging. Our findings, and other studies, suggest that the SPT using persulphates has no value in detecting patients with persulphate-induced respiratory disease, and it is important that a negative SPT alone is not used to exclude the diagnosis.

Persulphates are able to induce non-specific histamine release, and consequently both the scratch test and the HRT with persulphates produced positive reactions in a subsample of patients with persulphate-induced asthma and rhinitis, but also in some of the non-exposed controls. Therefore these tests cannot be used as diagnostic tools to detect persulphate-induced respiratory diseases.

In conclusion, the mechanisms of persulphate-induced respiratory diseases are still not completely understood, and the SIC remains crucial in diagnosing these diseases.

RESUMÉ (DANISH)

Frisører udsættes dagligt for diverse frisørprodukter indeholdende kemikalier med potentielt allergi-fremkaldende og irritative egenskaber. Særligt huden og luftvejene er udsat, og frisørerne er som følge heraf i risiko for at udvikle arbejdsbetingede hud- og luftvejssygdomme. Forekomsten af arbejdsbetingede hud- og luftvejssygdomme hos frisører tidligt i deres karriere – altså under frisøruddannelsen – er hidtil dårligt belyst og især prospektive studier, der følger frisørelever over en længere periode, mangler.

Flere studier peger på, at den primære årsag til astma og rhinitis hos frisører er inhalation af persulfater, som er små lavmolekylære salte, der findes i hårblegemidler. Mekanismen hvormed persulfater inducerer astma og rhinitis hos frisørerne er endnu ukendt, og dermed er udredningen af disse patienter en udfordring idet værdien af typisk allergitests, så som priktesten og histamin release testen, er usikker. Den specifikke inhalationstest, med provokation af patientens luftveje med persulfater, anses for at være gylden standard i udredningen af mistænkt persulfat-induceret astma og rhinitis, men den eksisterende validerede metode er desværre meget tidskrævende.

Denne afhandling består af tre studier, et epidemiologisk og to kliniske. De overordnede formål var: 1) at bestemme prævalensen og incidensen af hud- og luftvejssygdommen hos danske frisørelever og sammenligne disse med forekomsten hos unge fra baggrundbefolkningen, samt at undersøge hvor mange der dropper fra frisøruddannelse pga. disse, 2) at optimere diagnostikken af persulfat-induceret astma og rhinitis ved at undersøge om priktesten, ridsetesten og histamin release testen kan påvise persulfat-induceret astma og rhinitis, samt ved at etablere en mindre tidskrævende specifik inhalationstest end den hidtidigt validerede, som samtidig har en mere realistisk grad af eksponering for persulfater.

Et tværsnitsstudie og en prospektivt spørgeskemaundersøgelse blev foretaget blandt frisørelever og en kontrol-gruppe af unge danskere. Resultaterne viste at frisøreleverne havde en højere prævalens af håndeksem, kontakturticaria og rhinitissymptomer end andre unge fra baggrundsbefolkningen, og at forekomsten af disse steg med varigheden af udsættelse for frisørernes arbejdsmiljø. Incidensen af hudsygdommene og rhinitis-symptomer over en treårig periode var ligeledes signifikant forhøjet hos frisøreleverne. Hele 21.8 % af frisøreleverne havde valgt at forlade frisøruddannelsen, og mere end 70 % af disse angav at sygdom helt eller delvist var årsag til at de stoppede. Smerter i bevægeapparatet og hudsygdomme var de hyppigste årsager til at forlade faget, efterfulgt af luftvejssymptomer.

En gruppe frisører med mistænkt persulfat-induceret astma og rhinitis og en kontrolgruppe blev testet med priktesten, ridsetesten og histamin release testen samt med den specifikke inhalationstest med persulfater. Resultatet viste at histamin release testen og ridsetesten ikke kunne benyttes til at påvise persulfat-induceret astma og rhinitis, da persulfater inducerede uspecifik frigivelse af histamin hos en andel af frisører med luftvejssymptomer samt hos ikke eksponerede asymptomatiske individer. Priktesten med persulfater syntes ligeledes at have ringe værdi i påvisningen af disse sygdomme, idet ingen af deltagerene reagerede på testen. Vi etablerede en ny metode til at udføre den specifikke inhalationstest med persulfater, som var hurtigere at udføre end den hidtidige validerede metode og som samtidig havde en mere realistisk grad af eksponering for persulfater.

På baggrund af resultaterne fra det epidemiologiske studie kan det konkluderes at frisørelever har en højere risiko for at udvikle håndeksem, kontakturticaria og rhinitissymptomer end andre unge fra baggrundsbefolkningen, og at disse er en væsentlig årsag til at disponerede unge fravælger frisøruddannelsen som karrierevej eller hopper fra uddannelsen undervejs. På baggrund af de kliniske studier kan det konkluderes at hverken priktesten, ridsetesten eller histamin release testen kan benyttes til at påvise persulfat-induceret astma og/eller rhinitis og at den specifikke inhalationstest derfor bør benyttes til udredning af disse patienter.

SUMMARY (ENGLISH)

Hairdressers are exposed on a daily basis to various products with potentially allergenic and/or irritant properties. The skin and respiratory tract are particularly at risk and consequently hairdressers may have an increased susceptibility to occupational skin and respiratory diseases. The occurrence of these diseases in hairdressing apprentices during their training has not been extensively examined and prospective studies are in particular lacking.

Several studies suggest that hair bleaching products containing low-molecular weight persulphate salts are the major cause of occupational asthma and rhinitis in hairdressers. However, the mechanism behind persulphate-induced respiratory diseases is unclear and therefore diagnostics can be challenging because the value of common allergy tests, such as the skin prick test and the histamine release test, is uncertain. The specific inhalation challenge, which involves provocation of the patient's airways using persulphates, is considered the 'reference standard' for diagnosing these diseases. However, the currently validated method for performing the specific inhalation challenge with persulphates is very time consuming for both investigator and the patient.

This thesis consists of three studies; an epidemiologic study and two clinical studies. The overall aims were: 1) to determine the prevalence and incidence of skin and respiratory diseases in hairdressing apprentices and compare the results to those of young adults from the general population, and to examine whether hairdressing apprentices leave the trade because of these diseases, 2) to optimize diagnostics in persulphate-induced respiratory diseases by examining whether the skin prick test, the scratch test, and the histamine release test can detect persulphate-induced respiratory diseases, and by establishing a rapid specific inhalation challenge with an exposure level similar to those in the salons.

A prospective questionnaire study was conducted among hairdressing apprentices and young adults from the general population. Hairdressing apprentices had a higher prevalence of self-reported hand eczema, contact urticaria, and rhinitis symptoms compared with the general population, and the prevalence was higher in those who had been in the trade for longer. The incidence of the skin diseases and rhinitis symptoms occurring over a 3-year period was also significantly increased in the hairdressing apprentices. A total of 21.8% of the hairdressing apprentices had left the trade after three years, and more than 70.4% of those who left reported doing so, at least partly, because of disease. Musculoskeletal pain and skin diseases were the most frequently cited reasons for leaving, followed by respiratory symptoms.

A group of hairdressers with asthma and rhinitis symptoms and a control group were tested with the skin prick test, the scratch test, the histamine release test, and the specific inhalation challenge with persulphates. The results demonstrated that the histamine release test and the scratch test could not be used to detect persulphate-induced respiratory diseases because persulphates induce non-specific release of histamine in a fraction of both symptomatic and non-symptomatic individuals. The skin prick test also appeared to have little diagnostic value, since no participants reacted to the test. We established a rapid specific inhalation challenge with a more realistic level of persulphate exposure and a shorter duration than the currently validated method.

In conclusion, the epidemiologic study showed that hairdressing apprentices are at increased risk of hand eczema, contact urticaria, and rhinitis symptoms compared with young adults from the general population, and that a healthy worker effect with respect to skin and respiratory diseases exists causing sensitive individuals to discontinue training or to never begin in the first place. From the clinical studies we can conclude that the skin prick test, the scratch test, and the histamine release test should not be used to diagnose persulphate-induced respiratory diseases, and therefore the specific inhalation challenge remains crucial in diagnosing these diseases.

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