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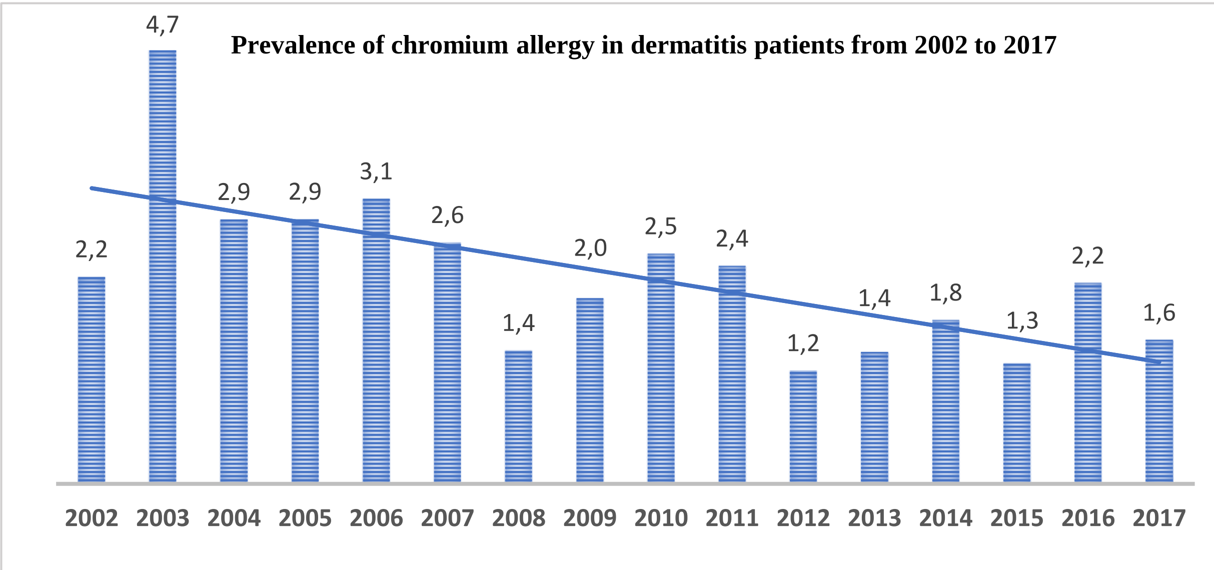

PhD Thesis

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PhD Title

Sensitization trends for chromium and cobalt and causative exposures

Supervisor: Jeanne Duus Johansen

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Sensitization Trends for Chromium and Cobalt and Causative Exposures

This PhD is the product of a scientific cooperation between:

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Preface

This thesis is based on cooperative work performed at the Danish National Allergy Research Centre, the Department of Dermatology and Allergy, Herlev-Gentofte Hospital, Denmark, and the Department of Chemistry, Division of Surface and Corrosion Science, KTH Royal Institute of Technology, Sweden, between 2018 and 2021. The project was funded by The Danish Environmental Protection Agency and the Aage Bang's Foundation, which are gratefully acknowledged.

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Farzad Alinaghi
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This PhD thesis is based on the following scientific papers:

I. Alinaghi F, Zachariae C, Thyssen JP, Johansen JD. *Temporal changes in chromium allergy in Denmark between 2002 and 2017.* Contact Dermatitis, 2019. 80 (3): 156-161.

II. Alinaghi F, Zachariae C, Thyssen JP, Johansen JD. *No immediate effect of regulatory reduction of chromium in leather among adult chromium allergic patients.* Submitted for publication in Contact Dermatitis January 2021.

III. Alinaghi F, Zachariae C, Thyssen JP, Johansen JD. *Causative exposures and temporal development of cobalt allergy in Denmark between 2002 and 2017.* Contact dermatitis, 2019. 81 (4): 242-248.

IV. Alinaghi F, Hedberg YS, Zachariae C, Thyssen JP, Johansen JD. *Metals in used and unused metalworking fluids: X-ray fluorescence spectrometry as a screening test.* Contact Dermatitis, 2020. 83 (2): 83-87.

Abbreviations

ACD	Allergic Contact Dermatitis
AD	Atopic Dermatitis
CEN	European Committee for Standardization
CI	Confidence Interval
Co	Cobalt
Cr	Chromium
DPC	DiPhenylCarbazide
DLQI	Dermatology Life Quality Index
ESCD	European Society of Contact Dermatitis
ESSCA	European Surveillance System on Contact Allergies
GFAAS	Graphite Furnace Atomic Absorption Spectroscopy
IQR	Inter-Quartile Range
IVDK	Information Network of Departments of Dermatology
MWFs	Metal-Working Fluids
NACDG	North American Contact Dermatitis Group
Ni	Nickel
OR	Odds Ratio
Pet.	Petrolatum
ROAT	Repeated Open Application test
TLRs	Toll-Like Receptors
XRF	X-Ray Fluorescens

Additional papers

- Alinaghi F, Zachariae C, Thyssen JP, Johansen JD. *Prevalence of contact allergy in the general population: A systematic review and meta-analysis.* Contact Dermatitis, 2019. 80 (2): 77-85.

- Alinaghi F, Friis UF, Deleuran MG, Zachariae C, Thyssen JP, Johansen JD. *Exposure analysis using X-ray fluorescence device and a cobalt spot test in four patients with cobalt allergy.* Contact Dermatitis, 2020. 82 (1): 67-69.

- Hoffmann S, Wennervaldt M, Alinaghi F, Simonsen AB, Johansen JD. *Aluminium contact allergy without vaccination granulomas: A systematic review and meta-analysis.* Submitted for publication in Contact Dermatitis January 2021.

Summary

Chromium and cobalt are common causes of contact allergy in the general population and among patients investigated for contact dermatitis. Since the 1990s, leather has become the single most important source of chromium exposure in Denmark. In March 2014, the EU commission adopted a regulation restricting the content of hexavalent chromium to a maximum of 3 mg/kg (ppm) in leather articles coming into contact with the skin. The regulation was expected to be 80% effective in reducing the incidence of allergic contact dermatitis due to hexavalent chromium in leather. Regarding cobalt allergy, the prevalence remains relatively high and largely stable across time with limited insight into relevant exposures. During recent years, a growing body of evidence has suggested that leather might constitute a more frequent exposure in cobalt allergic patients than hitherto recognized. In this thesis, we aimed at assessing whether the regulatory intervention against hexavalent chromium in leather entailed temporal changes in the epidemiology (manuscript I) of chromium allergy in Denmark and burden of disease (manuscript II) in those affected. Furthermore, trends in sensitization rates, clinical characteristics and causative exposures were explored across time for patients with cobalt allergy (manuscript III). Additionally, as previous studies have reported a potential association between metalwork - especially metalworking fluids - and metal allergy, we conducted a market survey exploring the presence of metal allergens in used and unused metalworking fluids collected from several metalworking plants in Copenhagen, Denmark (manuscript IV).

In manuscript I, we performed a retrospective cross-sectional registry study including 13,379 adult patients aged 18 to 99 with suspected allergic contact dermatitis patch tested at the Department of Dermatology and Allergy, Herlev-Gentofte Hospital, during 2002-2017. The overall prevalence of chromium allergy was 2.2%. We found a decreasing trend in the prevalence of chromium allergy (p_{trend}<0.001) and foot dermatitis (p_{trend}=0.01) among chromium allergic patients during 2002-2017. Leather was recorded as the relevant source of exposure in nearly half of the chromium allergic patients (48.3%). No cases of relevant cement exposure were recorded in patients with chromium allergy.

In manuscript II, a case-control questionnaire study was conducted including 172 adult dermatitis patients with chromium allergy and 587 age- and sex-matched dermatitis patients without chromium allergy. All patients were patch tested at the Department of Dermatology and Allergy,

Herlev-Gentofte Hospital, during 2003-2018. Compared to controls, chromium allergic patients were still more affected by current foot dermatitis (OR=3.82, 95% CI: 2.07-7.08) and hand dermatitis (OR=1.98, 95% CI: 1.13-3.49) during 2013-2018. As regards self-reported leather exposures causing dermatitis, no difference was found comparing chromium allergic patients during 2013-2018 versus 2003-2012 (66.1% vs. 71.0%, p=0.5). Furthermore, estimates on occupational performance and disease-severity were similar in patients with chromium allergy for 2013-2018 versus 2003-2012.

In manuscript III, we conducted a retrospective cross-sectional registry study including 13,475 adult patients aged 18 to 99 with suspected allergic contact dermatitis patch tested at the Department of Dermatology and Allergy, Herlev-Gentofte Hospital, during 2002-2017. The prevalence of overall and isolated cobalt allergy was, respectively, 3.3% and 1.5% and remained largely unchanged over time. The proportion of cobalt allergic patients with relevant leather exposure increased significantly from 3.7% in 2002-2009 to 8.3% 2010-2017 ($p_{trend}<0.001$). While exposures to jewellery (6.3%) and leather (6.0%) were most common in cobalt allergic dermatitis patients, a relevant source of exposure was recorded in only one in five patients.

In manuscript IV, the presence of metal allergens was examined in 80 used and unused metalworking fluids collected from eight metalworking plants in Copenhagen, Denmark. Elemental analysis with graphite furnace atomic absorption spectroscopy showed that 13 of 80 samples (16.3%) contained > 1 mg/kg nickel (range: 6.4-17.7 mg/kg), 3 of 80 (3.8%) contained > 1 mg/kg chromium (range: 1.4-3.1 mg/kg) and 1 of 80 (1.3%) contained 1.3 mg/kg cobalt. While nickel was found in both used and unused oils, chromium and cobalt were found in used ones only. The handheld x-ray fluorescens device was a poor screening instrument for these metals in metalworking fluids.

This thesis showed that the prevalence of chromium allergy has decreased, suggesting a positive effect of legislative actions against hexavalent chromium in leather. However, no sign of improvement was found regarding disease-burden nor in the proportion of self-reported leather exposures leading to dermatitis in patients with chromium allergy, thus implying an inadequate protection of those already sensitized. The prevalence of cobalt allergy remained unchanged and most cases could not be linked to a relevant exposure. It is currently not possible to assess the need and potential target for preventive measures. Lastly, considerable levels of metal allergens were detected in some used and unused metalworking fluids suggesting that these might constitute a relevant source of exposure in metalworkers with metal contact allergy.

Summary (Danish)

Krom og kobolt er hyppigt forekommende årsager til kontaktallergi i befolkningen og blandt patienter undersøgt for kontakteksem. Siden 1990'erne er læder blevet den vigtigste kilde til kromeksponering i Danmark. I marts 2014 vedtog EU-Kommissionen en regulering, der begrænser indholdet af hexavalent krom til maksimalt 3 mg/kg (ppm) i lædervarer, der kommer i kontakt med huden. Reguleringen forventedes at være 80% effektiv til at reducere forekomsten af allergisk kontakteksem udløst af hexavalent krom i læder. Vedrørende koboltallergi forbliver prævalensen relativt høj og stabil over tid med begrænset indsigt i relevante eksponeringer. I de senere år har et stigende evidensniveau indikeret, at læder muligvis udgør en hyppigere eksponering hos patienter med koboltallergi end hidtil antaget. I denne afhandling ønskede vi at vurdere, hvorvidt lovgivningen mod hexavalent krom i læder har medført tidsmæssige ændringer i epidemiologien (manuskript I) af kromallergi i Danmark samt sygdomsbyrde (manuskript II) hos de berørte. Desuden blev trends i forekomst, kliniske karakteristika og eksponeringskilder undersøgt over tid for patienter med koboltallergi (manuskript III). Da tidligere studier har vist en mulig sammenhæng mellem metalarbejde - særligt kølesmøremidler - og metalallergi, gennemførte vi en markedsundersøgelse med henblik på at undersøge forekomsten af metalallergener i brugte og ubrugte kølesmøremidler indsamlet fra metalvirksomheder i København, Danmark (manuskript IV).

I manuskript I udførte vi et retrospektivt tværsnitsstudie, der omfattede 13,379 voksne patienter i aldersgruppen 18 til 99 år lappetestet på mistanke om allergisk kontakteksem på Hud- og allergiafdelingen, Herlev-Gentofte Hospital, i perioden 2002-2017. Den samlede prævalens af kromallergi var 2.2%. Vi fandt en faldende trend i forekomsten af kromallergi (p_{trend} <0.001) og fodeksem (p_{trend} = 0.01) blandt patienter med kromallergi i perioden 2002-2017. Læder blev registreret som den relevante eksponeringskilde hos næsten halvdelen af kromallergikere (48.3%). Ingen tilfælde af relevant cementeksponering blev registreret hos patienter med kromallergi.

I manuskript II udførte vi en spørgeskemaundersøgelse, der omfattede 172 voksne eksempatienter med kromallergi samt 587 alders- og kønsmatchede eksempatienter uden kromallergi. Alle patienter blev lappetestet på Hud- og allergiafdelingen, Herlev-Gentofte Hospital, i løbet af 2003-2018. Sammenlignet med kontroller var kromallergiske patienter fortsat mere påvirket af aktuel fodeksem (OR = 3.82, 95% CI: 2.07-7.08) og håndeksem (OR = 1.98, 95% CI: 1.13-3.49) for perioden 2013-2018. Med hensyn til kontakteksem udløst af lædereksponeringer fandt vi ingen forskel ved

sammenligning af kromallergiske patienter i løbet af 2003-2012 versus 2013-2018 (71.0% vs. 66.1%, $p = 0.5$). Endvidere fandt vi sammenlignelige estimater for arbejdsevne og sygdomsgrad hos patienter med kromallergi for 2003-2012 versus 2013-2018.

I manuskript III gennemførte vi en retrospektiv tværsnitsundersøgelse, der omfattede 13,475 voksne patienter i alderen 18 til 99 år lappetestet på mistanke om allergisk kontakteksem på Hud- og allergiafdelingen, Herlev-Gentofte Hospital, i perioden 2002-2017. Forekomsten af alle og isolerede tilfælde af koboltallergi var henholdsvis 3.3% og 1.5%, og forblev uændret over tid. Andelen af koboltallergiske patienter med relevant lædereksponering steg markant fra 3.7% i 2002-2009 til 8.3% 2010-2017 ($p_{trend} <0.001$). Mens smykker (6.3%) og læder (6.0%) udgjorde de hyppigst registrerede eksponeringskilder hos patienter med allergisk kobolteksem, blev en relevant eksponering registreret i kun en ud af fem patienter.

I manuskript IV undersøgte vi forekomsten af krom, kobolt og nikkel i 80 brugte og ubrugte kølesmøremidler indsamlet fra otte metalvirksomheder i København, Danmark. Atomabsorptionsspektroskopi med grafitovn viste, at 13 af 80 prøver (16.3%) indeholdt > 1 mg/kg nikkel (interval: 6.4-17.7 mg/kg), 3 af 80 (3.8%) indeholdt > 1 mg/kg krom (interval: 1.4-3.1 mg/kg) og 1 ud af 80 (1.3%) indeholdt 1.3 mg/kg kobolt. Mens nikkel forekom i både brugte og ubrugte olier, blev krom og kobolt kun fundet i brugte kølesmøremidler. Det håndholdte røntgenfluorescens (XRF) apparat var et dårligt screeningsværktøj for disse metaller i kølesmøremidler.

Denne afhandling viste, at forekomsten af kromallergi er faldet, hvilket antyder en gunstig effekt af den nyligt vedtagne EU-lovgivning mod hexavalent krom i læder. Vi fandt dog ingen tegn på bedring i sygdomsbyrde eller i andelen af selvrapporterede lædereksponeringer, der førte til kontakteksem hos patienter med kromallergi, hvilket tyder på en utilstrækkelig beskyttelse af dem, der allerede er sensibiliserede. Forekomsten af koboltallergi forblev uændret, og de fleste tilfælde kunne ikke knyttes til en relevant eksponering. Det er således i øjeblikket ikke muligt at vurdere behovet og potentielle mål for forebyggende tiltag. Endelig blev der påvist betydelige niveauer af metalallergener i nogle brugte og ubrugte kølesmøremidler, hvilket indikerer, at disse kan udgøre en relevant eksponeringskilde hos metalarbejdere med metalkontaktallergi.

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1. Introduction

Industrialization, manufacturing of goods and consumerism have resulted in an increased risk of exposure to contact allergens for more than a century, both in an occupational setting and among consumers. Along with nickel (Ni), chromium (Cr) and cobalt (Co) constitute the main metal contact allergens in the environment. Both are transition metals occurring in several valences with Cr (III) and Cr (VI), and Co (II), being the most common within Cr and Co compounds. These ionic compounds have widespread industrial applications, including cement, alloys, leather, galvanized sheets, magnets, prosthetics, paint and costume jewellery, entailing an increased risk of cutaneous exposure to these metals which are recognized contact allergens capable of sensitization and eliciting allergic contact dermatitis (ACD) in those sensitized due to their solubility and ability to penetrate the skin. ACD is a debilitating skin disease with major public health and socioeconomic costs.[1] Accordingly, patients with Cr allergy are more commonly affected by occupational disability, e.g. sick-leave and change of job, and more burdened by disease as compared to patients without Cr allergy.[2] Disclosure of relevant sources of exposure to these compounds is a prerequisite for implementation of relevant primary, secondary and tertiary prevention strategies. The immunological mechanisms, diagnosis and clinical features, epidemiology, sources of exposure and legislative actions against Cr and Co contact allergy are reviewed below.

1.1 Immunology of contact allergy to chromium and cobalt

ACD is a T-cell mediated inflammatory skin condition involving both the innate and adaptive immune system. ACD emerges as a result of two phases: a clinically silent *sensitization phase* which primes the immune system for an allergic response, and a symptomatic *elicitation phase* appearing with itching, erythema, swelling and vesicles.[3] While the sensitization phase might last several weeks, the elicitation phase usually occurs within 24-72 hours. Contact allergens, including Cr and Co, are electrophilic haptens, i.e. small molecules capable of inducing contact allergy when intercalated to a large carrier within the skin, most often a protein. While Cr (VI) is the major biological transportable form of Cr and main practical challenge due to its strong oxidative capacity and higher penetration rates across the skin and cell membranes, Cr (III) is considered the actual hapten from an immunological perspective. Skin exposure to materials containing Cr and/or Co compounds on the surface might result in dissolution of free metal ions– unless these ions are already dissolved in an aqueous medium at exposure, e.g. wet cement.

Upon penetration of the skin, these ionic haptens bind to peptides/proteins yielding an allergen-conjugate capable of activating the innate immune system through antigen presenting cells present in the skin, i.e. Langerhans cells and Dendritic cells. In contrast to Co and Ni, which are capable of activating the innate immune system through direct ligation with human toll-like receptors (TLRs) present on the surface of the antigen presenting cells,[4] it is unclear how Cr generates this activation. Other allergens induce priming of TLRs indirectly via release of damage-associated molecular patterns, such as hyaluronic acid, acting as agonists on TLRs. While the latter might be the case for Cr, it is yet to be explored.[5] Upon activation of the innate immune system, the antigen presenting cells migrate to regional lymph nodes via lymphatic vessels. Here, they interact with naïve T-cells resulting in maturation and clonal proliferation of hapten-specific effector T-cells and memory T-cells which mark the induction of sensitization. The single most important factor for sensitization is the allergen dose per unit skin are ($\mu g/cm^2$) which depends on the concentration and solubility of the allergenic compound and the inherent chemical reactivity of the individual hapten. The level of sensitization varies and might increase with repeated exposures to the source of the culprit hapten. Upon re-exposure to the specific hapten, the aforementioned T-cells elicit a local dermal ACD reaction, which might spread to other skin areas. In case of persistent inflammation in the skin due to inadequate treatment of the initial dermatitis symptoms, continued skin exposure to the allergen source or the presence of other eczematous conditions such as irritant contact dermatitis or atopic dermatitis (AD), ACD might become chronic presenting with scaling, fissuring and desquamation.[3]

1.2 Allergic contact dermatitis to chromium and cobalt: Diagnosis and clinical features

1.2.1 The patch test procedure

Diagnosis of contact allergy is a holistic approach involving patch testing, clinical examination of dermatitis, patient history and exposure analysis. Jadassohn has been credited with the discovery of patch testing in 1895 which remains the golden standard technique in diagnosis of contact allergy. Patch testing is an *in vivo* test reproducing the elicitation phase of ACD following occluded skin exposure to a specific contact allergen. The test preparation, comprised of the allergenic compound dissolved in an aqueous medium, typically petrolatum (pet.) or aqua, is applied onto the skin using chambers made of aluminium or polypropylene. The skin of the back is the preferred anatomical site of patch testing given its flat surface facilitating occlusion and previous studies reporting higher skin reactivity in this area compared to other areas.[6-9] In order to capture as many reactions as possible, sequential readings are recommended on day 2, 3 or 4 and 7.[10] Reading of patch test reactions is based on an morphologic assessment. According to globally recognized criteria,[11] a weak test reaction (+), marked by the presence of erythema, infiltration and possibly papules, is discerned from strong (++) and extreme (+++) reactions based on an additional presence of, respectively, vesicles and coalescing vesicles. Furthermore, doubtful and irritant reactions might occur as well, complicating the interpretation of patch test reactions. Notably, weak positive reactions and doubtful reactions may be difficult to differentiate,[12] underscoring the importance of standardization of the patch testing procedure and training in readings. Doubtful reactions could be clinically relevant and further investigation might be warranted by patch testing with serial dilutions or use tests simulating real-life conditions, e.g. the repeated open application test (ROAT).[13]

1.2.2 Patch testing with chromium and cobalt

The first case of Cr ACD was diagnosed in a blueprint operator in 1925 based on skin testing with a 0.5% aqueous solution of potassium dichromate which produced a papulovesicular reaction within 24 hours.[14] This led to an increased awareness of the allergenic capacity of Cr and by 1931, patch testing with three different Cr (VI) compounds had been reported in the literature, including ammonium chromate 1%, potassium dichromate 0.5% and sodium dichromate 0.1%. Potassium dichromate (0.5% pet.) is still present in the European baseline series, whereas a lower test concentration (0.25% pet.) is applied in North America. In a previous study (1989) by the European Environmental and Contact Dermatitis Research Group, it was shown that patch testing with lower concentrations than 0.5% generated fewer irritant reactions though a significant proportion of true positive reactions was missed.[15] As regards capturing as many positive reactions as possible, previous studies have established the importance of several readings across time. While 3.4% of consecutive dermatitis patients reacted to potassium dichromate (0.5% pet.) based on readings on day 2 and 4, Shehade et al. (1991) reported that 18.9% of Cr allergic patients were negative on day 2.[16] Similarly, comparisons of readings on day 3 versus day 6 or 7 showed that 20.9% were negative initially.[17] Although Cr patch testing is performed with Cr (VI), it is to be noted that several studies have corroborated the allergenic capacity of Cr (III) per se, despite markedly higher skin concentrations of Cr (III), as compared to Cr (VI), is required to elicit ACD in patients with Cr allergy.[18] There seems to be no practical advantage in patch testing with Cr (III) given a previous study reporting that no Cr (VI) negative patients reacted to Cr (III).[19] However, in case of Cr (VI) positivity, an additional positive reaction to Cr (III) might point to leather as the causative exposure.[19]

Patch testing with Co is generally performed with cobalt chloride (1.0% pet.). Since the mid-1980s, patch testing in Sweden has been done with a lower test concentration (0.5% pet.) given reports of false positive results such as follicular, petechial and ‘poral’ test reactions, suggesting a lowering of the patch test concentration from 1.0% to 0.5%.[20, 21] However, it was recently shown that an unfavorably high proportion (>40%) of Co allergic cases was missed by testing with 0.5% only.[22] Accordingly, the Swedish Contact Dermatitis Research Group recommended to include the 1.0% test concentration in the Swedish baseline series.[22] Similar to Cr, it is pivotal with multiple reading days as late positive reactions are common when testing with Co 1.0%. Based on comparisons of positive reactions to Co 1.0% on day 3 versus day 6 or 7, Lidén et al. (2016) reported that 26 of 87 (29.9%) cases of Co allergy were missed at the initial reading.[17] Solitary patch test reactions to Co is common as well, comprising 41%-62% of all Co allergic cases.[23-25]

1.2.3 Clinical features

Patients with Cr allergy are more prone to dermatitis on hands and feet than patients without Cr allergy, whereas no difference has been reported regarding other locations.[2] In contrast to Co and Ni, Cr allergy is more common in dermatitis patients aged > 40 years.[26] Cr ACD is well-known as a severe, recurrent and therapeutic recalcitrant skin condition with poor prognosis. In a prospective cohort study (2009) examining the severity of hand dermatitis in consecutive dermatitis patients, Cr allergy was associated with the worst prognosis at six months follow-up as compared to patients without Cr allergy (OR=4.18, 95% CI: 1.42-12.28).[27] Similarly, Fregert (1975) found that only 7% of Cr ACD patients remained disease-free during a 10-year period, whereas 43% and 50% were, respectively, affected by intermittent and chronic dermatitis.[28] The persistent and severe nature of Cr allergy is a matter of speculation. While a high sensitization level combined with cutaneous exposure to unrecognized sources of Cr exposure in the environment might offer a plausible explanation, others have suggested that dietary ingestion of Cr may exacerbate dermatitis, at least in some patients with Cr allergy.[29-31] In a case report of a Cr allergic patient with intractable dermatitis, a complete resolution of dermatitis was observed after stopping daily intake of vitamin tablets containing 150 μg chromium chloride.[32]

Regarding patients with Co allergy, far too little is known. Due to concomitant sensitizations to other metal allergens, particularly Ni, patient characterization of Co allergic patients has resembled that of Ni allergic patients with a predominance of females and young age groups (<

40-50 years).[24, 33] The proportion of Ni allergy might be greater than 60% in dermatitis patients with Co allergy.[33] In a recent questionnaire study (2014) of Co allergic patients without concomitant Cr allergy, patients with Co allergy were more commonly affected by dermatitis on hands, arms and feet than those without Co and Cr allergy .[34] However, no adjustment was done for the presence of Ni allergy. During recent years, more attention has been shifted to patients with isolated Co allergy, i.e. without concomitant Ni and/or Cr allergy. While far more investigation is warranted, a previous study from Denmark reported a significantly higher median age in female patients with isolated Co allergy (without Ni allergy) as compared to those with Ni allergy.[33]

1.3 Allergic contact dermatitis to chromium and cobalt: Exposure analysis

1.3.1 Spot testing

Screening for the presence of metals and hence potential metal release from surfaces that come into contact with skin can be performed using spot tests. Spot testing provides an inexpensive and rapid semi-quantitative screening method which might be useful in establishing clinical relevance of metal exposures in dermatitis patients. These include the dimethylglyoxime (DMG) spot test for Ni,[35] the Co spot test,[36] and the diphenylcarbazide (DPC) spot test for Cr (VI).[37] Based on colorimetric assays, these tools detect metal release from a surface by means of a reagent that generates a visible color change upon contact with metal ions. Nevertheless, the analytical sensitivity of these methods varies for different materials yielding inaccuracies. Thus, the highest sensitivity and lowest limit of detection (LOD) of the Co and Cr spot tests have been reported for metallic surfaces, including a LOD of 8.3 ppm for Co and 0.5 ppm for Cr (VI). The Co spot test has shown poor sensitivity and specificity for leather, reported to be as low as 20% and 14%, respectively.[38] While the Cr spot test might be effective in determining release of Cr (VI) from leather, false positive results may occur, particularly from colored leather entailing a misinterpretation of color change. The potential damaging effect on leather at the spot where the reagent is applied combined with the fact that the Cr spot test needs to be kept frozen during storage, might complicate its use. Furthermore, the dynamic equilibrium governing the interconvertibility of Cr species could lead to false negative results at spot testing as Cr (VI) present on the surface is readily reduced to Cr (III) upon contact with humid air. There is currently no spot test available for quantification of Cr (III) release, which is another important limitation of the Cr spot test. While these spot tests offer a feasible tool for screening purposes, it is important to keep in mind that release patterns of metal ions might be different during conditions of relevance to skin exposure, which are considered in laboratory release tests, including the artificial sweat test, EN 1811, for metallic objects and the EN ISO17075 standard for leather items.

1.3.2 X-ray fluorescens

Portable x-ray fluorescence (XRF) devices are important screening instruments used to identify the elemental composition of a broad range of materials, including alloys, leather, powders and liquids. The XRF device captures element-specific fluorescent radiation emitted from excited atoms present in the material. Despite being a feasible, rapid and convenient method, the XRF device is solely a content test, providing information about the bulk composition, which is not a predictor of chemical release from the surface of a material.

1.3.3 Assessment of skin exposure

While spot testing, laboratory release tests and XRF screening present indirect measures of skin exposure, the metal skin dose, i.e. the deposited amount of metal per unit skin area ($\mu g/cm^2$), can be quantified by skin sampling techniques. Lidén et al. (2006) developed and validated the acid wipe sampling method for quantification of the metal skin dose, reporting recovery rates as high as 93%-103% for Ni, Cr and Co deposited on the skin.[39] In contrast, the sampling efficiency of the swap test was reported to be 46%.[40] In the acid wipe sampling technique, the skin is sampled by a cellulose wipe moistened with 0.5 mL 1% nitric acid followed by spectrometric analyses of the metal content in the wipe. In a recent study (2018) of Co skin exposure in 40 hard metal workers, deposited Co skin doses of 0.12-1.51 $\mu g/cm^2$ was reported after two hours of work using the acid wipe sampling technique, including a maximum Co skin dose of 28 $\mu g/cm^2$.[41]

1.4 Epidemiology and main sources of chromium and cobalt exposure

1.2.1 Chromium

Following Ni and Co, Cr is the most common metal allergen in the general population and in patients with allergic dermatitis. In the latter group, the prevalence of Cr contact allergy ranges from 1.7% to 3.7% in North America and Europe,[42, 43] while estimates as high as 13.5%-20.8% have been reported in Asia (Table 1).[44, 45] Historically, Cr allergy mainly affected the hands of male construction workers exposed to wet cement containing dissolved Cr (VI), mostly in the range of 10-20 mg/kg.[46] Due to highly efficient regulatory actions against Cr (VI) in cement in European countries, restricting its presence to a maximum of 2 mg/kg, Cr allergy is now mainly observed in consumers exposed to leather goods affecting both genders equally. In a Danish study of 16,228 patch tested dermatitis patients during 1985-2007, an increasing trend was noted in the prevalence of Cr allergy during 1995-2007, seemingly driven by a significant increase in the proportion of relevant leather exposures, mainly leather shoes, and foot dermatitis, being the anatomical site of dermatitis in nearly half of Cr allergic patients (47.7%).[47] The presence of Cr in leather is not surprising, as Cr tanning is the most common tanning technique worldwide, using a basic Cr (III) sulfate salt, $Cr(OH)SO_4$. The latter intercalates with proteins, mainly collagen fibers, in leather, creating a stable skeletal structure with high strength, durability and malleability. Enormous amounts of Cr (III) is used during tanning (10,000-80,000 mg/kg). Depending on several intrinsic and extrinsic factors of the leather, including presence of vegetable tannins, antioxidants, heat, humidity and wear during use, Cr (VI) might be formed and released onto the skin, posing a significant risk of sensitization and elicitation of dermatitis.[48] While legislation on cement has reduced the incidence of occupational dermatitis due to Cr, it is important to keep in mind that occupational Cr exposure might still be relevant. In an Italian multicenter study (2001) of 212 metalworkers with occupational ACD diagnosed during 1993-1998, 62 (29.2%) were allergic to Cr, seemingly driven by exposure to metalworking fluids (MWFs) as 48 of 212 (22.6%) cases also reacted positively when patch tested with these fluids.[49] As shown in Table 1, MWFs are commonly linked to occupational ACD in metalworkers although it remains unclear if and to what extent these fluids present a relevant metal exposure. While it is known that metal allergens might occur as contaminants in used MWFs due to machining operations, few studies have investigated the presence of metal allergens in MWFs.[50, 51]

1.2.2 Cobalt

Contact allergy to Co is common affecting 5.4%-6.2% of consecutively patch tested dermatitis patients in Europe and North America (Table 1). In a recent meta-analysis of the prevalence of contact allergy in the general population, a pooled Co prevalence of 2.7% was reported in 15,389 individuals.[52] The high frequency of Co allergy is not surprising, as Co has been classified as a strong skin sensitizer in animal studies, including the guinea pig maximization test and the murine local lymph node assay.[53, 54] Despite being the second most common metal allergen, causative exposures are rarely disclosed in patients with Co induced ACD. As a result, cross-reactivity with other sensitizing metals and false positive reactions in patch testing have been considered as possible explanations for the high prevalence of Co allergy. The idea of cross-reactivity emerged from a frequent observation of concomitant reactions to Ni and Cr in dermatitis patients with Co allergy. However, animals sensitized to Co did not react when challenged with Cr or Ni, supporting the notion of multiple exposures and co-sensitization rather than cross-reactivity.[54] Therefore, Co allergy and ACD in those sensitized constitute an undisputable challenge, further supported by epidemiological studies reporting largely unchanged and even increasing trends in the occurrence of Co allergy over time.[55, 56] Despite a lack of knowledge regarding the main causative exposures, there are several reports of Co allergic dermatitis in the literature, mostly in an occupational setting. These have traditionally included workers in the metal manufacturing and machining industries, pottery workers and construction workers. In a study from Taiwan including 166 cement workers, the prevalence of Co allergy was 4.2% and a Swedish study of 853 hard metal production workers reported a prevalence of 4.6%, including 2.8% with isolated Co allergy.[57, 58] Furthermore, in a study of consecutive patients with occupational ACD, the risk of Co allergy tended to be markedly higher in metalworkers exposed to MWFs as compared to men not working in the metal industry (7.6% vs. 4.1%, OR=1.9, 95% CI: 0.9-3.7).[59] Another important occupational exposure includes dental technicians handling dental alloys and tools.[60] In a Korean study (2001) of patients with occupational contact dermatitis, Co allergy was recorded in 12% of dental technicians.[60] Data on consumer exposure has generally been scarce. Available data include clothing items, jewellery, tattoo-inks, cosmetics, electronic devices, and more recently, leather has been suggested as an important exposure.[61] Co might be used as a dying agent in leather after tanning, but possibly has other functions as well. During the 2010s, several case studies have reported ACD due to Co exposure in leather goods,[62] and a recent Danish questionnaire study (2015) found that patients with Co allergy more often reported ACD due to non-occupational leather exposure.[34] It has been suggested that the proportion of Co-containing leather items might be as high as 15-20%.[63]

Table 1. A selection of recent epidemiological studies on chromium (Cr) and cobalt (Co) allergy among patch tested dermatitis patients.

	Reference	Region	Study population	Population size	Study period	Main exposures reported	Prevalence of Cr or Co allergy
General dermatitis patients*	Uter (2020)[43]	Europe (ESSCA)	Consecutive dermatitis patients	51914	2015-2018	N/A	Cr: 3.7% Co: 5.4%
	DeKoven (2018)[64]	North America (NACDG)	Consecutive dermatitis patients	5597	2015-2016		Cr: 1.7% Co: 6.2%
	Boyvat (2021)[65]	Turkey	Consecutive dermatitis patients	1309	2013-2019		Cr: 6.5% Co: 6.0%
	Boonchai (2014)[44]	China	Consecutive dermatitis patients	852	2000-2009		Cr: 20.8% Co: 16.0%
	Morrone (2014)[66]	Ethiopia	Consecutive dermatitis patients	480	2010-2011		Cr: 5.4% Co: 4.6%
	Toholka (2014)[67]	Australia	Consecutive dermatitis patients	5281	2001-2010		Cr: 10.0% Co: 11.0%
Occupational dermatitis	Schubert (2020)[68]	Europe (IVDK)	Metalworkers with dermatitis	3356	2010-2018	Metalworking fluids, metals, oils/greases	Cr: 2.8%-6.9% Co: 4.5%-7.5%
	Schubert (2019)[69]	Germany (OCCUDERM)	Metalworkers with dermatitis	230	2012-2017	Gloves, skin care creams and metalworking fluids	Cr: 3.4% Co: 3.4%
	Geier (2017)[70]	Europe (IVDK)	Construction workers with dermatitis	245	2009-2011	-	Cr: 15.1% Co: 9.0%
	Warshaw (2017)[71]	North America (NACDG)	Metalworkers with dermatitis	2732	1998-2014	Adhesives, metalworking fluids and coatings	Cr: 5.6% Co: 6.0%
	Crippa (2001)[49]	Italy	Metalworkers with dermatitis	212	1993-1998	Metalworking fluids	Cr: 29.2% Co: 22.6%
	Lee (2001)[60]	Korea	Dental technicians with dermatitis	49	-	Alloys and tools	Cr: 24.5% Co: 12.2%
	Papa (2000)[72]	Italy	Metalworkers with dermatitis	150	-	Metalworking fluids and metals	Cr: 4.0% Co: 5.3%

*: 5%-26% with occupational dermatitis.

1.5 Legislation and prevention of contact allergy to chromium and cobalt

In 1979, it was reported that adding an amount of 0.35% ferrous sulphate to cement was enough to reduce the level of soluble Cr (VI) below 2 ppm.[73] In 1983, a Danish legislation made the addition of ferrous sulphate compulsory to reduce the content of water-soluble Cr (VI) to no more than 2 ppm in dry cement.[74] The same legislation was implemented by Finland in 1987 and Sweden in 1989 followed by a EU legislation in 2005 (2003/53/EC) prohibiting the use or selling of cement and cement-containing preparations with a content of more than 2 ppm of Cr (VI).[75] Subsequently, a lower occurrence of ACD cases related to Cr (VI) in cement has been reported in several studies from Europe.[76, 77] Given the increasing prevalence of Cr allergy due to leather, The Federal Institute for Risk Assessment in Germany recommended limiting the content of Cr (VI) to a maximum of 3 mg/kg in leather goods which lead to a national legislation in 2010 (http://www.bfr.bund.de/cd/9575, last accessed March 14, 2021). The latter was preceded by a market survey conducted by the regulatory authorities reporting that 16% of 850 leather goods released more than 10 mg/kg Cr (VI). A similar Danish study (2009) of leather footwear found that 44% of 18 items released more than 3 mg/kg Cr (VI).[78] Based on these findings, Danish authorities submitted a proposal to the European Chemical Agency (ECHA) in 2012, resulting in adoption of a EU regulation on leather (No 301/2014) in March 2014.[79] Accordingly, leather articles and articles containing leather parts coming into contact with the skin shall not be placed on the market where they contain Cr (VI) concentrations equal to or greater than 3 mg/kg after May 2015. However, the legislation does not apply to marketing of second-hand articles in end-use before May 2015. The EU regulation was foreseen to be 80% effective in reducing the occurrence of new cases of ACD due to Cr (VI) in leather. Control of compliance regarding Cr (VI) release must be performed according to EN ISO17075, and only once before marketing. In the current version of this method, the conditioning of dry leather samples is done at 23 °C and 50% relative humidity for 24 hours. This is followed by cutting the samples into small pieces and storage in a dry and sealed container away from any source of heat. There is no information provided about storage time nor relative humidity during storage. After storage, the sample is immersed in a phosphate buffer (pH=8) for 3 hours and released amounts of Cr (VI) are measured using spectrometric analyses. While regulatory initiatives are applauded, it is pivotal with research addressing the effect of regulations and further assessing whether adjustments are required.

There is currently no regulation restricting the use of Co for prevention of sensitization and ACD in those sensitized. Disclosure of the main exposures causing Co contact allergy is necessary before regulatory restrictions can be introduced.

2. Thesis objectives

2.1 Manuscript I and II

- To examine temporal trends in sensitization rates, clinical characteristics and causatives exposures in adult dermatitis patients with Cr allergy patch tested during 2002-2017 in a cross-sectional registry study (manuscript I).
- To assess whether burden of disease changed in adult dermatitis patients with Cr allergy during 2003-2018 in a case-control questionnaire study (manuscript II).

2.2 Manuscript III

- To examine temporal trends in sensitization rates, clinical characteristics and causatives exposures in adults dermatitis patients with Co allergy patch tested during 2002-2017 in a cross-sectional registry study.

2.3 Manuscript IV

- To determine the amount of Ni, Cr and Co in large samples of used and unused MWFs collected from metalworking plants in Denmark in a cross-sectional market survey.
- Further, to evaluate a handheld XRF device as a screening instrument for these metals in MWFs.

3. Materials and methods

3.1 Data extraction from the Danish National Database for Contact Allergy (manuscripts I, II and III)

Alongside with the foundation of The National Allergy Research Centre in 2001, The National Database for Contact Allergy was established the same year with the overall aim of monitoring the occurrence of contact allergies to chemicals and a characterization of those affected. The database is a collaboration across health-care sectors containing data on patch test results, clinical relevance of positive patch test reactions, suspected causative exposures, demographics and clinical characteristics, including the MOAHLFA-index (Male, Occupational dermatitis, history of Atopic dermatitis, Hand eczema, Leg dermatitis, Facial dermatitis, and Age > 40 years).[80]

3.2 Patch testing procedure (manuscripts I, II and III)

Patch testing was performed with the European baseline series [Trolab allergens (Hermal, Reinbek, Germany) and Allergeaze allergens since April 2016)] with Finn Chambers® (8 mm; Epitest Ltd, Oy, Finland) on Scanpor® tape (Norgesplaster A/S, Alpharma, Vennesla, Norway). Potassium dichromate (0.5% pet.) and cobalt chloride (1.0% pet.) were used for testing. Dosing of the chamber was performed with 20 mg of the test preparation. Patch test readings were done by trained nurses and performed according to the recommendations of the European Society of Contact Dermatitis (ESCD),[10] with an exposure time of 48 hours and readings being performed on day 2, 3 or 4, and 7. Patch test reactions designated as 1+, 2+ or 3+ were interpreted as positive reactions. Irritant responses and doubtful (+?) or negative readings were interpreted as negative responses. Isolated Co allergy was defined as a positive reaction to Co while reacting negatively to Cr and Ni. Co allergy implied a positive patch test reaction to cobalt chloride (1.0% pet.) with possible but not necessarily concomitant positive reactions to other allergens.

3.3 Assessment of clinical relevance (manuscript I and III)

The clinical relevance of positive patch test reactions was assessed by the consulting physician; "current relevance" was recorded when a patient presented with ACD combined with current skin exposure to a source of Cr or Co; "past relevance", when a patient had a positive patch test reaction to either metal and a history of past ACD caused by skin exposure to a source of Cr or Co; and finally, "unknown relevance" or "missing" was recorded when ACD due to Cr or Co could not be linked to a relevant current or past exposure, or when no evaluation had been performed by the physician. Furthermore, recording of relevant causative exposures was based on the notes made by the physician in medical charts, as well as use of spot tests, XRF screening and in selected cases chemical analysis.

3.4 Dermatology Life Quality Index (manuscript II)

The Dermatology Life Quality Index (DLQI) is a 10-item questionnaire developed in 1994 by Finlay and Khan to estimate the impact of skin disease on quality of life during the last week.[81] The questionnaire targets adults (≥ 18 years) and collects data regarding feelings, daily activities, leisure, work, school, personal relationships and treatment. The DLQI score was estimated according to published instructions resulting in a score between 0 and 30, with higher scores indicating a lower quality of life. The validated official version in Danish was used. Permission to use the scale was obtained from http://www.cardiff.ac.uk/dermatology.

3.4 Sample collection and chemical analysis (manuscript IV)

3.4.1 Sample collection

A consultant from the Danish Union of Metalworkers provided a list on plants assumed to use MWFs. Twenty plants were contacted. A metalworking plant was defined as a factory working with metals to create individual parts, assemblies or large-scale structures. Participating plants were visited and samples of both used and unused MWFs were collected. Used samples included MWFs which had been in use for metalworking such as stamping, grinding and milling. Furthermore, we recorded the name of the plant, numbered the samples chronologically and retrieved the safety data sheet for the MWFs. Materials processed at the plants included steel, stainless steel, aluminum alloys, brass, iron, copper alloys, palladium alloys, silver alloys, chromium-nickel alloys and plastic alloys (manuscript IV, Supplementary Table 1).

3.4.2 Chemical analysis

Prior to quantitative analysis, all samples were digested by means of a microwave digestion system (Multiwave GO Plus, Anton Paar, Graz, Austria). Quantitative analysis was performed using graphite furnace atomic absorption spectroscopy (GFAAS) (Perkin Elmer AAnalyst 800) KTH Royal Institute, Stockholm. The calibration curve was based on 1% HNO_3 (0 µg/L) and standards with known concentrations, including 10, 30 and 60 µg/L for Ni, 10, 30, 60, and 80 µg/L for Cr and 10, 30, 60 and 90 µg/L for Co. All samples were shaken by a vortex shaker before analysis. Results were presented as an average of three replicate readings. Furthermore, the measured metal concentrations of blank samples were subtracted from the metal concentrations found in the MWFs. The LOD was estimated as three times the standard deviation (SD) of the blank solutions. Hence, the LOD was 2.1 μg/L for Ni, 0.6 μg/L for Cr and 0.4 μg/L for Co. The quality control samples spiked with 10 μg/L of either metal showed acceptable recoveries of 107% for Ni, 101% for Cr and 101% for Co.

4. Main results

This section summarizes the main findings of each manuscript included. The original manuscripts are included at the end of the thesis.

4.1 Temporal changes in chromium allergy in Denmark between 2002 and 2017

In this cross-sectional registry study, we intended to determine whether EU regulation against Cr (VI) in leather entailed changes in the prevalence of Cr contact allergy, sources of Cr exposure and clinical characteristics among consecutive adult dermatitis patients patch tested between 2002 and 2017 at the Department of Dermatology and Allergy, Herlev-Gentofte Hospital, Denmark.

In total, 13,379 adult dermatitis patients aged 18-99 years were patch tested during 2002-2017. A positive patch test reaction to Cr was recorded in 2.2% (296/13,3379), equally affecting both sexes (2.2%) (manuscript I, Table 1 and 2). Adjusted analyses based on a multivariate logistic regression model with Cr allergy as the dependent dichotomous categorial variable and sex, age group, patch test year, hand dermatitis, foot dermatitis, face dermatitis and AD as independent variables showed an increased risk of foot dermatitis (OR=7.4, 95% CI: 5.7-9.8) and hand dermatitis (OR=2.3, 95% CI: 1.8-3.0) in Cr allergic patients as compared to dermatitis patients without Cr allergy. Furthermore, dermatitis patients in the youngest age group (18-40 years) were less often affected by Cr allergy as compared to those aged $>$ 60 years (OR=0.5, 95% CI: 0.3-0.7); Cr allergy was more common in dermatitis patients patch tested during 2002-2005 as compared to those tested during 2014-2017 (OR=2.2, 95% CI: 1.6-3.1). Similarly, trend analysis using the linear-to-linear chi-square association test showed a decreasing trend in the prevalence of Cr allergy from 3.2% in 2002-2005 to 1.9% and 1.7% in, respectively, 2010-2013 (P_{trend}=0.002) and 2014-2017 (P_{trend}<0.0001) (manuscript I, Figure 1). As regards clinical characteristics of Cr allergic patients (n=296), we observed a significantly decreasing trend in the prevalence of foot dermatitis from 51.6% in 2006-2009 to 29.9% in 2014-2017 (P_{trend}=0.01) (manuscript I, Table 3). As depicted in manuscript I, Table 4 and Table 5, leather was the most single most important source of exposure recorded in 48.3% (143/296) of patients with Cr allergy while other sources of Cr exposure constituted 3.0% (9/296). The main leather exposures included leather shoes (36.8%), leather gloves (12.8%), and other leather exposures (7.4%) i.e.

furniture, watch straps, cars wheels and clothing. We did not record any cases of Cr ACD related to cement exposure.

4.2 No immediate effect of regulatory reduction of chromium in leather among adult chromium allergic patients

In this case-control questionnaire study, we aimed at assessing whether EU regulation against Cr (VI) in leather entailed changes in perception of disease-severity and leather exposures causing dermatitis in adult Cr allergic patients patch tested between 2003 and 2018 at the Department of Dermatology and Allergy, Herlev-Gentofte Hospital, Denmark. Overall, 12,792 consecutive adult dermatitis patients aged 18-99 years had been patch tested during 2003-2018; a questionnaire was sent to 1,241 dermatitis patients, including 237 Cr allergic cases and 1004 controls without Cr allergy. The overall response rate was 61.2% (759/1241), including 72.5% (172/237) for cases and 58.5% (587/1004) for controls.

Cr allergic patients diagnosed in 2003-2012 were younger than those diagnosed in 2013-2018 (50.0 +/- 11.3 years vs. 56.0 +/- 14.7 years, p=0.003). Comparing Cr allergic patients diagnosed in 2003-2012 versus 2013-2018, we found no difference regarding the self-reported prevalence of current hand dermatitis (72.0% vs. 63.1%, p=0.2) nor current foot dermatitis (46.0% vs. 40.0%, p=0.4) (manuscript II, Table 1). Measures of self-reported perception of disease-severity are presented as median (IQR) in manuscript II, Table 2. No difference was found in DLQI score comparing Cr allergic patients diagnosed in 2003-2012 versus 2013-2018 (2.0 vs. 2.0, p=0.8). Similarly, no difference was found as regards the number of anatomical locations with dermatitis, current dermatitis, worst-case dermatitis, effect on work and effect on spare-time. Regarding the aforementioned outcomes, trend analysis with the Jonckhkeere-Terpstra test did not yield any significant temporal changes for Cr allergic patients within the most recent study period (2013-2018). Regarding self-reported exposures causing dermatitis, the proportions of Cr allergic patients reporting leather induced dermatitis were comparable (66.2% vs 71.0%, p=0.5) for those diagnosed during 2003-2012 versus 2013-2018 (manuscript II, Table 4).

Case-control comparisons for those diagnosed in 2013-2018 showed that patients with Cr allergy were still more commonly affected by current hand dermatitis (OR=1.98, 95% CI: 1.13-3.49) and current foot dermatitis (OR=3.82, 95% CI: 2.07-7.08) (manuscript II, Table 1). Whilst Cr allergic patients reported more severe disease as compared to controls in 2003-2012, these differences were not found for those diagnosed in 2013-2018 (manuscript II, Table 2).

4.3 Causative exposures and temporal development of cobalt allergy in Denmark between 2002 and 2017

In this cross-sectional registry study, we aimed at exploring temporal changes in Co contact allergy prevalence, causative exposures and clinical characteristics in consecutive dermatitis patients patch tested across 2002 and 2017 at the Department of Dermatology and Allergy, Herlev-Gentofte Hospital, Denmark.

Overall, 13,475 adult dermatitis patients aged 18-99 years were patch tested between 2002 and 2017 at the Department of Dermatology and Allergy, Herlev-Gentofte Hospital, Denmark. A positive patch test reaction to Co was found in 3.3% (447/13,475) whereas an isolated reaction without the concomitant presence of Cr and/or Ni allergy was recorded in 1.5% (206/13,475) (manuscript III, Table 1). The proportion of Co allergic patients with either Ni or Cr allergy was, respectively, 38.0% and 21.0%. Adjusted analysis based on a multivariate logistic regression model with either Co allergy or isolated Co allergy as the dependent variable showed that Co allergy was more common in female dermatitis patients (OR=2.1, 95% CI: 1.6-2.6), those aged 18-40 years (as compared to > 60 years) (OR=1.4, 95% CI: 1.0-1.9), and patients patch tested in 2006-2009 (OR=1.6, 95% CI: 1.2-2.0). Furthermore, Co allergic patients were more commonly affected by foot dermatitis (OR=2.1, 95% CI: 1.6-2.9) and hand dermatitis (OR=2.1, 95% CI: 1.6-2.6) as compared to dermatitis patients without Co allergy. Similarly, isolated Co allergy was more common in female dermatitis patients (OR=1.6, 95% CI: 1.2-2.3) and those tested during 2006-2009 (as compared to 2014-2017) (OR=2.2, 95% CI: 1.5-3.3). Whilst no association was found with foot dermatitis, patients with isolated Co allergy were more commonly affected by hand dermatitis (OR=2.9, 95% CI: 2.1-4.0) and facial dermatitis (OR=1.6, 95% CI: 1.1-2.2) as compared to dermatitis patients without isolated Co allergy. Furthermore, trend analysis by means of linear-to-linear chi-square association testing showed an increasing trend in the prevalence of facial dermatitis among patients with isolated Co allergy across 2003-2017 (P_{trend}=0.01, manuscript III, Table 3). Hence, the prevalence increased from 13.3% during 2002-2005 to 40.0% and 33.3% during 2014-2017. While the prevalence of Co allergy remained largely stable across the entire study period (P_{trend}=0.1, manuscript III, Figure 1b), we found a decreasing trend in the prevalence of isolated Co allergy across 2003-2017 (P_{trend}=0.03, manuscript III, Figure 1a), decreasing from 2.4% in 2006-2009 to 1.1% in 2014-2017 (P_{trend}<0.001).

As regards causative exposures, leather and jewellery were the most common sources of exposure, established in 6.3% and 6.0% of Co allergic patients, respectively (manuscript III, Table 4). Nonetheless, current clinical relevance of a positive patch test reaction to Co was low (20.1%) and a relevant source of exposure was unrecorded in 83.9% of Co allergic patients (manuscript III, Table 5).

4.4 Metals in used and unused metalworking fluids: X-ray fluorescence spectrometry as a screening test

In this market survey, we intended to examine the occurrence of metal allergens, i.e. Ni, Cr and Co, in used and unused MWFs collected from metalworking plants in Denmark.

Eight of 20 (40%) plants contacted participated in the study. Overall, 80 samples of MWFs were collected, including 61 used and 19 unused ones (manuscript IV, Supplementary Table 1). XRF-screening showed that 9 of 80 (11.2%) contained either Ni, Cr or Co (manuscript IV, Table 1). Of 8 samples with Ni content (range: 2.5-15.5 mg/kg), only one sample with 3.0 (±0.5) was shown to contain 9.9 (±0.02) by GFAAS analysis. Two samples with Co content, 6.0 (±1.5) mg/kg and 5.0 (±1.5) mg/kg, were shown to contain 0.1 (±0.01) and 0.08 (±0.01) by GFAAS analysis. No Cr was found by XRF-screening. For contents ≥ 1mg/kg, Ni, Cr or Co were detected in 17 of 80 samples (21.3%) in elemental analysis by GFAAS; 13 of 80 (16.3%) contained 6.4-17.7 mg/kg Ni, 3 of 80 (3.8%) contained 1.4-3.1 mg/kg Cr and one sample (1.3%) contained 1.3 mg/kg Co. Ni was found in both used (n=6, range: 6.4-17.7 mg/kg) and unused (n=7, range: 9.1-17.3 mg/kg) MWFs while Cr and Co were found in used samples only. For contents ≥ 1mg/kg, the XRF device was a poor screening instrument for these metals in undigested MWFs which is illustrated by the poor correlation between findings from XRF-screening and GFAAS in manuscript IV, Figure 1. The sensitivity and specificity were 7.7% and 89.4% for Ni. Zero sensitivity was found for Cr and Co. The specificity was, respectively, 96.3% and 97.4%.

5. General discussion and considerations on methodology

5.1 Sensitization trends and burden of disease in contact allergy to chromium (manuscript I and II)

In manuscript I, we reported decreasing trends in the prevalence of chromium allergy for the study periods 2010 to 2013 (p_{trend}=0.002) and 2014 to 2017 (p_{trend}<0.001) in our patients as compared to previous years. The accompanying decline in foot dermatitis in Cr allergic patients during 2014-2017 suggests a favorable effect of the regulatory reduction of Cr (VI) in leather (manuscript I, Table 3). According to our data, a decline in cases already began in 2012 (manuscript I, Figure 1), the same year in which the EU regulation was in a hearing, before adoption (2014) and implementation (2015). While the decrease in the occurrence of Cr allergy during the most recent study period may be an effect of the EU regulation, the preceding decrease since 2012 may mirror the efficiency of independent national initiatives against Cr (VI) and pre-emptive manufacturers favouring the use of alternative Cr-free tanning techniques, e.g. oil tanning, vegetable tanning or aldehyde tanning or other techniques reducing the level of free Cr. Accordingly, the Federal Institute of Risk Assessment in Germany already recommended limiting the content of Cr (VI) in leather to a maximum of 3 mg/kg in 2007. The aforementioned actions might already have entailed reduced amounts of Cr (VI) in leather, both domestically and on foreign markets, years before the collective implementation of the EU regulation in May 2015. Interestingly, in the latest report (2018) from the North American Contact Dermatitis Group (NACDG), the prevalence of a positive patch test reaction to potassium dichromate (0.25% pet.) was significantly lower during 2015-2016 versus 2005-2014 (OR=0.55, 95% CI: 0.44-0.68, p<0.001).[64] As no regulation yet exists against Cr (VI) in leather in North America, this decline has been ascribed to collateral effects of regulatory actions in Europe. In the latest report (2021) by the European Surveillance System on Contact Allergies (ESSCA),[56] including 48,610 patch tested individuals with suspected ACD across Europe during 2015-2018, a largely unchanged prevalence of Cr allergy was found (3.7%) as compared to those patch tested in a similar study during 2004 (4.4%).[82]. Furthermore, in another large-scale trinational study (2020) in Central Europe by the Information Network of Departments of Dermatology (IVDK) including 125,436 patients patch tested with the baseline series during 2007-2018, an unexpected increase was noted for the prevalence of Cr allergy from 3.3% (95% CI: 3.1-3.5) in 2011-2014 to 4.5% (95% CI: 4.3-4.7) in 2015-2018.[83] While these prevalence estimates are not stratified according to exposure type, a decline in the overall prevalence of Cr allergy is to be expected, as leather has been the single most important causative exposure in Europe since the regulation on cement in 2005. In our patients, leather was recorded as the main source of exposure in nearly half (48.3%) of Cr allergic patients.

Thus, there are divergent results concerning trends in Europe. This may at least partly relate to methodology; while our study (manuscript I) was fairly small compared to the big networks,[43, 64, 83] the data in our study was from one department with a - as far as possible - consistency in patch testing techniques over the years. The departments included in the large studies were only partly identical across the years, implying a possible bias, when looking at trends. All the studies rely on patient data, which means that selection mechanisms for patients in seeking medical attention and structural barriers may vary over time and across countries. Nevertheless, the discipline of clinical epidemiology in contact allergy has repeatedly proven its value, with largely consistent results between department/networks in Europe e.g. concerning the methylisothiazolinone epidemic, Ni allergy etc.[83, 84] It could be of value in the future to have a more uniform stratification of data across studies for better comparison. The true outcome measure concerning effects of regulation of Cr (VI) in leather would be ACD related to leather exposure. Another indicator of effects of regulation could be changing exposures to Cr. In a recent study (2019) the content and release of Cr (VI) from leather products available on the Danish market was conducted.[85] It was found that 10 of 94 (10.6%) leather samples exceeded the limit value of 3 mg/kg with the highest values found in handbags (11 mg/kg – 28 mg/kg), whereas 12.2% of 41 footwear items released more than 3 mg/kg Cr (VI). In a similar study, but only focusing on shoes, was conducted in 2009 prior to regulation, Cr (VI) was extracted from 44% of 18 footwear products, with the highest value of 62 mg/kg.[78] These studies are not directly comparable but may indicate a reduced but still in some cases significant exposure, which may both sensitize and elicit ACD.

As regards elicitation of ACD, it is plausible that reduced amounts of Cr (VI) in leather might result in less disease-experience in those already sensitized. However, we did not find significant improvements in measures of disease-severity nor a decrease in the proportion of self-reported leather exposures leading to dermatitis in Cr allergic patients during 2003-2012 versus 2013-2018 (manuscript II). The latter findings suggest that leather articles in end-use among consumers and workers remain important causative exposures in those sensitized despite legislative actions against Cr (VI) in leather. In general, a lower amount of the culprit hapten is needed for elicitation as compared to the sensitization phase. As mentioned above, leather articles on the market might still contain and release critically high levels of Cr (VI). According to previous patch test dose-response studies, Cr (VI) levels as low as 1 mg/kg cause ACD in 10% of Cr allergic patients,[86] corroborating the allergenic capacity of levels lower than the LOD of the reference method, EN ISO 17075. The 10% minimum elicitation threshold might be even lower during prolonged real-life exposure conditions, as suggested by previous use tests with Cr-tanned leather bracelets.[87] Other factors of importance include second-hand leather articles in end-use before May 2015, leather articles bought outside the EU, changing fashion trends and the presence of Cr (III) which is not covered by the regulation. Whether Cr (III) in leather poses a significant risk of elicitation (and sensitization) is debated. The eliciting capacity of Cr (III) has already been elucidated in previous dose-response patch test studies and a 10% minimum elicitation threshold of 6 mg/kg has been reported.[86] Although markedly higher amounts of Cr (III) are required to elicit ACD as compared to Cr (VI), the released amounts of Cr (III) from leather over time is persistently higher (> 10-fold) than that of Cr (VI).[88] To investigate if prolonged and repeated exposures to Cr-tanned leather with only Cr (III) release (313 mg/kg) could elicit ACD, 10 Cr allergic patients and 22 controls were enrolled for a 3-week use test study with leather bracelets for 12 hours a day. ACD was elicited in 4 of 10 (40%) Cr allergic patients whereas none reacted in the control group.[89] This finding is noteworthy, as much higher amounts of Cr (III) may be released from leather (>3000 mg/kg).[48] While released Cr (III) per se might constitute a more major cause of ACD than hitherto recognized, it is essential to be aware of the dynamic equilibrium that governs the interconvertibility of Cr species, implying that Cr (III) present in leather might oxidize to form Cr (VI). Hence, a negative test result (< 3mg/kg) of Cr (VI) by the ISO 17075 at one timepoint, as required before marketing, does not guarantee a low release of Cr (VI) throughout the lifecycle of the leather. It has previously been reported that total Cr release is highest for new leather due to the migration of unbound Cr (III) whereas Cr (VI) might be formed and released during use among consumers and workers depending on both intrinsic factors in the leather - e.g. presence of antioxidants, vegetable tannins and fatty acids - and environmental parameters.[48] During a stimulated use study with sequential leather exposures to storage conditions and subsequent immersions in phosphate buffer (pH=8.0) during 8 months, it was found that dry air (relative humidity < 35%), UV irradiation and alkaline pH increased the formation of Cr (VI).[90] Interestingly, Cr (VI) comprised an increasing proportion of total Cr released over time driven by an increase in Cr (VI) release and a concurrent decrease of Cr (III). However, the leather used in this study was unfinished (non-coated) intended for gloves, hence not containing large amounts of antioxidants. As Cr (VI) formation is a function of the antioxidant content in the leather, future investigations need to elucidate whether these substances are leached during use and ageing.

5.2 Sensitization trends and causative exposures in cobalt allergy (manuscript III)

In manuscript III, we found that the overall prevalence of Co allergy remained largely stable in men and women across the entire study period (2002-2017) (manuscript III, Figure 1). This is in line with a recent study (2020) of 125,436 patch tested dermatitis patients in Central Europe during 2007-2018, in which similar prevalence estimates were reported for 2015-2018 (5.5%) and 2007-2010 (5.3%).[83] We did, however, observe an unexplainable peak during 2006-2009 which seemed driven by women with isolated Co allergy (manuscript III, Table 2). Historically, isolated sensitization to Co was deemed an occupational issue, as it was mostly diagnosed in pottery workers and metal workers.[91] In our data, no association was found between isolated Co allergy and occupational dermatitis in a fully adjusted regression model; instead, significant associations were found for being a female (OR=1.6), hand dermatitis (OR=2.9) and facial dermatitis (OR=1.6). Interestingly, an increasing trend was noted for the presence of facial dermatitis among isolated Co allergic patients (manuscript III, Table 3). Similarly, Ruff and Belsito reported an increased risk of dermatitis on the lips in patients with isolated Co allergy.[92] The combination of facial dermatitis and female sex suggest that cosmetics might constitute an important source of isolated Co allergy. The latter is further supported by previous studies reporting critically high levels of metals, including Co, in eye-shadows.[93, 94] However, it is not known if Co is in a bioavailable state in such products. The relatively high and stable occurrence of Co allergy along with difficulties in establishing relevant causative exposures, has led to speculations about misinterpretation of false positive reactions as genuine positive reactions. In a Swedish study, reactivity at patch testing to Co in two concentrations (0.5% and 1.0% pet.) and development of reactions were analysed. It was shown that a significant proportion of Co allergy cases was missed by testing with 0.5% as compared with 1.0%. The proportion of doubtful or irritant reactions, in relation to positive reactions, was of equal magnitude for 0.5% and 1.0%.[17] In addition, patch testing with Co (1.0% pet.) generated a markedly lower proportion of irritant and doubtful reactions as compared to Cr (0.5% pet.).[17] The latter findings suggest that Co acts no different than other metal allergens at patch testing and that more focus should be given to identification of relevant exposures, especially in cases of isolated cobalt allergy.

Following regulatory actions against consumer Ni exposure in Europe during the 1990s, it was debated whether Ni would be replaced by Co in costume jewellery, leading to an increase in the occurrence of Co allergy. While an epidemic has not emerged, jewellery remains an important exposure in patients with Co allergy. In our patients, jewellery was the most recorded source of exposure (6.3%). Several market studies have explored the content and released amounts of Co from jewellery, mainly from unused and inexpensive ones. Migration analysis of costume jewellery items according to the artificial sweat test, EN 1811, showed that 38 of 87 (43.7%) unused items had at least one part releasing Co, including 22% of post assemblies releasing more than 0.2 μg/cm^2/week, which is the regulatory migration limit for Ni in post assemblies.[95] However, by means of a Co spot test with a detection limit of 8.3 mg/kg, Haman et al. reported that only four of 557 (0.7%) unused inexpensive earrings bought in China and Thailand released Co.[96] Similarly, only four of 354 (1.1%) unused inexpensive consumer items from Denmark released Co.[97] In contrast, examination of Co release from used consumer items showed a positive spot test in 206 of 551 (37.4%) in Thailand.[98] It is important to note that consumer items in use among consumers might show a different release pattern due to long-term corrosion, wear and ageing. Furthermore, as Co is a potent allergen, it is conceivable that released amounts lower than the detection limit of the spot test may elicit ACD in those already sensitized. It has previously been suggested that dark-coloured inexpensive jewellery might be an important source of Co end-use.[96, 97, 99] However, due to its white silvery appearance and high market price, it has been suggested that Co might occur in more expensive and light-coloured items as well.[100] Accordingly, in a Korean market study comparing 193 branded and 202 non-branded jewellery, the majority of positive cobalt spot tests was found in branded light-appearing jewellery items.[100] The latter finding needs to be further investigated in larger studies.

In our study leather was recorded as the causative source of exposure in 28 of 447 (6.0%) Co allergic patients, with leather shoes being the most common (3.4%) (manuscript III, Table 4). In a recent case-control questionnaire study (2015) from Denmark including 126 Co allergic dermatitis patients without concomitant Cr allergy, a significant association was reported between non-occupational leather exposure and Co allergy (OR=2.49, 95% CI: 1.49-4.17).[34] Moreover, Co allergic patients were more often affected by current foot dermatitis as compared to controls, suggesting a potential role for leather shoes in Co allergy. A Swedish study (2009) reported a Co content of <0.3-16 mg/kg in 21 shoes bought from different countries and Hamann et al. (2018) reported contents of more than 400 mg/kg in leather shoes and gloves.[101] While several studies have corroborated the presence of Co in various leather articles, few have examined the extent of Co release – at least not according to a standardized protocol specifically devised for quantification of Co release from leather. Most studies have used different acid extraction methodologies, including the artificial sweat immersion test, combined with spectrometric analyses. Hence, a recent Danish study (2019) of Co release from marketed leather items found that 6 of 10 samples released 0.07-0.44 μg/cm^2/week with the highest levels found in a brown shoe and a black bracelet.[85] Another study of 29 leather samples collected from two different tanneries in Nicaragua found released Co amounts of 1.0-4.7 mg/kg, mainly from one of the tanneries, using the EN ISO17075 method for migration analysis.[102] The significant difference in Co release from the two tanneries indicates that different dye compositions might be used during leather production. Furthermore, the low levels of Co found in the aforementioned study might be due to poor solubility of Co in the alkaline phosphate buffer solution used in the EN 1S017075 analysis, entailing an underestimation of Co. The latter highlights the need for a specific protocol for quantification of Co release from leather. Nonetheless, there is a growing body of evidence indicating that leather contains and releases Co, posing a significant risk of sensitization and elicitation of ACD.

5.3 Metalworking fluids as a source of chromium and cobalt contact allergy (manuscript IV)

In manuscript IV, we investigated the occurrence of Ni, Cr and Co in a large sample of used and unused MWFs collected from several metalworking plants in Denmark. Despite the overall occurrence of these metal allergens was low (< 1 mg/kg in 63 of 80), considerable levels (> 1 mg/kg in 17 of 80) were found in some used and unused MWFs (manuscript IV, Table 1). Metals are not used in manufacturing of MWFs, but might occur as contaminants from machining operations, particularly grinding and cutting operations, depending on the elemental composition of the workpiece processed. In a study (1978) of Co dissolution from hard metal alloys (containing 5-10% Co) by nine commercial MWFs, the authors reported a concentration range of 7 mg/kg – 552 mg/kg Co in solution after five days of use with the highest solubility rate occurring during the first day.[103] Likewise, the maximum Co content was as high as 217 mg/kg after 14 days in an oil used for grinding of a tungsten carbide alloy (10% Co).[50] While high amounts of Co might occur in MWFs, minor amounts of Co were found in our study with only one used sample containing 1.3 mg/kg, suggesting a low prevalence of clinically relevant (≥1 mg/kg) Co exposure from MWFs in metalworkers. The latter finding is not surprising as none of the participating metalworking plants reported processing of hard metal alloys or other Co-based alloys (Supplementary Table 1, manuscript IV). Furthermore, due to its high market price - as compared to Cr and Ni – and its valuable application in superalloys used for production of gas turbines and engines, dental implants and prosthetics, it is conceivable that Co end-use has become more exclusive. Nonetheless, Co residues in MWFs might be a clinically relevant exposure in patients with Co allergy, as shown in a recent case report of a marine engineer with isolated Co allergy affected by severe hand dermatitis after exposure to 2.4-2.7 mg/kg Co in a machine oil.[104]

In manuscript II, we noted that Cr allergic patients more often reported dermatitis due to metalwork as compared to those without Cr allergy (OR=3.39, 95% CI: 1.44-7.97) (manuscript II, Table 4). However, we did not have data on the specific type of metalwork nor about exposure to MWFs in our patients. The latter is pivotal in interpretation of patch test data and clinical relevance of these fluids, as not all metalworkers are exposed to MWFs or the extent of exposure might vary between occupational subgroups.[105] While no Cr was found in unused MWFs, 1.4-3.1 mg/kg Cr was found in 3 of 61 (4.9%) of the used ones. As no speciation analysis was performed, we were not able to determine the valence state of the Cr content. Since MWFs tend to be alkaline, it is likely that most of the Cr content may be present as Cr (VI). Given a 10% minimum elicitation threshold of 1 mg/kg for Cr (VI),[86] the aforementioned amounts might elicit ACD in those already sensitized. The transdermal penetration enhancing effects of lipids and fatty acids present in the MWFs combined with the skin irritant effects of the alkaline pH might further augment the allergenic capacity of the Cr contents found in the fluids. Although Cr levels found in this study may be sufficient to elicit dermatitis in a considerable proportion of Cr allergic metalworkers exposed to MWFs, the sensitizing capacity of these levels are unknown. In a previous study (2004) of sensitization patterns in metalworkers exposed to MWFs, Geier et al. reported that the occurrence of Cr allergy tended to be lower in the latter group as compared to men not working in the metal industry (3.5% vs. 5.1%).[59]

As shown in Figure 1 (manuscript IV), a poor correlation was found comparing findings from a handheld XRF device and GFAAS for Ni contents above 1 mg/kg in the MWFs. However, it is to be noted that XRF screening was performed on undigested samples which are known to contain several organic components that might interfere with the solubility of the metals, thus affecting the readings of the device. Moreover, it has previously been reported that the presence of bulky particles in the fluids, which was the case in the undigested samples, might further reduce the accuracy of the XRF measurements.

6. Conclusion

In this thesis, we found a decreasing trend in the occurrence of Cr allergy and dermatitis, particularly foot dermatitis, during 2002-2017, which could mirror a favourable effect of regulation against Cr (VI) in leather. Moreover, we did not find significant improvements in burden of disease, including occupational performance and quality of life, nor a decrease in the proportion of leather induced dermatitis in those already sensitized from 2003 to 2018. The current regulation on leather may have to be revised for better protection of consumers, workers and those already sensitized, both regarding a further reduction of the current permissible levels of Cr (VI) and especially the unregulated presence of Cr (III) which might be involved in sensitization and elicitation of ACD. Lastly, used MWFs might constitute a clinically relevant Cr exposure in metalworkers, particularly for those already sensitized.

Co allergy remains a fairly common issue among dermatitis patients given the largely unchanged prevalence of overall and isolated Co allergy during 2002-2017. While the majority of cases with Co allergy could not be linked to a relevant causative exposure, the increasing proportion of leather induced dermatitis across time suggest that leather may constitute a more important exposure in these patients than hitherto recognized. In addition, the low prevalence of clinically relevant amounts of Co found in the MWFs indicate that these fluids, in general, rarely cause Co contact allergy in metalworkers, but probably depend on the type of metalwork. Currently, the sources of Co allergy and its clinical impact are in most cases unknown. This will have to be elucidated in order to assess the need and target for prevention.

7. Future perspectives

7.1 Chromium

Despite the significant decline in the occurrence of Cr contact allergy in our first study, much still remains to be done; particularly for those already sensitized as shown by the lack of improvement in disease-burden and unchanged proportion of Cr allergic patients reporting leather-induced dermatitis across time. Also, a decline is not found in European network studies, the reason for this need to be further scrutinized. The persistent issue of Cr allergy and ACD due to leather exposure may be driven by several aspects: induction and elicitation of dermatitis by lower levels of Cr (VI) than the regulated limit value of 3 mg/kg, the allergenic potency of Cr (III) per se and formation of Cr (VI) from Cr (III) depending on the tanning and manufacturing process, leather ageing and environmental factors during the service life of the leather. As regards future studies, it is necessary to quantify the released amounts of Cr (VI) from a wide range of leather articles on the market using the colorimetric reference method EN ISO17075. The analytical LOD of this method is currently too high and should be reduced as far as possible or more exact methods developed. In addition, it is necessary with clear instructions with respect to storage time and relative humidity during storage prior to immersion in the phosphate buffer, as exposure to relative humidity above 35% during storage increases the likelihood of test values below the restriction limit. Furthermore, a negative test result (< 3 mg/kg) for Cr (VI) at one point does not guarantee a low release of Cr (VI) at another timepoint, e.g. during use. Thus, Cr (III) is mostly released from newly manufactured leather whereas Cr (VI) tends to be released to a higher extent from aged leather, mainly due to wash-out of antioxidants, acids and colors.[88] Therefore, investigation of Cr species released from marketed leather items and in use among consumers and workers is warranted in order to attain a more accurate insight into the real-life levels of exposure to Cr species in Cr allergic patients. Importantly, the regulation may have to be revised for better protection of those already sensitized, especially regarding Cr (III) which is currently unregulated. Despite possessing poor sensitizing capacity, several studies have corroborated its release from leather and capability of elicitation.

7.2 Cobalt

The main issue concerning Co allergy arises from the difficulty in linking positive patch test reactions to relevant causative exposures. False positive reactions in patch testing or cross-reactivity with other metal allergens do not seem to explain the fairly high prevalence of Co allergy, thus underscoring the immediate need for continued research and disclosure of the main sources of exposure. As regards costume jewellery items, more studies are needed addressing the significance of the price and colour. Moreover, as used and aged items might exhibit a different release pattern, investigation of used items and use test studies with Co containing items are warranted. Since early 2010s, leather has been proposed as a possibly important and hitherto overlooked source of exposure in Co allergic patients based on several case reports.

While there is a growing number of studies corroborating the presence and release of Co from leather products, more extensive market studies are needed to elucidate the content, and more importantly, released amounts of Co from marketed leather products, thus adding to the current body of evidence. Currently, the EN ISO17075 protocol presents the golden standard method for quantification of leached metal ions from leather. However, it was originally prepared by the Chemical Test Commission of the International Union for Leather Technologists and Chemists Societies (IULTCS), in collaboration with the European Committee for Standardization (CEN), for sampling and testing of Cr (VI) in leather. The test operates with alkaline conditions which may pose a risk for underestimation of Co ions leached from leather as the solubility of Co compounds rapidly decrease for $pH > 7$. Therefore, a new protocol, specifically devised for chemical determination of Co release from leather is warranted in the future. Lastly, amounts of Co deposited onto the skin during leather contact should be investigated in consumers and workers, e.g. by the acid wipe method.

8. Manuscripts

8.1 Temporal changes in chromium allergy in Denmark between 2002 and 2017

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WILEY CONTACT DERMATITIS

ORIGINAL ARTICLE

Temporal changes in chromium allergy in Denmark between 2002 and 2017

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Background: In 2012, Danish authorities submitted a proposal to the European Chemical Agency restricting the content of hexavalent chromium to a maximum of 3 ppm in leather goods. Following its adoption, this proposal was implemented in 2015 as a directive in the EU.
Objectives: To examine the temporal trend of chromium contact allergy in adult dermatitis patients patch tested between 2002 and 2017, and to determine clinical characteristics and causative exposures in these patients.
Methods: All adult dermatitis patients patch tested between 2002 and 2017 were included. Patch test data were reviewed retrospectively. Comparisons were performed with the χ^2 test and logistic regression analysis.
Results: A total of 13 379 adults aged 18 to 99 years were patch tested between 2002 and 2017. The overall prevalence of chromium allergy was 2.2%. An overall decreasing trend was found for the prevalence of chromium allergy (P_{trend} = 0.00002). Specifically, a significant difference was found for the study periods 2010 to 2013 (P_{trend} = 0.002) and 2014 to 2017 (P_{trend} < 0.0001) as compared with 2002 to 2005. Leather remained the most important single cause of allergic contact dermatitis caused by chromium. The proportion of clinically relevant leather exposures increased from 42.3% during 2002 to 2009 to 54.8% during 2010 to 2017 (P = 0.04).
Conclusions: The prevalence of chromium allergy is decreasing. The EU Directive restricting the use of hexavalent chromium in leather goods is thought to be playing a central role in this change.

KEYWORDS
chromium, contact allergy, hexavalent, leather, prevalence, regulation, trend

1 | INTRODUCTION

Chromium is a transition metal that is capable of causing severe allergic contact dermatitis (ACD). It has several oxidation states, of which only trivalent chromium and hexavalent chromium are sufficiently stable to act as haptens.[1] Historically, cement has been the most important source of exposure for the induction of chromium allergy, particularly among men.[2,3] However, Zachariae et al[4] reported that leather items had become the most frequent sources of chromium allergy in dermatitis patients patch tested during 1989 to 1994 in Gentofte, Denmark. In a cross-sectional follow-up study including >16 000 dermatitis patients, Thyssen et al[5] found a significant increase in clinically relevant leather exposure when they compared 1989 to 1994 data and 1995 to 2007 data in Danish patients with suspected ACD, and a simultaneous significant increase in the prevalence of chromium allergy.

In a Danish market survey from 2009, it was found that nearly all of 60 randomly evaluated leather footwear items contained chromium, with a median content of 1.7%, emphasizing a significant source of chromium exposure.[6] Notably, hexavalent chromium was released from 44% of 18 products evaluated with the ISO 17075 standard method, which has a detection limit of 3 ppm. To address the growing leather problem, the Danish government submitted a dossier in January 2012 to the European Chemical Agency elucidating the chromium problem. Subsequently, in May 2015, a legislative initiative was implemented by the EU prohibiting a content of more than 3 ppm of hexavalent chromium in leather products.[7]

In the present study, we provide an updated epidemiological analysis of the prevalence and exposure sources of chromium allergy in adult Danish patients with suspected ACD and who were patch tested at the Department of Dermatology and Allergy, Herlev-Gentofte Hospital over a 16-year period.

2 | METHODS

This study was performed in accordance with the "strengthening the reporting of observational studies in epidemiology" (STROBE) statement.[8] Data were extracted from the National Database for Contact Allergy in Denmark. All adult patients (≥18 years) with suspected ACD who were patch tested during 2002 to 2017 at the Department of Dermatology and Allergy, Herlev-Gentofte Hospital were included. Through a retrospective review of patient data regarding patch testing (test year, reading day, and test results, ie, the presence or absence of chromium allergy), the clinical relevance of chromium allergy (current, past, or unknown), the MOAHLFA index (male, occupational dermatitis, history of atopic dermatitis [AD], hand eczema, leg dermatitis, facial dermatitis, and age > 40 years),[9] the presence of foot eczema and sources of exposure were retrieved. Permission was obtained from the Danish data Protection Agency.

2.1 | Definitions

The clinical relevance of positive patch test reactions to chromium was assessed by the consulting physician: "current relevance" was registered when a patient presented with a dermatitis reaction in combination with a history of current skin exposure to a source of chromium; "past relevance" was registered when a patient had a positive patch test reaction to chromium in combination with a history of a past dermatitis reaction caused by skin exposure to a source of chromium; and "unknown relevance" or "missing" was registered when patients had dermatitis and chromium allergy, and no relationship with current or past chromium exposure could be assessed, or no evaluation had been performed by the physician. Relevant causative exposures were based on the physician's notes in medical charts.

2.2 | Patch testing

Patch testing was performed with the European baseline series by the use of Trolab allergens (Hermal, Reinbek, Germany) and Allergeaze allergens from April 2016 with Finn Chambers (8 mm; Epitest, Tuusula, Finland) on Scanpor tape (Epitest). Dosing of the chamber was performed with 20 mg of the test preparation. Potassium dichromate 0.5% pet. was used for testing. Patch test readings were performed according to ESCD recommendations,[10] with an exposure time of 48 hours and readings on day (D) 2, D3 or D4, and D7. Patch test reactions designated as +, ++ or +++ were interpreted as positive reactions. Irritant responses and doubtful (?+) or negative readings were interpreted as negative responses.

2.3 | Statistical analysis

Data analysis was performed with SPSS for Windows (release 22.0). The χ^2 test of independence was used to test for associations between categorical variables, including age groups (18-40, 41-60 and >60 years), patch test year (2002-2005, 2006-2009, 2010-2013, and 2014-2017), MOAHLFA index, clinical relevance of positive reactions, and relevant sources of exposure (leather shoes, leather gloves, "other leather" exposure, chemicals, and tools). Fisher's exact test was applied when the expected value in any of the cells of the contingency table was <5. As the registration of foot dermatitis first began in 2004, we were not able to estimate its prevalence during the initial study period of 2002 to 2005. Linear-to-linear association tests and χ^2 trend tests were used for trend analysis across test years.

Furthermore, a logistic regression model was applied with "chromium allergy" as the dependent dichotomous categorical variable, and with sex, patch test year, age group, AD, foot dermatitis, hand eczema and face dermatitis as independent categorical variables. Interactions between the main covariates were evaluated with the Wald test. All results were expressed as adjusted odds ratios (ORs). A significance level of 5% was applied, and 95% confidence intervals (CIs) were constructed.

3 | RESULTS

The baseline characteristics of the included dermatitis patients are shown in Table 1. A total of 13 379 (9013 women) adults aged 18 to 99 years were patch tested in 2002 to 2017. The mean age at diagnosis was 48.7 ± 16.8 years. Chromium allergy was found in 296 (2.2%) of all patients: in 96 (2.2%) men and in 200 (2.2%) women. In crude analyses, patients with chromium allergy were frequently aged >40 years (78.7% vs 67.6%, P = 0.00005), and were more frequently affected by foot eczema (31.8% vs 5.0%, P < 0.00001) and hand eczema (62.8% vs 37.6%, P < 0.00001). Face dermatitis was more common in dermatitis patients without chromium allergy (16.6% vs 24.0%, P = 0.003). Furthermore, adjusted regression analysis showed significant associations between chromium allergy and, respectively, foot eczema (OR = 7.4, 95%CI: 5.7-9.8), hand eczema (OR = 2.3, 95% CI: 1.8-3.0), and AD (OR = 1.5, 95%CI: 1.1-2.1). However, the significant association with AD disappeared (OR = 1.4, 95%CI: 0.8-2.4) when an interaction term was inserted between AD and test year (P = 0.02). Specifically, AD was more prevalent in chromium-allergic patients during the study period 2010 to 2013. Patients from the lowest age group, that is, 18 to 40 years, were less likely to have chromium allergy (OR = 0.5, 95%CI: 0.3-0.7) than patients in the age group >60 years. Furthermore, dermatitis patients from the study period 2002 to 2005 were more likely to have chromium allergy than those from the study period 2014 to 2017 (OR = 2.2, 95%CI: 1.6-3.1). No significant association was found with sex or face dermatitis in regression analysis. We found no interaction between test year and foot eczema (P = 0.08) or sex (P = 0.8).

Table 2 shows the prevalence of chromium allergy in both sexes stratified by age group and test year. The prevalence was similar in men and women aged 41 to 60 years (2.7% vs 2.6%, P = 0.7), in men

TABLE 1 Baseline characteristics of included dermatitis patients (n = 13 379) during 2002 to 2017 at Gentofte, Denmark

Characteristics	Total	Patients with chromium allergy	Patients without chromium allergy	*P*-value*
All patients, % (n)	100 (13 379)	2.2 (296)	97.8 (13 083)	-
Age ± SD (years)	48.7 ± 16.8	52.8 ± 15.1	48.8 ± 16.9	-
Male, % (n)	32.6 (4366)	32.4 (96)	32.6 (4270)	-
Female, % (n)	67.4 (9013)	67.6 (200)	67.4 (8813)	-
Age > 40 y, % (n)	67.8 (9072)	78.7 (233)	67.6 (8839)	**0.00005**
Foot eczema, % (n)	5.6 (743)	31.8 (94)	5.0 (649)	**<0.00001**
Hand eczema, % (n)	38.2 (5111)	62.8 (186)	37.6 (4925)	**<0.00001**
Atopic dermatitis, % (n)	17.6 (2361)	20.9 (62)	17.6 (2299)	0.1
Facial dermatitis, % (n)	23.8 (3184)	16.6 (49)	24.0 (3135)	**0.003**
Occupational dermatitis, % (n)	18.7 (2496)	17.9 (53)	18.7 (2443)	0.7

The bold values represent the level of significance is 0.05 (5%).
*χ^2 test comparing dermatitis patients with and without chromium allergy.

TABLE 2 The prevalence of chromium allergy among 13 379 adult dermatitis patients stratified by age group and patch test year

Characteristic[a]	Total, % (n)	Men, % (n)	Women, % (n)
All patients	100 (13379)	32.6 (4366)	67.4 (9013)
Chromium allergy	2.2 (296)	2.2 (96)	2.2 (200)
Age group (years)			
18-40	1.5 (66/4456)	1.1 (15/1325)	1.6 (51/3131)
41-60	2.6 (142/5442)	2.7 (49/1801)	2.6 (93/3641)
>60	2.5 (88/3481)	2.6 (32/1240)	2.5 (56/2241)
Year of patch test			
2002-2005	3.2 (99/3136)	2.9 (32/1112)	3.3 (67/2024)
2006-2009	2.3 (62/2671)	2.7 (24/903)	2.1 (38/1768)
2010-2013	1.9 (68/3665)	1.7 (19/1103)	1.9 (49/2562)
2014-2017	1.7 (67/3907)	1.7 (21/1248)	1.7 (46/2659)

[a] No significant difference was found by sex.

and women aged >60 years (2.6% vs 2.5%, $P = 0.9$), and in the youngest age group of 18 to 40 years (1.1% vs 1.6%, $P = 0.2$, respectively). The overall prevalence of chromium allergy decreased from 2002 to 2017 ($P_{trend} = 0.00002$) (Figure 1). Specifically, a decreasing trend was seen from 3.2% in 2002 to 2005 to 1.7% in 2014 to 2017 ($P_{trend} < 0.0001$), and a decreasing trend was also observed from 2002 to 2005 to 2010 to 2013 ($P_{trend} = 0.002$). Table 3 shows the different clinical variables stratified by test year in dermatitis patients with chromium allergy. The prevalence of foot dermatitis decreased after a peak during 2006 to 2009 (2014-2017 vs 2006-2009, $P_{trend} = 0.01$). Regarding AD, an increasing trend was found during 2010 to 2013 as compared with 2002 to 2005 ($P_{trend} = 0.003$) and 2006 to 2009 ($P_{trend} = 0.005$). After this, the prevalence of AD remained stable. Regarding present or past clinical relevance, an increasing trend was noted for 2010 to 2013 as compared with 2002 to 2005 ($P_{trend} = 0.005$).

Table 4 shows the characteristics of all patients with chromium allergy. Whereas occupational dermatitis more frequently occurred in men with chromium allergy (29.2% vs 12.5%, $P = 0.0005$), AD was significantly more common in women with chromium allergy than in men (25.5% vs 11.5%, $P = 0.005$). Dermatitis was most often localized on the hands (62.8%), the feet (32.4%), and the face (16.6%). The clinical relevance of positive patch reactions to chromium was generally high, as current or past relevance was registered in 56.1% and 32.4% of patients, respectively. There was no significant difference between men and women regarding the clinical relevance of positive chromium test reactions. Leather was the most common relevant source of exposure in our patients (47.6%); leather shoes (36.8%), leather gloves (12.8%), and "other leather" items (7.4%), including furniture, watch straps, car wheels, and clothing. Leather gloves were more common sources of exposure in men than in women (24.1% vs 5.4%, $P < 0.00001$). Table 5 shows the sources of chromium exposure in dermatitis patients from 2002 to 2009 as compared with those from 2010 to 2017. The relative proportion of leather exposures as the cause of chromium allergy increased significantly from 42.3% during 2000 to 2009 to 54.8% during 2010 to 2017 ($P = 0.04$); leather shoe exposures increased from 27.8% to 40.0% ($P = 0.02$), leather glove exposures increased from 9.3% to 15.6% ($P = 0.1$), and "other leather" exposures increased from 2.5% to 13.3% ($P = 0.00008$). The proportion of unknown exposure sources decreased significantly from 57.1% to 44.4% ($P = 0.03$).

4 | DISCUSSION

This cross-sectional study showed that the overall prevalence of chromium allergy decreased for adult male and female dermatitis patients

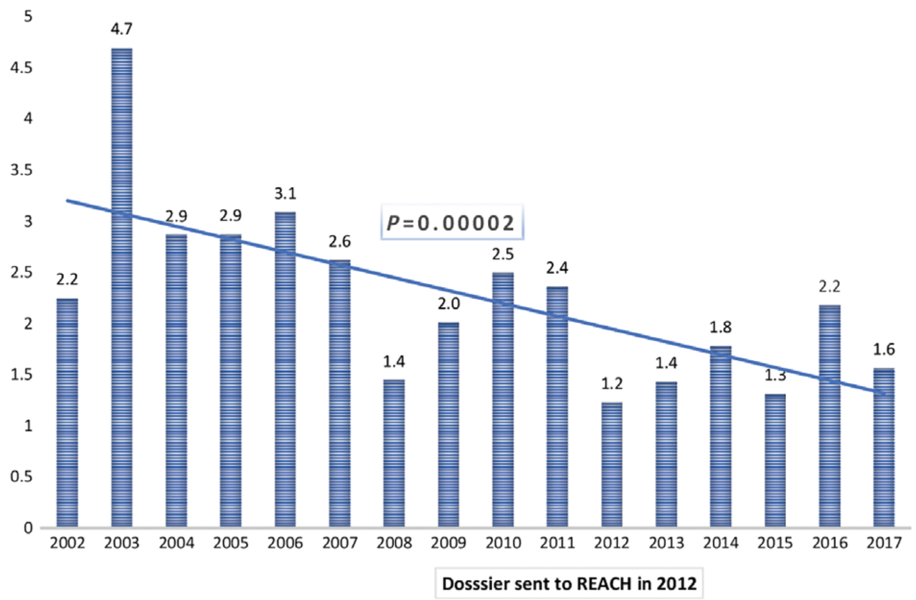

FIGURE 1 Prevalence of chromium allergy in dermatitis patients during 2002 to 2017. Decreasing trends were found for the study periods 2010 to 2013 vs 2002 to 2005 (P_{trend} = 0.002) and 2014 to 2017 vs 2002 to 2005 (P_{trend} < 0.0001)

TABLE 3 Profile of dermatitis patients with chromium allergy stratified by patch test year (n = 296)

Characteristics	2002-2005, % (n)	2006-2009, % (n)	2010-2013, % (n)	2014-2017, % (n)	P_{trend}[a]
Age group (years)					
18-40	22.2 (22/99)	19.4 (12/62)	29.4 (20/68)	17.9 (12/67)	0.9
41-60	44.4 (44/99)	54.8 (34/62)	44.1 (30/68)	50.7 (34/67)	0.6
>60	33.3 (33/99)	25.8 (16/62)	26.5 (18/68)	31.3 (21/67)	0.7
Men	32.3 (32/99)	38.7 (24/62)	27.9 (19/68)	31.3 (21/67)	0.6
Women	67.7 (67/99)	61.3 (38/62)	72.1 (49/68)	68.7 (46/67)	0.6
Hand eczema	54.5 (54/99)	74.2 (46/62)	69.1 (47/68)	58.2 (39/67)	0.5
Foot dermatitis[b]	-	51.6 (32/62)	41.2 (28/68)	29.9 (20/67)	**0.01**
Facial dermatitis	14.1 (14/99)	12.9 (8/62)	16.2 (11/68)	23.9 (16/67)	0.1
Atopic dermatitis	14.1 (14/99)	12.9 (8/62)	33.8 (23/68)	25.4 (17/67)	**0.009**
Occupational dermatitis	12.1 (12/99)	24.2 (15/62)	22.1 (15/68)	16.4 (11/67)	0.4
Clinical relevance[c]	58.6 (58/99)	71.0 (44/62)	79.4 (54/68)	73.1 (49/67)	**0.01**

The bold values represent the level of significance is 0.05 (5%).
[a] Across the entire study period.
[b] The registration of foot dermatitis started in 2004.
[c] Present or past relevance.

during 2002 to 2017 (Table 2). Notably, a decreasing trend was observed for foot dermatitis during the most recent years (2014-2017), indicating that the frequency of clinical disease has also been reduced (Table 4). In the remaining cases of chromium allergy, relevance increased and leather was still the single most important cause of ACD.

The decreasing trend of chromium allergy is noteworthy, as it probably reflects the effect of the regulatory initiatives restricting the content of hexavalent chromium to a maximum of 3 ppm in leather articles and articles containing leather parts used by consumers or workers. Historically, allergic chromium dermatitis mainly affected the hands of construction workers because of cement exposure, resulting in a high degree of occupational disability.[11] However, prompted by a highly effective regulatory intervention in 2005 by the EU,[12] preceded by Denmark in 1983[13] and Sweden in 1989, the addition of ferrous sulfate to cement became compulsory to reduce the amount of water-soluble hexavalent chromium to no more than 2 ppm. We found significantly decreasing trends in the prevalence of chromium allergy for the study periods 2010 to 2013 and 2014 to 2017 in our patients as compared with previous years. The decreasing trend during the last period is likely to be a consequence of the adoption and enforcement of the new EU regulatory directive against leather during 2014 to 2015. The EU regulation on leather is expected to be 80%[7] effective in reducing the incidence of hexavalent chromium ACD, according to the regulatory text. However, an allergic response may be elicited at lower concentrations than the present limit of 3 ppm in already sensitized individuals.[14] This highlights the need for sustained efforts to reach the ultimate goal of complete removal of hexavalent

TABLE 4 Characteristics of 296 adults with chromium allergy during 2002 to 2017 stratified by sex

Variable	Total, % (n)	Men, % (n)	Women, % (n)	*P*-value*
Clinical profile				
Males	32.4 (96)	-	-	-
Occupational dermatitis	17.9 (53)	29.2 (28)	12.5 (25)	**0.0005**
Atopic dermatitis	20.9 (62)	11.5 (11)	25.5 (51)	**0.005**
Hand dermatitis	62.8 (186)	65.6 (63)	61.5 (123)	0.5
Leg dermatitis	6.1 (18)	5.2 (5)	6.5 (13)	0.7
Facial dermatitis	16.6 (49)	13.5 (13)	18.0 (36)	0.3
Age > 40 years	78.7 (233)	85.4 (82)	75.5 (151)	0.05
Foot dermatitis	32.4 (96)	33.3 (32)	32.0 (64)	0.8
Relevance of positive patch test reactions				
Current relevance	56.1 (166)	61.5 (59)	53.5 (107)	0.2
Past relevance	32.4 (96)	30.2 (29)	33.5 (67)	0.6
Unknown relevance	30.7 (91)	36.6 (59)	23.7 (32)	**0.02**
Relevant exposures				
Leather shoes	36.8 (109)	32.3 (31)	39.0 (78)	0.3
Leather gloves	12.8 (38)	26.0 (25)	6.5 (13)	**<0.00001**
Other leather exposure[a]	7.4 (22)	7.3 (7)	7.5 (15)	0.9
Chemicals	1.4 (4)	3.1 (3)	0.5 (1)	0.1

The bold values represent the level of significance is 0.05 (5%).
*χ^2 test comparing men and women.
[a]Furniture, watch straps, car wheels, and clothing.

TABLE 5 Sources of chromium exposure in dermatitis patients with chromium allergy during 2002 to 2009 and 2010 to 2017 at Gentofte Hospital, Denmark

Exposure sources	2002-2009 (N = 161), % (n)	2010-2017 (N = 135), % (n)	*P*-value[a]
Leather shoes	34.8 (56)	37.8 (51)	0.6
Leather gloves	9.3 (15)	15.6 (21)	0.1
Other leather sources[a]	2.5 (4)	13.3 (18)	**0.0004**
Leather (total)	42.3 (69)	54.8 (74)	**0.04**
Other sources of chromium	1.9 (3)	4.4 (6)	0.3
Unknown	57.1 (92)	44.4 (60)	**0.03**

The bold values represent the level of significance is 0.05 (5%).
**P*-value of χ^2 test comparing 2000 to 2009 and 2010 to 2017.
[a]Furniture, watch straps, car wheels, and clothing.

chromium from leather. Interestingly, no effect of regulatory interventions on leather was noted in the latest report by the European Surveillance System on Contact Allergies during the study period 2013 to 2014, including 31 689 patients from 12 European countries,[15] as a largely stable prevalence of chromium allergy (3.2%) was found as compared with a similar analysis in 2004. However, the sample of participating European departments was only partly identical with that used in the 2004 analysis. The decreasing trend observed in our patients already began during 2010 to 2013, more specifically in 2012, and could be explained by proactive producers and independent national initiatives against chromium before the collective implementation by the EU during 2014 to 2015. In line with this, the Federal Institute for Risk Assessment in Germany already proposed restricting the content of hexavalent chromium in leather to a maximum of 3 ppm in 2007 (http://www.bfr.bund.de/cd/9575, last accessed August 18, 2018). Such initiatives might already have affected both domestic and foreign markets years before the EU regulation, favouring the use of chromium-free tanning and other available techniques to considerably reduce or even completely remove hexavalent chromium from leather. Interestingly, the North American Contact Dermatitis Group (NACDG) reported a decrease in the prevalence of chromium allergy from 4.8% during 2005 to 2006 to 1.6% and 2.2% during 2011 to 2012 and 2013 to 2014, respectively.[16] The participating departments in the NACDG were only partly identical over the years, introducing a potential bias. However, this decrease has been partially ascribed to secondary effects of chromium regulation in other countries, as no regulation on chromium salts in leather yet exists in North America. Nevertheless, the prevalence of chromium allergy remains high in countries in which no regulation regarding this metal yet exists.[17–19]

We observed no cases of chromium allergy resulting from cement exposure during the study period; instead, we found a relative increase in leather exposures as a cause of chromium allergy, from 42.3% to 54.8%, during the study periods 2002 to 2009 and 2010 to 2017, respectively (P = 0.04) (Table 5). This increase seemed to be explained by a marked increase in exposures from "other leather" items (P = 0.0004). The latter finding might also explain the significant proportional decrease in "unknown sources of exposure" between these two study periods (57.1% vs 44.4%, P = 0.03). Furthermore, the overall proportion of "unknown sources of exposure" during 2002 to 2017 was high (51.4%), emphasizing the need for continued registration and search for potentially new sources of exposure causing chromium allergy.

In an extensive systematic review and meta-analysis regarding the association between AD and contact allergy, Hamann et al[20] reported a significant association between chromium allergy and AD

(OR = 1.3, 95%CI: 1.0-1.6). In our regression model, interaction analysis showed that AD was more prevalent in chromium-allergic patients than in those without chromium allergy only during 2010 to 2013. We have no explanation for this, and it might be a chance finding.

Concerning strengths and limitations, the relative increase in leather as a source of exposure might have been partially biased by an increased focus on leather as the main source among our physicians. We were unable to estimate the prevalence of foot dermatitis in chromium-allergic patients during 2002 to 2005, as its registration in the database began in 2004. Strengths of this study include the standardized and similar reading techniques for patch test reactions throughout the entire study period. Other strengths include the large number of dermatitis patients included, the unchanged patch test methods over the years, the wide timespan, facilitating trend analysis, and the fact that clinical disease was diagnosed by physicians.

5 | CONCLUSION

This study shows that the overall prevalence of chromium allergy, and of foot dermatitis, among chromium-allergic individuals has decreased significantly in recent years. This sudden change strongly suggests a positive effect of the regulatory discussion and initiatives against hexavalent chromium in leather goods. Future studies, both nationally and internationally, are required to continuously monitor the effect of regulatory directives.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

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8.2 No immediate effect of regulatory reduction of chromium in leather among adult chromium allergic patients

No immediate effect of regulatory reduction of chromium in leather among adult chromium allergic patients

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Conflict of interests

None declared.

Abstract

Background
In March 2014, the European Commission issued a new regulation restricting the content of hexavalent chromium in leather to no more than 3 mg/kg. We previously performed a questionnaire study in January 2014 to characterize our patients with chromium contact allergy prior to regulatory intervention.

Objectives
To assess whether clinical characteristics, self-reported sources of chromium exposure and burden of disease changed in chromium allergic patients over time.

Methods
A questionnaire study was sent to 172 adult dermatitis patients with chromium allergy and 587 age- and sex-matched dermatitis patients without chromium allergy. Questionnaires were sent to all dermatitis patients patch-tested from 2003 to 2018 on August 2019.

Results
The overall response rate was 61.2% (759/1241). Patients with chromium allergy were still more commonly affected by current foot dermatitis [odds ratio (OR)=3.82, 95% confidence interval (CI): 2.07-7.08] and hand dermatitis (OR=1.98, 95% CI: 1.13-3.49) compared to controls during 2013-2018. The proportion of chromium allergic patients reporting dermatitis caused by leather exposure did not change during 2003-2012 versus 2013-2018 (71.0% vs. 66.2%, p=0.5). Furthermore, estimates on occupational performance and disease-severity, e.g. current dermatitis, number of anatomical locations with dermatitis, worst-case dermatitis and effect on work were similar in patients with chromium allergy for 2003-2012 versus 2013-2018.

Conclusion
No immediate sign of improvement was found in patients with chromium allergy concerning severity of disease and dermatitis from leather exposures five years after adoption of regulation against hexavalent chromium in leather. The regulation may have to be revised for better protection of those already sensitized.

Key words: Allergic contact dermatitis, chromium, disease-severity, leather, regulation

Introduction

Historically, the most common cause of chromium (Cr) sensitization has derived from skin contact with hexavalent Cr [Cr (VI)] in wet cement, primarily affecting construction workers.[1] Prompted by a highly effective regulatory intervention in 2005 by EU,[2] preceded by Sweden in 1989 and Denmark in 1983,[3] the addition of ferrous sulphate to wet cement became compulsory to reduce the amount of water-soluble Cr (VI) to no more than 2 ppm. Subsequently, numerous studies have reported a substantial decline in cases of Cr allergic contact dermatitis (ACD) due to cement.[4, 5] Currently, leather is considered the most common source of exposure in patients with Cr-induced ACD.[6] In May 2015, a regulation was enforced by EU restricting the content of Cr (VI) to no more than 3 ppm in leather articles coming into contact with the skin.[7] In a Danish questionnaire case-control study conducted prior to the regulation, including 121 cases with Cr-induced ACD and 443 age- and sex-matched dermatitis patients acting as controls during 2003-2012, respectively, 66.1% and 12.6% reported a history of dermatitis from leather exposure.[8] Furthermore, Cr allergic patients had a lower quality of life and were more burdened by dermatitis compared to the control group.

In the preamble of the regulation, it was foreseen to be 80% effective in reducing the incidence of ACD due to Cr (VI) in leather.[7] A recent study showed a significantly decreasing trend in the prevalence of Cr allergy for all exposures during 2002-2017,[6] which might be an effect of the regulation on leather. In this questionnaire study we aimed at assessing whether an improvement occurred in self-reported measures of disease-severity and leather exposures leading to dermatitis in Cr allergic patients diagnosed during 2003-2018.

Materials and methods

Study population

From 1 January 2003 to 31 December 2018, a total of 12,792 adults aged 18 to 99 with suspected ACD were patch-tested at the Department of Dermatology and Allergy, Herlev-Gentofte Hospital, Copenhagen, Denmark. All adult patients (≥ 18 years) with a positive patch test reaction to potassium dichromate (0.5% pet.) were age- and sex-matched in a 1:4 ratio to a control group of dermatitis patients with a negative patch test reaction to potassium dichromate (0.5% pet.), but possibly positive test reactions to other allergens. All data were retrieved from the National Database for Contact Allergy in Denmark. Their home addresses were obtained from the Danish central personal register which is a unique register of social information and health services.[9] Patients were excluded if they did not wish to be contacted for research purposes, had unknown addresses or were no longer alive. Permission was obtained from The Danish Data Protection Agency (VD-2018-132/I-Suite number: 6375).

Patch testing

Patch testing was performed with the European baseline and extended patch test series [Trolab allergens (Hermal, Reinbek, Germany) and Allergeaze allergens (SmartPractice, Canada) since April 2016)] with Finn Chambers® (8 mm; Epitest Ltd, Oy, Finland) on Scanpor® tape (Norgesplaster A/S, Alpharma, Vennesla, Norway). Dosing of the chamber was performed with 20 mg of the test preparation. Potassium dichromate (0.5% pet.) was used for testing. Patch test readings were performed according to the recommendations of the European Society of Contact Dermatitis (ESCD),[10] with an exposure time of 2 days and readings being performed on day 2, 3 or 4, and 7. Patch test reactions designated as 1+, 2+ or 3+ were interpreted as positive reactions. Irritant responses and doubtful (+?) or negative readings were interpreted as negative responses.

Questionnaire

We used the same questionnaire as in the previous study[8] (supplementary material) to identify differences between the study groups regarding clinical characteristics (age, sex, atopic dermatitis (AD), initial location of dermatitis, current location of dermatitis and the presence of other contact allergies), sources of exposure leading to dermatitis at present workplace, previous workplace or in spare-time (leather, jewellery, work tools, metalwork, cement, word preservation and mobile phone) and burden of disease [impact on occupational performance, medical needs over the last 12 months, personal perception of disease-severity ('current dermatitis', 'worst-case dermatitis', 'effect on work' and 'effect on spare-time') on a visual analogue scale (VAS) and an estimate of their quality of life using the Dermatology Life Quality Index (DLQI)]. The DLQI score was estimated according to published instructions resulting in a score between 0 and 30, with higher scores indicating a lower quality of life.[11] The validated official version in Danish was used. Permission to use the scale was obtained from http://www.cardiff.ac.uk/dermatology. Furthermore, a diagnosis of AD was defined according to the UK working party's diagnostic criteria.[12] Thus, the patient must have had an itchy skin condition during the past 12 months plus three or more of the following: (i) onset before the age of 2 years, (ii) a history of flexural involvement, (iii) a history of generally dry skin, and (iv) a personal history of other atopic diseases. Questionnaires were sent to all dermatitis patients patch-tested during 2003-2018 on August 2019; 4 weeks later, a reminder was sent to the non-respondents and the study was closed for entry after another 4 weeks.

Data management

Data-analysis was done using the Statistical Products and Service Solution package (SPSS) for Windows (release 22.0). We stratified the respondents into two study cohorts; those patch-tested between 2003-2012 and those tested between 2013-2018. This was done due to our previous study including patients during 2003-2012, but also as the regulation of Cr (VI) in leather was in a hearing in 2012, prior to adoption (2014) and implementation (2015). The χ^2-test of independence - or Fischer's exact test when the expected value in any of the cells of the contingency table was below 5 - was used to compare groups. All results are presented as odds ratios (OR) with 95% confidence intervals (CI). As DLQI data and VAS scores followed a non-parametric distribution, the Mann-Whitney *U*-test was performed to determine differences between groups and the results were presented as median values with an interquartile range (IQR). The Jonckhkeere-Terpstra test was used for trend analysis of non-parametric continuous variables whereas the linear-to-linear χ^2-test was used for trend analysis of categorical variables. Two-sample t-tests were used for normal data. Testing of data for normality was done with the Shapiro-Wilk test.

The level of statistical significance was set at α=0.05 for a single test. As a large number of independent tests (m=108) were performed, the Benjamini-Hochberg (BH) method was applied to control the type I error rate and to generate adjusted p-values.[13] The BH-method uses the false discovery rate (FDR) - i.e. expected proportion of false-positives among all significant results - to deflate the type I error rate. In the BH-method, p-values from all tests performed are ranked in an ascending order and adjusted p-values are calculated using the formula (I/m)*FDR, where I denotes the rank of a p-value and m the total number of tests performed. The FDR was set to 5%.

Results

The overall response rate was 61.2% (759/1241), including 72.6% (172/237) in the case group and 58.5% (587/1004) in the control group. Female patients were the predominant gender comprising 68.6% of the respondents.

Characteristics of Cr allergic patients over time

The mean age of Cr allergic patients diagnosed during 2003-2012 versus 2013-2018 was 50.0 +/- 11.3 years and 56.0 +/- 14.7 years (p=0.003) (table 1). The presence of other contact allergies than Cr was comparable in Cr allergic patients during 2003-2012 versus 2013-2018 (63.6% vs. 72.3%, p=0.2). No significant difference was found regarding the self-reported prevalence of hand dermatitis (72.9% vs. 70.8%, p=0.9) or foot dermatitis (54.2% vs. 50.8%, p=0.8) as the initial location of dermatitis comparing Cr allergic patients during 2003-2012 versus 2013-2018. Similarly, no difference was recorded in the prevalence of current hand dermatitis (72.0% vs. 63.1%, p=0.2) or foot dermatitis (46.0% vs. 40.0%, p=0.4).

Regarding the initial location of dermatitis, Cr allergic patients were more often affected by hand dermatitis (OR=2.54, 95% CI: 1.58-4.09, p<0.001) and foot dermatitis (OR=4.78, 95% CI: 3.02-7.58, p<0.001) compared to controls during 2003-2012. A similar case-control pattern was found during 2013-2018 for initial hand dermatitis (OR=2.36, 95% CI: 1.30-4.27, p<0.001) and initial foot dermatitis (OR=5.18, 95% CI: 2.85-9.43, p<0.001). Furthermore, Cr allergic patients more often reported current hand dermatitis (OR=3.52, 95% CI: 2.20-5.64, p<0.001) and current foot dermatitis (OR=5.80, 95% CI: 3.56-9.46, p<0.001) compared to controls during 2003-2012. The corresponding estimates during 2013-2018 were OR=1.98 (95% CI: 1.13-3.49, p<0.001) for hand dermatitis and OR=3.82 (95% CI: 2.07-7.08, p<0.001) for foot dermatitis.

Severity and QoL

DLQI scores and measures of disease severity presented as median (IQR) are summarized in Table 2. The median (IQR) DLQI score reported by Cr allergic patients during 2003-2012 versus 2013-2018 was 2.0 (0-7.0) and 2.0 (1.0-6.5), respectively (p=0.8). As regards personal perception of disease severity using VAS, comparisons of Cr allergic patients during 2003-2012 versus 2013-2018 did not show any significant difference concerning ‘current dermatitis’ [3.4 (1.0-5.6) vs. 2.4 (1.0-5.0), p=0.5], ‘worst-case dermatitis’ [9.0 (7.5-9.5) vs. 8.7 (7.4-9.5), p=0.2], ‘effect on work’ [5.0 (2.4-7.4) vs. 4.0 (1.8-7.1), p=0.2] and ‘effect on spare-time’ [5.1 (2.4-7.4) vs. 4.5 (1.7-6.1), p=0.1]. Additionally, we found no difference with respect to the initial [2.0 (1.0-3.0) vs. 3.0 (1.0-4.0), p=0.13] or current number [2.0 (1.0-3.0) vs. 2.0 (1.0-3.0), p=0.92] of anatomical locations with dermatitis in Cr allergic patients through 2003-2012 versus 2013-2018. Trend analysis of Cr allergic patients across 2013-2018 did not show any change regarding DLQI scores (p_{trend}=0.8), ‘current dermatitis’ (p_{trend}=0.05), ‘worst-case dermatitis’ (p_{trend}=0.9), ‘effect on work’ (p_{trend}=0.2) and ‘effect on spare-time’ (p_{trend}=0.7).

Compared to controls, Cr allergic patients during 2003-2012 reported a significantly higher DLQI score [2.0 (0-7.0) vs. 1.0 (0-3.0), p<0.001], while no difference was found for the corresponding case-control comparison during 2013-2018 (p=0.08). Compared to controls, Cr allergic patients during 2003-2012 reported more severe disease with respect to ‘current dermatitis’ [3.4 (1.0-5.6) vs. 1.8 (0.5-4.7), p=0.002], ‘worst-case dermatitis’ [9.0 (7.5-9.5) vs. 8.0 (6.5-9.4), p<0.001], ‘effect on work’ [5.0 (2.4-7.4) vs. 3.7 (0.8-6.6), p=0.007] and ‘effect on spare-time’ [5.1 (2.4-7.4) vs. 3.9 (1.1-6.1), p=0.002]. However, no difference was found for the latter-mentioned measures of severity comparing cases and controls during 2013-2018.

Occupational status

No significant difference was found comparing Cr allergic patients during 2003-2012 versus 2013-2018 regarding 'loss of job' (7.5% vs. 6.2%, p=1.0), 'change of job' (19.6% vs. 13.8%, p=0.3) or 'sick-leave from job' (28.0% vs. 16.9%, p=0.1) (Table 3). Compared to controls, Cr allergic patients during 2003-2012 reported more 'sick-leave from job' (OR=2.35, 95% CI: 1.40-3.93, p=0.001) whereas no case-control difference was found during 2013-2018.

Relevant exposure sources

Table 4 presents self-reported sources of exposure leading to dermatitis. As shown, leather exposure causing dermatitis was significantly more common in cases compared to controls within both study periods ($OR_{2003\text{-}2012}$=13.51, 95% CI: 8.14-22.42, p<0.001 and $OR_{2013\text{-}2018}$=17.51, 95% CI: 8.95-34.23, p<0.001). Leather exposure as a causative factor of dermatitis was reported by similar proportions of Cr allergic patients diagnosed during 2003-2012 versus 2013-2018 (71.0% vs. 66.2%, p=0.5). Additionally, an unchanged trend (p_{trend}=0.7) was found for self-reported leather exposure causing dermatitis in patients with Cr allergy during 2013-2018. In contrary to 2003-2012, exposure to metalwork as a cause of dermatitis was more common in cases compared to controls during 2013-2018 (OR=3.23, 95% CI: 1.44-9.96, p=0.008).

Discussion

Main findings

In this post-regulatory questionnaire study, examination of Cr allergic patients over time did not show any significant change in perception of disease-severity nor in the proportion of self-reported leather exposures causing dermatitis.

Interpretation

In this study, we did not find a decline in the occurrence of foot dermatitis – nor hand dermatitis or other locations – in Cr allergic patients over time. Apart from reducing the occurrence of new cases of Cr-induced ACD, it is likewise pertinent to evaluate if the EU regulative has entailed an improvement in perception of disease-severity in patients with Cr allergy over time. It is conceivable that restricted levels of Cr (VI) in leather articles on the market might result in less disease-experience in already sensitized patients. However, we did not find any significant change in measures of disease-severity comparing Cr allergic patients diagnosed during 2003-2012 with those diagnosed during 2013-2018 (Table 2). In spite of this, we did notice similar levels in personal perception of disease-severity comparing cases and controls during 2013-2018 as opposed to 2003-2012 (Table 2). The latter observation might suggest a diminished burden of disease in the most recently diagnosed Cr allergic patients. However, an increasing level of disease-burden in controls might explain the lack of case-control difference observed which might partly be due to the methylisothiazolinone epidemic outbreaking in 2010, peaking in 2013.[14] The lack of improvement in disease-severity over time may also be attributed to the tertiary selection of our study population, hence mainly capturing the most severe cases of Cr allergy. Nevertheless, our Cr allergic patients reported very low levels of DLQI yielded by a median DLQI score of 2.0 during 2003-2012 and 2013-2018 (Table 2).

The self-reported prevalence of leather exposure as a causative factor of dermatitis was comparable for Cr allergic patients during 2003-2012 versus 2013-2018 (71.0% vs. 66.2%, p=0.5); and is further supported by an unchanged trend in the self-reported proportion of leather exposures causing dermatitis in Cr allergic patients across 2013-2018 (p_{trend}=0.7). This is noteworthy as it might suggest that Cr-induced ACD due to leather exposure remains a substantial issue among those with Cr allergy despite legislative actions against Cr (VI) in leather. In a recent market survey on leather articles performed by The Environmental Protection Agency in Denmark, 10 out of 94 leather samples (10.6%) exceeded the limit value of 3 mg/kg. The highest measured values (11 mg/kg-28 mg/kg) were found in handbags.[15] Despite a markedly lower proportion of leather articles on the Danish market exceeding 3 mg/kg Cr (VI) compared to a market survey from 2009 (44%),[16] these levels might partially explain the insignificant differences found regarding dermatitis from leather exposure and severity of disease comparing cases during 2003-2012 and 2013-2018. Other factors include changing fashion trends, leather articles bought outside the EU and second-hand leather articles in end-use before 1 May 2015 which are not covered by the regulation. Furthermore, previous dose-response patch test studies have reported a 10% minimum elicitation threshold as low as 1 mg/kg for Cr (VI), hence elucidating the allergic potential of Cr (VI) levels lower than the regulatory limit value of 3 mg/kg.[17] Ideally, no Cr (VI) should be present in leather. However, the reference method EN ISO 17075 used for quantification has a detection limit of 3 mg/kg. Additionally, it is important to note that the regulation on leather targets Cr (VI), thus not covering the presence of trivalent Cr despite several studies suggesting its capability of eliciting ACD in patients with Cr allergy.[17-19] Furthermore, trivalent Cr present in the leather after tanning might undergo oxidation to Cr (VI) depending on various factors including pH, vegetable retanning, ammonia treatment, thermal and photo-ageing, use of fatliquors and reducing agents.[20] Despite a seemingly decreasing trend in the occurrence of Cr (VI) on the Danish market, more market studies with a wide-range inclusion of leather products accompanied by epidemiological studies are warranted to address the efficiency of the EU regulation, both regarding leather-marketing and patient outcomes.

Strength and limitations

Strengths of this study include the high response rate, inclusion of a sex- and age-matched control group and controlling the type I error rate. This study was limited by selection bias as our dermatitis patients were included from a tertiary clinic, hence potentially having more severe dermatitis compared to patients seen at the general practitioner or at a dermatological practice. Recall bias was of importance as well possibly entailing an underestimation of dermatitis appearing years ago. Lastly, this study was limited by a potential increase in disease-burden in patients without Cr allergy, thus not acting as an ideal reference group.

Conclusion

No immediate sign of improvement was found in patients with Cr allergy concerning severity of disease and dermatitis from leather exposures five years following adoption of regulation against Cr (VI) in leather.

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Table 1 – Baseline and clinical characteristics of the respondents (n=759).

Clinical characteristic	Cases 2003-2012 % (n=107)	Controls 2003-2012 % (n=358)	OR* (95% CI)	Cases 2013-2018 % (n=65)	Controls 2013-2018 % (n=229)	OR* (95% CI)	Cases 2003-2012 % (n=107)	Cases 2013-2018 % (n=65)	OR* (95% CI)
Age – mean (+/-SD)	50.0 (+/-11.3)	50.1 (+/-12.1)	-	56.0 (+/-14.7)	55.8 (+/- 13.0)	-	50.0 (+/-11.3)	56.0 (+/-14.7)	-
Sex – female	69.2 (74)	70.7 (253)	-	69.2 (45)	65.1 (149)	-	69.2 (74)	69.2 (45)	-
Atopic dermatitis	16.8 (18)	12.0 (43)	1.48 (0.81-2.70)	13.8 (9)	12.2 (28)	1.15 (0.52-2.59)	16.8 (18)	13.8 (9)	1.27 (0.53-3.03)
Other contact allergies - overall	64.0 (68)	33.8 (121)	**3.42 (2.18-5.36)**	72.3 (47)	33.6 (77)	**5.15 (2.81-9.47)**	64.0 (68)	72.3 (47)	0.67 (0.34-1.30)
Nickel	10.3 (11)	14.5 (52)	0.67 (0.34-1.34)	20.0 (13)	7.4 (17)	**3.12 (1.43-6.82)**	10.3 (11)	20.0 (13)	0.46 (0.19-1.10)
MCI/MI	4.7 (5)	2.0 (7)	2.46 (0.76-7.91)	9.2 (6)	3.9 (9)	2.49 (0.85-7.26)	4.7 (5)	9.2 (6)	0.48 (0.14-1.64)
Initial location of dermatitis									
Face	17.8 (19)	29.9 (107)	**0.51 (0.29-0.87)**	21.5 (14)	35.4 (81)	0.50 (0.26-0.96)	17.8 (19)	21.5 (14)	0.79 (0.36-1.69)
Hands	72.9 (78)	51.4 (184)	**2.54 (1.58-4.09)**	70.8 (46)	50.7 (116)	**2.36 (1.30-4.27)**	72.9 (78)	70.8 (46)	1.11 (0.56-2.22)
Feet	54.2 (58)	19.8 (71)	**4.78 (3.02-7.58)**	50.8 (33)	16.6 (38)	**5.18 (2.85-9.43)**	54.2 (58)	50.8 (33)	1.15 (0.62-2.13)
Other	57.0 (61)	68.7 (246)	0.60 (0.39-0.94)	73.8 (48)	71.6 (164)	1.12 (0.60-2.09)	57.0 (61)	73.8 (48)	0.47 (0.23-0.92)
Current location of dermatitis									
Face	18.7 (20)	23.5 (84)	0.75 (0.44-1.29)	13.8 (9)	31.4 (72)	**0.35 (0.16-0.75)**	18.7 (20)	13.8 (9)	1.43 (0.61-3.33)
Hands	72.0 (77)	42.2 (151)	**3.52 (2.20-5.64)**	63.1 (41)	46.3 (106)	**1.98 (1.13-3.49)**	72.0 (77)	63.1 (41)	1.49 (0.78-2.86)
Feet	46.7 (50)	13.1 (47)	**5.80 (3.56-9.46)**	40.0 (26)	14.8 (34)	**3.82 (2.07-7.08)**	46.7 (50)	40.0 (26)	1.32 (0.70-2.44)
Other	54.2 (58)	58.1 (208)	0.85 (0.55-1.32)	64.6 (42)	57.6 (132)	1.34 (0.76-2.38)	54.2 (58)	64.6 (42)	0.65 (0.34-1.22)

*: Chi-square or Fisher's exact test (if < 5). Significant results (FDR-adjusted) are illustrated in bold.

Table 2 – Dermatology life quality index (DLQI) and disease-severity.

	Cases 2003-2012 (n=107)	Controls 2003-2012 (n=358)	P-value*	Cases 2013-2018 (n=65)	Controls 2013-2018 (n=229)	P-value*	Cases 2003-2012 (n=107)	Cases 2013-2018 (n=65)	P-value*
DLQI score Median (IQR) Range	2.0 (0-7.0) 0-30	1.0 (0-3.0) 0-27	**<0.001**	2.0 (1.0-6.5) 0-27	2.0 (0-5.0) 0-27	0.08	2.0 (0-7.0) 0-30	2.0 (1.0-6.5) 0-27	0.78
Current dermatitis (VAS) Median (IQR) Range	3.4 (1.0-5.6) 0-10	1.8 (0.5-4.7) 0-10	**0.002**	2.4 (1.0-5.0) 0-9.7	2.9 (0.7-5.0) 0-10	0.96	3.4 (1.0-5.6) 0-10	2.4 (1.0-5.0) 0-9.7	0.45
Worst-case dermatitis (VAS) Median (IQR) Range	9.0 (7.5-9.5) 0.5-10	8.0 (6.5-9.4) 0-10	**<0.001**	8.7 (7.4-9.5) 0.2-10	8.2 (7.0-9.5) 0-10	0.32	9.0 (7.5-9.5) 0.5-10	8.7 (7.4-9.5) 0.2-10	0.18
Effect on work (VAS) Median (IQR) Range	5.0 (2.4-7.4) 0-10	3.7 (0.8-6.6) 0-10	**0.007**	4.0 (1.8-7.1) 0-10	4.5 (1.0-7.3) 0-10	0.97	5.0 (2.4-7.4) 0-10	4.0 (1.8-7.1) 0-10	0.22
Effect on spare time (VAS) Median (IQR) Range	5.1 (2.4-7.4) 0-10	3.9 (1.1-6.1) 0-10	**0.002**	4.5 (1.7-6.1) 0-10	4.0 (1.5-6.5) 0-10	0.86	5.1 (2.4-7.4) 0-10	4.5 (1.7-6.1) 0-10	0.12
Initial number of anatomical locations with dermatitis Median (IQR) Range	2.0 (1.0-3.0) 0-11	2.0 (1.0-3.0) 0-10	0.21	3.0 (1.0-4.0) 0-10	2.0 (1.0-4.0) 0-10	0.03	2.0 (1.0-3.0) 0-11	3.0 (1.0-4.0) 0-10	0.13
Current number of anatomical locations with dermatitis Median (IQR) Range	2.0 (1.0-3.0) 0-10	1.0 (1.0-2.0) 0-10	**<0.001**	2.0 (1.0-3.0) 0-10	1.0 (1.0-3.0) 0-10	0.14	2.0 (1.0-3.0) 0-10	2.0 (1.0-3.0) 0-10	0.92

IQR, interquartile range; VAS, visual analogue scale.
*: Mann-Whitney *U*-test. Significant results (FDR-adjusted) are illustrated in bold.

Table 3 – Occupational status and medical needs due to dermatitis during the last 12 months.

	Cases 2003-2012 % (n=107)	Controls 2003-2012 % (n=358)	OR* (95% CI)	Cases 2013-2018 % (n=65)	Controls 2013-2018 % (n=229)	OR* (95% CI)	Cases 2003-2012 % (n=107)	Cases 2013-2018 % (n=65)	OR* (95% CI)
Occupational status									
Loss of job	7.5 (8)	5.6 (20)	1.37 (0.58-3.20)	6.2 (4)	5.7 (13)	1.09 (0.34-3.46)	7.5 (8)	6.2 (4)	1.23 (0.36-4.35)
Change of job	19.6 (21)	12.0 (43)	1.79 (1.01-3.18)	13.8 (9)	7.0 (16)	2.14 (0.90-5.10)	19.6 (21)	13.8 (9)	1.52 (0.65-3.57)
Sick-leave from job	28.0 (30)	14.2 (51)	**2.35 (1.40-3.93)**	16.9 (11)	16.6 (38)	1.02 (0.49-2.14)	28.0 (30)	16.9 (11)	1.92 (0.88-4.17)
Medical needs due to dermatitis during the last 12 months									
General practitioner consultation	29.9 (32)	24.0 (86)	1.35 (0.84-2.18)	40.0 (26)	26.6 (61)	1.84 (1.03-3.27)	29.9 (32)	40.0 (26)	0.64 (0.33-1.22)
Dermatologist consultation	23.4 (25)	18.7 (67)	1.32 (0.79-2.23)	43.1 (28)	26.6 (61)	**2.08 (1.18-3.69)**	23.4 (25)	43.1 (28)	**0.40 (0.21-0.78)**
No treatment	18.7 (20)	34.4 (123)	**0.44 (0.26-0.75)**	6.2 (4)	22.7 (52)	**0.22 (0.08-0.64)**	18.7 (20)	6.2 (4)	3.45 (1.14-11.11)
Emollients	61.7 (66)	40.5 (145)	**2.37 (1.52-3.68)**	55.4 (36)	46.7 (107)	1.42 (0.81-2.46)	61.7 (66)	55.4 (36)	1.30 (0.69-2.44)
Topical corticosteroid	60.7 (65)	36.6 (131)	**2.68 (1.72-4.18)**	69.2 (45)	45.0 (103)	**2.75 (1.53-4.95)**	60.7 (65)	69.2 (45)	0.69 (0.36-1.32)
Systemic corticosteroid	12.1 (13)	3.1 (11)	**4.36 (1.89-10.05)**	13.8 (9)	2.6 (6)	**5.97 (2.04-17.48)**	12.1 (13)	13.8 (9)	0.86 (0.35-2.13)
Immunosuppressive drugs	3.7 (4)	4.7 (17)	0.78 (0.26-2.37)	16.9 (11)	5.2 (12)	**3.68 (1.54-8.80)**	3.7 (4)	16.9 (11)	**0.19 (0.06-0.63)**

*: Chi-square or Fisher's exact test (if n < 5). Significant results (FDR-adjusted) are illustrated in bold.

Table 4 – Sources of exposure causing dermatitis.

Self-reported exposure sources*	Cases 2003-2012 % (n=107)	Controls 2003-2012 % (n=358)	OR** (95% CI)	Cases 2013-2018 % (n=65)	Controls 2013-2018 % (n=229)	OR** (95% CI)	Cases 2003-2012 % (n=107)	Cases 2013-2018 % (n=65)	OR** (95% CI)
Leather	71.0 (76)	15.4 (55)	**13.51 (8.14-22.42)**	66.2 (43)	10.0 (23)	**17.51 (8.95-34.23)**	71.0 (76)	66.2 (43)	1.25 (0.65-2.44)
Work tools	13.1 (14)	2.0 (7)	**7.55 (2.96-19.24)**	9.2 (6)	3.1 (7)	3.23 (1.04-9.96)	13.1 (14)	9.2 (6)	1.47 (0.54-4.00)
Metalwork	8.4 (9)	4.2 (15)	2.10 (0.89-4.95)	16.9 (11)	5.7 (13)	**3.39 (1.44-7.97)**	8.4 (9)	16.9 (11)	0.45 (0.18-1.15)
Wood preservation	14.0 (15)	1.7 (6)	**9.57 (3.61-25-34)**	7.7 (5)	4.4 (10)	1.83 (0.60-5.54)	14.0 (15)	7.7 (5)	1.92 (0.68-5.56)
Cement	15.9 (17)	1.7 (6)	**11.08 (4.25-28.91)**	9.2 (6)	3.1 (7)	3.23 (1.04-9.96)	15.9 (17)	9.2 (6)	1.85 (0.69-5.00)

*: Present workplace', 'Earlier workplace' and 'In spare-time' combined.
**: Chi-square or Fisher's exact test (if < 5). Significant results (FDR-adjusted) are illustrated in bold.

8.3 Causative exposures and temporal development of cobalt allergy in Denmark between 2002 and 2017

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Causative exposures and temporal development of cobalt allergy in Denmark between 2002 and 2017

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Abstract

Background: Cobalt contact allergy is common, but clinical relevance is often difficult to determine.

Objectives: To examine the aetiology, prevalence and clinical characteristics of cobalt-allergic patients who were patch tested between 2002 and 2017 at the Department of Dermatology and Allergy, Herlev-Gentofte Hospital.

Methods: Patch test data, along with patient characteristics and causative exposures, from all adult dermatitis patients seen and tested between 2002 and 2017 were analysed. Associations were tested with the χ^2 test and logistic regression.

Results: A total of 13 475 adults aged 18 to 99 years were patch tested. The overall prevalence of cobalt allergy and the prevalence of isolated cobalt allergy were 3.3% and 1.5%, respectively. The prevalence of isolated cobalt allergy decreased from 2.4% in 2006 to 2009 to 1.1% in 2014 to 2017 (P_{trend} = 0.00003). Leather exposure as a relevant cause of allergic cobalt dermatitis increased from 3.7% in 2002 to 2009 to 8.3% in 2010 to 2017 (P = 0.04). The current clinical relevance of positive patch test reactions, that is, a positive reaction to cobalt combined with a history of current skin exposure to a source of cobalt, was 20.1%.

Conclusions: We conclude that cobalt allergy is relatively common, but causative exposures are largely unknown, and the proportion of positive patch test reactions with clinical relevance is low. It is therefore currently unclear how we can better protect consumers and workers from cobalt exposure.

KEYWORDS

cobalt, contact allergy, exposure, leather, prevalence, relevance, trend

1 | INTRODUCTION

Cobalt is a strong skin sensitizer,[1] and an estimated 5.9% and 7.4% of patch tested dermatitis patients in Europe and North America, respectively, are cobalt-allergic.[2,3] However, clinical relevance is often difficult to determine, and previous identification of occupational and consumer sources of cobalt seems to be insufficient. According to a Danish study, only 25% of positive patch test reactions to cobalt have clinical relevance, as exposure sources were largely unknown.[4]

Historically, occupational cobalt exposure has mainly been observed in metal workers, bricklayers, and pottery workers.[5] The hard metal industry is believed to represent the main source of occupational cobalt exposure, particularly in the EU and North America, as almost 15% of the worldwide production of cobalt is used for hard metal production.[6] Dental tools and alloys have also been reported to contain and release high levels of cobalt.[7] Consumer exposure sources have also been described, including jewellery and, recently, leather items.[8] Whereas piercing jewellery may indeed contain and release cobalt, a large study of inexpensive earrings showed that very few contained cobalt.[9] Also, a North American study showed that cobalt allergy was not independently associated with piercings.[10] We have observed single cases of allergic cobalt dermatitis caused by leather items,[11,12] but suspect that leather might be a more important source of cobalt exposure than hitherto realized. In this respect, a recent questionnaire case-control study from our clinic showed that leather was the most frequently reported exposure source causing dermatitis in patients with cobalt allergy without concomitant allergy to hexavalent chromium.[13]

In the present retrospective database study, we examined the development of cobalt allergy prevalence over a 16-year period, and investigated the causative exposures and clinical characteristics of cobalt-allergic dermatitis patients in our clinic.

2 | METHODS

2.1 | Study population

This study was performed in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.[14] Data were extracted from the National Database for Contact Allergy in Denmark. All adult patients (≥18 years) with suspected allergic contact dermatitis (ACD) who were patch tested January 2002 to December 2017 at the Department of Dermatology and Allergy, Herlev-Gentofte Hospital were included. Through a retrospective review of patient data regarding patch testing (test year, reading day and test results, that is, presence or absence of allergy to cobalt, nickel, or chromium), the clinical relevance of cobalt allergy (current, past, or unknown), MOAHLFA index (Male, Occupational dermatitis, history of Atopic dermatitis, Hand eczema, Leg dermatitis, Facial dermatitis, and Age > 40 years),[15] and sources of exposure were retrieved. Permission was obtained from The Danish Data Protection Agency.

2.2 | Definitions

The clinical relevance of positive patch test reactions to cobalt was assessed by the consulting physician: "current clinical relevance" was registered when a patient presented with a dermatitis reaction in combination with a history of current skin exposure to a source of cobalt; "past clinical relevance" was registered when a patient had a positive patch test reaction to cobalt in combination with a history of a past dermatitis reaction caused by skin exposure to a source of cobalt; and "unknown clinical relevance" or "missing" was registered in patients with dermatitis and cobalt allergy when no relationship with current or past cobalt exposure could be assessed, or when no registrations had been performed by the physician. Relevant causative exposures were based on the physician's notes in medical charts, on the use of spot tests, and, in selected cases, on chemical analysis. Isolated cobalt allergy was defined as a positive reaction to cobalt but negative patch test results with chromium and nickel. Cobalt allergy referred to a positive patch test reaction to cobalt independently from concomitant positive reactions to chromium, nickel or other metals, and allergens.

2.3 | Patch testing

Patch testing was performed with the European baseline series by the use of Trolab allergens (Hermal, Reinbek, Germany) and Allergeaze allergens after April 2016, with Finn Chambers (8 mm; Epitest, Tuusula, Finland) on Scanpor tape (Norgesplaster, Vennesla, Norway). Dosing of the chamber was performed with 20 mg of the test preparation. Cobalt chloride 1.0% pet. was used for testing during the entire period. Patch test readings were performed according to the recommendations of the European Society of Contact Dermatitis,[16] with an exposure time of 2 days and readings being performed on day (D) 2, D3 or D4, and D7. Patch test reactions designated as +, ++ or +++ were interpreted as positive reactions. Irritant responses and doubtful (?+) or negative readings were interpreted as negative responses.

2.4 | Statistical analysis

Data analysis was performed with SPSS for Windows (release 22.0). The χ^2 test of independence was used to test for associations between categorical variables. For small sample sizes (<5), Fisher's exact test was applied. Linear-to-linear association tests were used for trend analysis across test years. Two logistic regression models were applied with either "cobalt allergy" or "isolated cobalt allergy" as the dependent variable, and with sex, patch test year, age group, atopic dermatitis (AD), occupational dermatitis, foot dermatitis, hand eczema and facial dermatitis as explanatory variables. All results were expressed as adjusted odds ratios (aORs). A significance level of 5% was applied, and 95% confidence intervals (CIs) were determined.

3 | RESULTS

Baseline characteristics are shown in Table 1. In total, 13 475 (9085 women) adults with suspected ACD were included. The prevalence of cobalt allergy and the prevalence of isolated cobalt allergy were 3.3% and 1.5%, respectively. In crude data analyses, patients with cobalt allergy tended to be aged <40 years (62.4% vs 68.1%, P = 0.0004), and were more often affected by foot eczema (12.5% vs 5.5%, P < 0.00001), hand eczema (57.5% vs 37.8%, P < 0.00001) and occupational contact dermatitis (26.8% vs 18.4%, P < 0.00001) than those without cobalt allergy. Furthermore, patients with isolated cobalt allergy were more often affected by hand eczema (61.2% vs 37.8%, P < 0.00001), AD (23.3% vs 17.6%, P = 0.03) and occupational contact dermatitis (25.7% vs 18.4%, P = 0.007) than those without cobalt allergy.

Table 2 shows patients with isolated cobalt allergy stratified by sex, age group, and patch test year. Isolated cobalt allergy more frequently affected women than men in the age group 18 to 40 years (1.8% vs 1.1%, P = 0.007) and in the test years 2006 to 2009 (2.8% vs 1.4%, P = 0.02). Fully adjusted logistic regression showed that cobalt

TABLE 1 Baseline characteristics of included dermatitis patients (N = 13 475) in 2002 to 2017 at Gentofte, Denmark

Characteristics	Total	Patients with cobalt allergy	Patients with isolated cobalt allergy	Patients without cobalt allergy
All patients, % (n)	100 (13 475)	3.3 (447)	1.5 (206)	96.7 (13 028)
Age (y) ± SD	48.8 ± 16.8	46.2 ± 15.6	48.0 ± 16.1	48.9 ± 16.8
Male, % (n)	32.6 (4390)	20.8* (93)	23.8** (49)	33.0 (4297)
Female, % (n)	67.4 (9085)	79.2* (354)	76.2** (157)	67.0 (8731)
Age > 40 y, % (n)	67.9 (9156)	62.4* (279)	67.0 (138)	68.1 (8877)
Foot eczema, % (n)	5.7 (770)	12.5* (56)	8.3 (17)	5.5 (714)
Hand eczema, % (n)	38.4 (5177)	57.5* (257)	61.2** (126)	37.8 (4920)
Atopic dermatitis, % (n)	17.7 (2381)	19.5 (87)	23.3** (48)	17.6 (2294)
Facial dermatitis, % (n)	23.8 (3207)	23.5 (105)	29.1 (60)	23.8 (3102)
Occupational dermatitis, % (n)	18.6 (2511)	26.8* (120)	25.7** (53)	18.4 (2391)
Chromium allergy, % (n)	2.2 (296)	21.0* (94)	-	1.6 (202)
Nickel allergy, % (n)	10.9 (1467)	38.0* (170)	-	10.0 (1297)

*P < 0.05: χ^2 test comparing patients with and without cobalt allergy. **P < 0.05: χ^2 test comparing patients with isolated cobalt allergy and patients without cobalt allergy.

TABLE 2 The prevalence of isolated cobalt allergy among 13 475 adult dermatitis patients stratified by age group and patch test year

Characteristics	Total (N = 13 475), % (n)	Men (N = 4390), % (n)	Women (N = 9085), % (n)
Isolated cobalt allergy	1.5 (206)	1.1 (49)	1.7 (157)
Age group (y)			
18-40	1.6 (72/4469)	1.1 (14/1325)	1.8* (58/3144)
41-60	1.4 (79/5490)	1.2 (21/1815)	1.6 (58/3675)
>60	1.6 (55/3516)	1.1 (14/1250)	1.8 (41/2266)
Test year			
2002-2005	1.4 (45/3159)	1.3 (15/1116)	1.5 (30/2043)
2006-2009	2.4 (64/2698)	1.4 (13/907)	2.8* (51/1791)
2010-2013	1.5 (55/3691)	0.9 (10/1114)	1.7 (45/2577)
2014-2017	1.1 (42/3927)	0.9 (11/1253)	1.2 (31/2674)

*P < 0.05: χ^2 test comparing men and women.

allergy was significantly associated with female sex (aOR 2.1, 95%CI: 1.6-2.6), hand eczema (aOR 2.1, 95%CI: 1.6-2.6), foot eczema (aOR 2.1, 95%CI: 1.6-2.9), being aged 18 to 40 years (aOR 1.4, 95%CI: 1.0-1.9), and being tested in 2006 to 2009 (aOR 1.6, 95%CI: 1.2-2.0). When "isolated cobalt allergy" was inserted as the dependent variable, significant associations were found with female sex (aOR 1.6, 95%CI: 1.2-2.3), hand eczema (aOR 2.9, 95%CI: 2.1-4.0), facial eczema (aOR 1.6, 95%CI: 1.1-2.2), and being tested in 2006 to 2009 (aOR 2.2, 95% CI: 1.5-3.3).

No trend was found for the prevalence of cobalt allergy in 2002 to 2017 (P_{trend} = 0.1) (Figure 1A). Likewise, no change in the trend of cobalt allergy was found for either men (P_{trend} = 0.1) or women (P_{trend} = 0.3) across the entire study period. Regarding isolated cobalt allergy, we found a significantly decreasing trend from 2002 to 2017 (P_{trend} = 0.03) (Figure 1B). Hence, the prevalence decreased from 2.4% in 2006 to 2009 to 1.1% in 2014 to 2017 (P_{trend} = 0.00003); similarly, the prevalence decreased from 2.4% in 2006 to 2009 to 1.5% in 2010 to 2013 (P_{trend} = 0.01). No significantly decreasing or increasing trends were found for men (P_{trend} = 0.2) or women (P_{trend} = 0.08).

Table 3 shows the characteristics of dermatitis patients with isolated cobalt allergy stratified by test year. An overall increasing trend was found for facial dermatitis (P_{trend} = 0.01). Hence, the proportion of patients with facial dermatitis increased from 13.3% in 2002 to 2005 to 40.0% in 2010 to 2013. However, no trends were found for the other clinical variables. Table 4 shows the clinical characteristics, including exposure status, in cobalt-allergic dermatitis patients stratified by sex. Hand eczema (68.8% vs 54.5%, P = 0.02) and leg ulcers (6.5% vs 2.0%, P = 0.02) were more common in men, whereas AD was more frequently registered in women (10.8% vs 21.8%, P = 0.02). Furthermore, male patients were more often aged >40 years than females (76.3% vs 58.8%, P = 0.002). Current clinical relevance of any cobalt allergy was found in 20.1% of the patients, with no difference by sex (18.4% in women and 26.9% in men, P = 0.08). Past clinical relevance was registered in 25.8% of women and 10.8% of men (P = 0.002), yielding an overall estimate of past clinical relevance of 22.4%. Regarding causative exposures, jewellery (6.3%) and leather items (6.0%), that is, leather shoes (3.4%), leather gloves (1.3%), and "other leather items" (1.8%), were most commonly registered. Exposure to leather gloves was more frequently registered in men than in women (4.3% vs 0.6%, P = 0.02). Moreover, exposure to tools/metals and exposure to "other sources of cobalt" were registered in 2.2% and 2.0% of the patients, with a tendency for there

FIGURE 1 (**A**), Prevalence of cobalt allergy in 2002 to 2017. (**B**), Prevalence of isolated cobalt allergy in 2002 to 2017

TABLE 3 Profile of dermatitis patients with isolated cobalt allergy stratified by patch test year (N= 206)

Characteristics	2002-2005, % (n)	2006-2009, % (n)	2010-2013, % (n)	2014-2017, % (n)
Age group (y)				
18-40	28.9 (13/45)	34.4 (22/64)	40.0 (22/55)	35.7 (15/42)
41-60	44.4 (20/45)	40.6 (26/64)	32.7 (18/55)	35.7 (15/42)
>60	26.7 (12/45)	25.0 (16/64)	27.3 (15/55)	28.6 (12/42)
Men	33.3 (15/45)	20.3 (13/64)	18.2 (10/55)	26.2 (11/42)
Women	66.7 (30/45)	79.7 (51/64)	81.8 (45/55)	73.8 (31/42)
Hand eczema	64.4 (29/45)	68.8 (44/64)	47.3 (26/55)	64.3 (27/42)
Foot dermatitis[a]	-	10.9 (7/64)	7.3 (4/55)	9.5 (4/42)
Facial dermatitis	13.3 (6/45)	28.1 (18/64)	40.0 (22/55)	33.3* (14/42)
Atopic dermatitis	24.4 (11/45)	21.9 (14/64)	21.8 (12/55)	26.2 (11/42)
Occupational dermatitis	15.6 (7/45)	31.3 (20/64)	25.5 (14/55)	28.6 (12/42)
Clinical relevance[b]	13.3 (6/45)	42.2 (27/64)	40.0 (22/55)	33.3 (14/42)

*P_{trend} = 0.01.
[a]The registration of foot dermatitis started in 2004.
[b]Present or past relevance.

to be higher estimates in men than in women, although the differences were not significant.

Table 5 shows a comparison of the exposure sources between the study periods 2002 to 2009 and 2010 to 2017 in patients with cobalt allergy. Total leather exposure increased significantly from 3.8% in 2002 to 2009 to 8.4% in 2010 to 2017 (*P* = 0.04). This was mainly driven by an increase in exposure to leather gloves from 0% to 2.6% (*P* = 0.03). Exposure to “other leather sources” increased from 0.5% in 2002 to 2009 to 3.0% in 2010 to 2017 (*P* = 0.07). Exposure to tools/metals and jewellery remained largely stable over the study period.

TABLE 4 Characteristics of 447 adults with cobalt allergy in 2002 to 2017 stratified by sex

Variable	Total, % (n)	Men, % (n)	Women, % (n)
Clinical profile			
Males	20.8 (93)	-	-
Occupational dermatitis	26.8 (120)	30.1 (28)	26.0 (92)
Atopic dermatitis	19.5 (87)	10.8 (10)	21.8 (77)*
Hand dermatitis	57.5 (257)	68.8 (64)	54.5 (193)*
Leg ulcers	2.9 (13)	6.5 (6)	2.0 (7)*
Facial dermatitis	23.5 (105)	18.3 (17)	24.9 (88)
Age > 40 years	62.4 (279)	76.3 (71)	58.8 (208)*
Foot dermatitis	12.5 (56)	17.2 (16)	11.3 (40)
Relevance of positive patch test reactions			
Current	20.1 (90)	26.9 (25)	18.4 (65)
Past	22.4 (100)	10.8 (10)	25.4 (90)*
Unknown	64.7 (289)	67.7 (63)	63.8 (226)
Exposure sources			
Jewellery	6.3 (28/447)	4.3 (4/93)	6.8 (24/354)
Leather shoes	3.4 (15/447)	5.4 (5/93)	2.8 (10/354)
Leather gloves	1.3 (6/447)	4.3 (4/93)	0.6* (2/354)
Other leather items[a]	1.8 (8/447)	2.2 (2/93)	1.7 (6/354)
Leather (total)	6.0 (27/447)	9.7 (9/93)	5.1 (18/354)
Tools/metal	2.2 (10/447)	4.3 (4/93)	1.7 (6/354)
Other sources of cobalt[b]	2.0 (9/447)	4.3 (4/93)	1.4 (5/354)

*$P < 0.05$: χ^2 test comparing men and women.
[a]Furniture, clothing, and other leather items.
[b]Cutting oil, mobile phones, chemicals, cement, and paints.

TABLE 5 Sources of cobalt exposure in dermatitis patients with cobalt allergy in 2002 to 2009 and in 2010 to 2017 at Gentofte Hospital, Denmark

Exposure sources	2002-2009 (N = 217), % (n)	2010-2017 (n = 230), % (n)
Leather shoes	3.2 (7)	3.5 (8)
Leather gloves	0 (0)	2.6 (6)*
Other leather sources[a]	0.5 (1)	3.0 (7)
Leather (total)	3.7 (8)	8.3 (19)*
Jewellery	6.5 (14)	6.1 (14)
Tools/metals	2.3 (5)	2.2 (5)
Other sources of cobalt[b]	2.8 (6)	1.3 (3)
Unknown	84.8 (184)	83.0 (191)

*$P < 0.05$: χ^2 test comparing 2002 to 2009 with 2010 to 2017.
[a]Furniture, clothing, and other leather items.
[b]Cutting oil, mobile phones, chemicals, cement, and paints.

4 | DISCUSSION

4.1 | Main findings

In this cross-sectional database study, the overall prevalence of cobalt allergy and the prevalence of isolated cobalt allergy were 3.3% and 1.5%, respectively, during 2002 to 2017. Although leather and jewellery were clinically relevant causes of cobalt allergy, most cobalt-allergic cases could not be linked to a causative exposure. Current clinical relevance of positive cobalt patch test reactions was therefore identified in only one of five patients.

4.2 | Interpretation

On the basis of a clinical case of allergic cobalt dermatitis caused by prolonged contact with leather furniture, we suggested that cobalt release from leather might be responsible for hitherto unrecognized cases of ACD.[11] Recently, Haman et al examined 131 leather swatches from different companies producing leather furniture in the United States.[17] When screened with a handheld X-ray fluorescence (XRF) device, 20 swatches were shown to contain cobalt; six swatches contained more than 5 wt.% cobalt. When subsequently assessed with inductively coupled plasma mass spectrometry, all six swatches were shown to contain >300 ppm, which is, depending on the rate of release of cobalt ions from the leather, a critically high level, given that concentrations of 30.8 to 259 ppm will elicit a positive patch test reaction in 10% of cobalt-allergic patients.[18] After the tanning stage, leather might be dyed with pigments. This typically involves two classes of premetallized acid dye, that is, 1:1 and 1:2 metal complexes, in which, respectively, one or two dye molecules form strong coordination complexes with the metal ion, usually chromium or cobalt.[19] In support of the latter being a relevant exposure that may cause cobalt allergy, Rui et al reported a significant association between having isolated cobalt allergy and being a textile and leather worker (odds ratio 1.9, 95%CI: 1.1-3.1) in a cross-sectional study including 12 492 patients with suspected ACD from units of dermatology or occupational medicine in 1997 to 2004.[20] Moreover, we found that leather shoes were the most common putative sources of leather exposure (3.4%), with a seemingly higher occurrence in men, although this was not significantly different from that in women (5.6% vs 2.9%, $P = 0.2$) (Table 4). Cobalt was also found in 21 shoes randomly analysed for metal content in Sweden (range: <0.3 to 16 ppm).[21] In a recent study, we also identified >1 wt.% and > 5 wt.% cobalt in samples of leather gloves and leather shoes, respectively.[22] Furthermore, cobalt has been reported to be the third most common shoe allergen (12.9%), preceded by hexavalent chromium (31.5%) and *p-tert*-butylphenol formaldehyde resin (17.1%).[23] The latter correlation between cobalt and shoes is supported by the significant association between foot eczema and cobalt allergy in our study (aOR 2.1, 95%CI: 1.6-2.9). Similarly, in a case-control study from Denmark including 126 cobalt-allergic patients without concomitant hexavalent chromium allergy, a significant association was shown between current foot eczema, presumably caused by leather shoes, and cobalt allergy (aOR 1.9, 95% CI:1.1-3.3).[13] Collectively, these data show that cobalt from leather causes allergic cobalt dermatitis, and that leather exposure should be evaluated in patients with cobalt allergy. Indeed, the increased attention in our clinic may explain the increasing relevance of leather during the study period. However, we suspect that many patients with dermatitis, for example, atopics, could become sensitized to cobalt without knowing it. This may, to a certain degree, explain the low proportions of clinical relevance.

Jewellery was the most common single source of exposure (6.4%). In spite of this, inconsistent results have been reported regarding the content and release of cobalt from jewellery products around the world. In a recent market survey from Thailand, Boonchai et al found, using spot testing, that 206 of 551 (37.4%) costume jewellery items released cobalt.[24] Similarly, a study from Germany showed that 38 of 87 (43.7%) earrings and piercing jewellery items had at least one part releasing cobalt as assessed with EN1811:2011.[25] Nonetheless, cobalt release was detected in only four of 557 (0.7%) inexpensive earrings bought in Thailand and China.[26] In support of this, only four of 354 (1.1%) inexpensive consumer items from Denmark, including 170 earrings, released cobalt when examined with the cobalt spot test.[27] This discrepancy in results has created an ongoing debate regarding jewellery as an essential putative source of exposure to cobalt. Remarkably, in a recent study from Korea including 193 branded and 202 non-branded jewellery items, branded belts and branded hairpins more frequently released cobalt than the non-branded ones.[28] This finding, combined with the negligible levels of cobalt found in inexpensive jewellery by several studies, might partially be attributed to the higher price of cobalt than of nickel, and outlines a potential source of cobalt allergy in branded and more expensive jewellery items. Furthermore, previous studies have reported that dark-coloured jewellery items more frequently contain and release cobalt than lighter-coloured ones, but this needs to be confirmed in larger studies.[24,27]

The proportion of patients in whom current clinical relevance could be established (20%) was low as compared with other allergens; for example, the corresponding estimate for chromium allergy was 60% in another study from our clinic.[29] The low degree of relevance for cobalt allergy has led to speculations concerning the adequacy of the patch test preparation. In a recent study by Lidén et al focusing on these technical aspects, it was shown that patch testing with cobalt chloride 1.0% caused a significantly lower proportion of doubtful and irritant reactions than patch testing with potassium dichromate 0.5%.[30] Moreover, the number of doubtful or irritant reactions and the number of positive reactions were of equal magnitude for cobalt 1% and 0.5%.[30] In the study by Uter et al in 2014, trends in the frequency of positive reactions to cobalt were statistically unaffected by excluding the weakest positive reactions (+) from the analysis.[31] In our study, we found decreases over time in both isolated positive patch test reactions to cobalt chloride (statistically significant) and positive patch test reactions to cobalt chloride in combination with nickel and chromium. Neither of these results can be explained by non-specific reactivity during patch testing. Patch testers should carefully evaluate the clinical relevance of patch test results, which, so far, has proven especially difficult for cobalt. The cobalt spot test has considerably improved our ability to screen materials, particularly metallic surfaces, for cobalt release, owing to its high positive predictive value.[32] However, the test is regarded to be poor for screening leather products for cobalt release, because of low estimates for sensitivity and specificity of 20% and 14%, respectively.[17] The XRF device has shown great feasibility and accuracy in determining the metallic compositions of various materials, including leather products. Nonetheless, the XRF device is not able to assess the profile of metal release from the surface of a product, making it insufficient for evaluating the allergic capacity of a material. The synthetic sweat immersion method, that is, EN1811, and EN ISO 17075 are gold standards for determining release of chemicals from metallic objects and leather goods, respectively. Currently, these methods are expensive, time-consuming, and might destroy the test item. Ideally, modifications of these assessment methods to create more rapid, feasible and less expensive versions, combined with the XRF device, might result in a more homogeneous and systematic approach to the cobalt problem all over the world, potentially resulting in the detection of new putative sources of exposure.

4.3 | Strengths and limitations

The relative increase in leather as a source of exposure might partially have been affected by detection bias towards leather. We were unable to estimate the prevalence of foot dermatitis in cobalt-allergic patients during 2002 to 2005, as its registration in the database began in 2004. A strength of this study comprises the standardized reading techniques for patch test reactions throughout the entire study period. Other strengths include the large number of dermatitis patients included, the fact that patch test methods were unchanged over the years, the long period covered, facilitating trend analysis, and the fact that clinical disease was diagnosed by physicians.

5 | CONCLUSION

The exposure profile of cobalt-allergic patients remains poorly understood, emphasizing the need for a continued search for important sources of exposure. A better understanding of the cobalt end-use combined with more feasible screening methods for cobalt release are strongly needed in order to detect putative sources of exposure to this metal.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

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8.4 Metals in used and unused metalworking fluids: X-ray fluorescence spectrometry as a screening test

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ORIGINAL ARTICLE

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Metals in used and unused metalworking fluids: X-ray fluorescence spectrometry as a screening test

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Abstract

Background: Exposure to metalworking fluids (MWFs) is a well-known cause of occupational contact dermatitis.

Objectives: We aimed to (1) determine the amount of nickel, chromium, and cobalt in large samples of used and unused MWFs collected from metalworking plants in Denmark, and (2) evaluate a handheld x-ray fluorescence (XRF) device as a screening instrument for metals in MWFs.

Methods: A handheld XRF device was used to screen for metals in MWFs. All samples were also analyzed for concentrations of nickel, chromium, and cobalt using graphite furnace atomic absorption spectroscopy (GFAAS).

Results: GFAAS analysis showed that 13 of 80 samples (16.3%) contained >1 mg/kg (ppm) nickel (range: 6.4-17.7 mg/kg), 3 of 80 (3.8%) contained >1 (range: 1.4-3.1) mg/kg chromium, and 1 of 80 (1.3%) contained 1.3 mg/kg cobalt. XRF-screening detected nickel in eight samples (range: 2.5-15.5 mg/kg), but only one sample with 3.0 (±0.5) mg/kg was found subsequently to contain 9.9 (0.02) mg/kg nickel by GFAAS. Although no chromium was found by XRF analysis, cobalt was found in two samples with 6 (±1.5) mg/kg and 5 (±1.5) mg/kg, subsequently found to contain 0.1 (±0.01) mg/kg and 0.08 (±0.01) mg/kg by GFAAS. Similar concentrations of nickel were found in used (N = 6, range: 6.4-17.7 mg/kg) and unused MWFs (N = 7, range: 9.1-17.3 mg/kg).

Conclusion: Considerable levels of nickel, chromium, and cobalt were found in some used and unused MWFs indicating that these might represent a source of metal allergy. The XRF device is a poor screening test for these metals in MWFs.

KEYWORDS
allergic contact dermatitis, chromium, cobalt, metals, metalworking fluids, nickel, X-ray fluorescence

1 | INTRODUCTION

Occupational contact dermatitis (OCD), mainly irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD), is estimated to constitute 90%-95% of all cases of occupational skin diseases.[1] In Europe, OCD has an estimated incidence of 0.5 to 1 per 1000 workers annually and is generally associated with major socioeconomic impacts.[1]

Metals, including nickel (Ni), chromium (Cr), and cobalt (Co), are well-recognized occupational allergens. According to British occupational surveillance schemes, Cr and Co caused, respectively, 6% and 4%, respectively, of all OCD cases recorded during 1993-2004.[2] Exposure to metalworking fluids (MWFs) among metal workers is a well-known cause of occupational skin diseases.[3] MWFs consist of various chemicals and fall into classes of straight (mineral oil, neat), soluble (emulsion of oil and water), semisynthetic (lower oil concentrations), and synthetic (no mineral oil) MWFs.[4] In metal manufacturing processes, MWFs are applied as coolants and lubricants sprayed on the metal surfaces to reduce friction and heat generated with the machining, grinding, and fabrication of metal products. In a Finnish study including 1027 metalworking machinists, 279 cases of occupational skin diseases were recorded, of which 144 (53%) were ICD and 107 (39%) were ACD.[5] Previous studies from the 1970s have shown the presence of metals in MWFs, elucidating a potential important source of exposure causing ACD.[6,7]

In the present survey, we determined and quantified the metallic composition in a large sample of MWFs from several metalworking plants located in Copenhagen, Denmark. Furthermore, we evaluated the benefit of a handheld x-ray fluorescence (XRF) device as a screening instrument for metals in MWFs.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Twenty metalworking plants were contacted in Copenhagen, Denmark. A consultant from the Danish Union of Metalworkers provided a list on plants assumed to use MWFs. A metalworking plant was defined as a factory working with metals to create individual parts, assemblies, or large-scale structures. Participating plants were visited and samples of both used and unused MWFs were collected (Table SS1). Used samples consisted of MWFs that had been used for metalworking processes such as stamping, grinding, and milling. Furthermore, we recorded the name of the plant, numbered the samples chronologically, and retrieved the safety data sheet for the MWFs. Materials processed at the plants included steel, stainless steel, aluminum alloys, brass, iron, copper alloys, palladium alloys, silver alloys, chromium-nickel alloys, and plastic alloys (Table SS1).

2.2 | XRF screening

A handheld XRF device (X-MET8000 Series, Uedem, Germany) was used to measure the content of Ni, Cr, and Co in predesigned polyethylene sample cups. The XRF device bombards the material with high-energy x-ray beams, capturing the emitted secondary characteristic radiation of each element contained in the material. The manufacturer recommended applying the analytical mode "FP-Plastic" to screen for metals in mg/kg (ppm) using an energy source of 50 kV and 60.5 seconds of measuring time. All samples were shaken manually for 10 seconds before XRF screening. The results were presented as an average of two replicates.

2.3 | Digestion

Prior to elemental analysis, all samples were digested using a microwave digestion system (Multiwave GO Plus, Anton Paar, Graz, Austria), digesting up to 12 samples simultaneously. Then 350 μL of each sample was pipetted into a sealed vessel and the weight was recorded. Furthermore, 400 μL of 30% ultrapure H_2O_2 and 7 mL of 65% ultrapure HNO_3 were added to the vessel before starting the digestion for 55 minutes at 190°C. Subsequently, the digested samples were transferred to test tubes and diluted with ultrapure water (resistivity of 18.2 MΩ cm), and the total volume was noted. The final volume in milliliters (mL) was divided by the initial weight in grams (g) to obtain the individual dilution factor for each sample. Except for regular samples, known amounts of Ni, Cr, and Co were added to unused MWFs as quality controls and blank samples (for no added metal). These were digested and treated as the regular samples.

2.4 | Elemental analysis

Quantitative elemental analysis was done by graphite furnace atomic absorption spectroscopy (GFAAS, μg/L range) (Perkin Elmer AAnalyst 800) at KTH Royal Institute, Stockholm. The calibration curve was based on 1% HNO_3 (0 μg/L) and standards with known concentrations: 10, 30, and 60 μg/L for Ni; 10, 30, 60, and 80 μg/L for Cr; and 10, 30, 60 ,and 90 μg/L for Co. All samples were shaken by a vortex shaker for 10 seconds before elemental analysis. All results were presented as an average of three replicate readings. Furthermore, the measured metal concentrations of blank samples were subtracted from the metal concentrations found in the MWFs. The limit of detection (LOD) was estimated as three times the standard deviation (SD) of the blank solutions. Accordingly, the LOD was 2.1 μg/L for Ni, 0.6 μg/L for Cr, and 0.4 μg/L for Co. The quality control samples spiked with 10 μg/L of either metal showed acceptable recoveries of 107% for Ni, 101% for Cr, and 101% for Co. Consequently, there were no matrix effects (systematic analytical errors induced by other components in the MWFs) or interferences detected.

3 | RESULTS

Eight metalworking plants were included yielding a response rate of 40%. Overall, 80 samples were collected, including 61 used and 19 unused samples. Table SS1 presents an overview of the MWFs and materials processed at each metalworking plant. Table 1 provides a summary of the main findings from XRF screening and GFAAS analysis.

3.1 | XRF screening

According to the XRF screening, 9 of 80 samples (11.2%) contained Ni, Cr, or Co. Despite detecting Ni in eight samples (range: 2.5-15.5 mg/kg), only one sample with 3.0 (±0.5) mg/kg was subsequently found to contain 9.9 (0.02) mg/kg Ni by GFAAS while no Cr was found by XRF analysis, Co was found in two samples with 6 (±1.5) mg/kg and 5 (±1.5) mg/kg subsequently found to contain 0.1 (±0.01) mg/kg and 0.08 (±0.01) mg/kg by GFAAS.

TABLE 1 All samples with metal content ≥1 mg/kg analyzed by GFAAS and XRF screening

MWF type	Used/unused	Sample number	XRF (±SD)[a] mg/kg			GFAAS (±SD)[b] mg/kg		
			Ni	Cr	Co	Ni	Cr	Co
Semisynthetic	Unused	1	-	-	-	11.2 (1.5)	-	-
Semisynthetic	Used	2	-	-	-	10.0 (0.3)	-	-
Semisynthetic	Used	3	-	-	-	11.1 (0.4)	-	-
Semisynthetic	Used	4	3.0 (0.5)	-	-	9.9 (0.02)	-	-
Semisynthetic	Used	5	-	-	-	9.9 (0.5)	-	-
Neat	Unused	6	-	-	-	9.4 (1.5)	-	-
Neat	Unused	7	-	-	-	9.1 (0.9)	-	-
Neat	Unused	8	-	-	-	10.6 (2.0)	-	-
Semisynthetic	Used	9	3.5 (1)	-	-	0.01 (0.02)	-	-
Soluble	Used	17	2.5 (0.5)	-	-	-	-	1.3 (0.04)
Semisynthetic	Used	32	-	-	-	17.7 (0.4)	-	-
Soluble	Unused	33	-	-	-	17.3 (1.9)	-	-
Neat	Unused	34	-	-	-	17.1 (0.6)	-	-
Soluble	Unused	35	-	-	-	14.7 (1.8)	-	-
Semisynthetic	Used	44	-	-	-	-	1.4 (0.3)	-
Soluble	Used	49	2.5 (0.5)	-	-	-	3.1 (0.03)	-
Soluble	Used	54	15.5 (1.5)	-	6 (1.5)	0.8 (0.01)	-	0.1 (0.01)
Soluble	Used	56	5.5 (1)	-	-	-	-	-
Soluble	Used	60	-	-	5 (1.5)	-	-	0.1 (0.01)
Soluble	Used	64	-	-	-	-	1.8 (0.2)	-
Soluble	Used	68	-	-	-	6.4 (0.2)	-	-
Semisynthetic	Used	73	3 (0.5)	-	-	-	-	-
Semisynthetic	Used	76	3 (1)	-	-	-	-	-

[a]Mean and standard deviation of two replicate measurements.
[b]Mean and standard deviation of triplicate measurements.

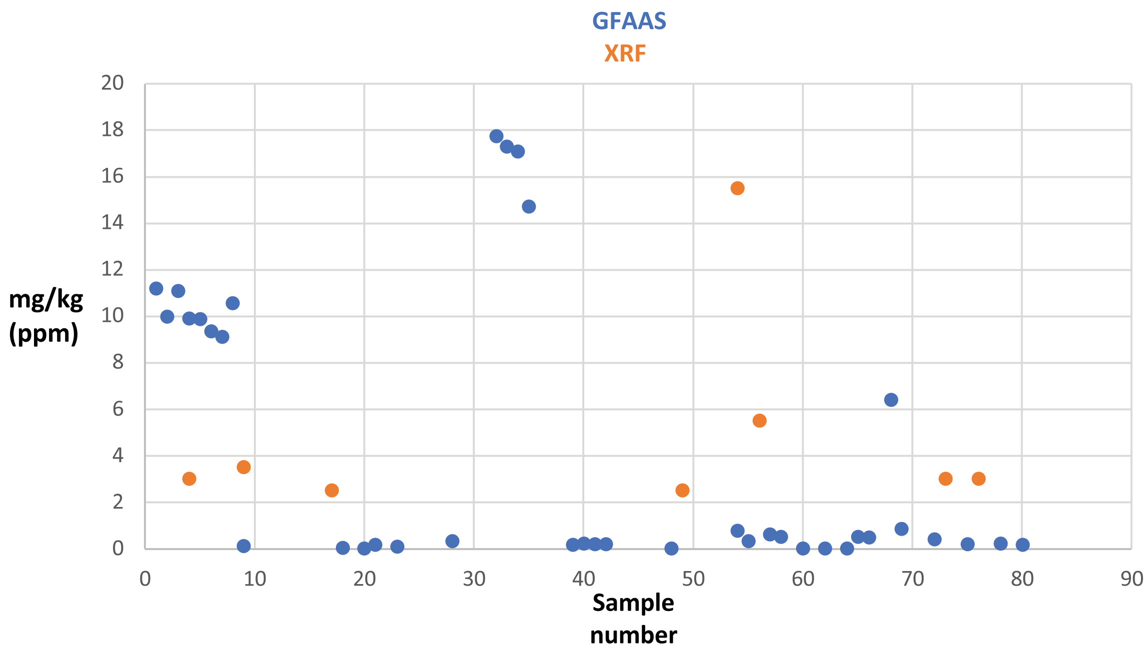

FIGURE 1 Scatter plot illustrating the poor relationship between XRF screening and GFAAS analysis for Ni contents (mg/kg) in all samples. Samples with amounts below LOD are not shown

3.2 | GFAAS analysis

GFAAS analysis showed that 13 of 80 samples (16.3%) contained >1 mg/kg Ni (range: 6.4-17.7 mg/kg), 3 of 80 (3.8%) contained >1 mg/kg Cr (1.4-3.1 mg/kg), and 1 of 80 (1.3%) contained 1.3 mg/kg Co. Overall, the mean concentrations of Ni, Cr, and Co were 2.0 (±0.2), 0.22 (±0.02), and 0.05 (±0.01) mg/kg, respectively. Ni was found in both used (N = 6, range: 6.4-17.7 mg/kg) and unused MWFs (N = 7, range: 9.1-17.3 mg/kg), whereas Cr and Co were found only in used ones (Table 1). Overall, 17 of 80 samples (21.3%) contained ≥1 mg/kg of Ni, Cr, or Co.

3.3 | Sensitivity and specificity calculations

Applying a cut-off value of ≥1 mg/kg for GFAAS, XRF screening detected 1 true positive and had 12 false negatives for Ni, yielding a sensitivity of 7.7%, whereas the specificity was 89.4% based on 59 true negatives and 7 false positives. Regarding Cr and Co, no true-positive event was recorded, yielding zero sensitivity. Furthermore, the specificity of the XRF screening was 96.3% for Cr based on 77 true negatives and 3 false positives, whereas it was 97.4% for Co based on 76 true negatives and 2 false positives. Figure 1 demonstrates the poor correlation between XRF-screening and GFAAS analysis for Ni.

4 | DISCUSSION

All three metals were found in in the MWFs, with Ni being the most prevalent. Cr and Co were found only in used oils, whereas Ni occurred in unused ones as well. Furthermore, the XRF device was a poor screening instrument for metals in MWFs.

Despite the overall low amounts of metals found in the MWFs, it is important to highlight that these levels might induce ACD. Fischer et al assessed the elicitation threshold in 20 nickel-allergic patients, reporting that 16.7% of the individuals reacted to an Ni dose of 0.035 $\mu g/cm^2$ (15.8 mg/kg) applied twice daily during 3 weeks of a repeated open application test.[8] Furthermore, 1% and 10% reacted to 0.048 $\mu g/cm^2$ (1.6 mg/kg) and 0.78 $\mu g/cm^2$ (26 mg/kg) Ni, respectively, through patch testing.[8] The latter findings illustrate the allergic capacity of the Ni levels found in our study, with 13 of 80 of MWFs containing 6.4-17.7 mg/kg. Regarding trivalent and hexavalent Cr, previous dose–response patch test studies have reported minimum 10% elicitation thresholds of 0.18 $\mu g/cm^2$ (6 mg/kg) and 0.03 $\mu g/cm^2$ (1 mg/kg), respectively,[9] elucidating the allergic potential of the Cr levels found in 3 of 80 samples (range: 1.4-3.1 mg/kg). Generally, very low levels of Co were found in our study, with only one sample containing more than 1 mg/kg (1.3 mg/kg), suggesting that this hapten occurs rarely in MWFs. The low levels of Co might also be due to the lack of Co as an alloying element in the materials processed (Table SS1). In a recent case report involving a patient with severe hand dermatitis, a machine oil was assessed as the causative hapten containing 2.4-2.7 mg/kg Co.[10] In line with this, previous studies detected Co levels at 300-550 mg/kg in MWFs used for processing of hard metal alloys, thus indicating that high levels of cobalt might occur in MWFs.[6,7] In addition, the oils and additives in the MWFs might act as surfactants and irritants, disturbing the skin barrier and thus facilitating the penetration of the metals, and resulting in lower sensitization and elicitation thresholds. The ability of some oils to enhance transdermal penetration has been described previously.[11,12] The risk of ACD might further be increased because many metal workers refuse to use protective gloves due to reduced dexterity and risk of accidents, entailing an increased risk of microtraumatic skin lesions that might facilitate the passage of metal particles or ions.

The most common causes of occupational ACD in machinists due to MWFs have been ascribed to alkanolamines, formaldehyde, and colophonium.[13] However, among metals, Ni has been suggested as the most prevalent hapten.[14] The occurrence of metals in MWFs has been attributed to contamination from machining operations, entailing a dissolution of metals in the fluids from the workpiece. Stainless steel and Ni-Cr alloys were processed at some plants, which might explain the occurrence of Ni and Cr in the used MWFs. Nevertheless, we found Ni in unused MWFs containing concentrations similar to those of the used ones. The latter finding suggests that the contamination might originate from a source other than machining operations. In some plants, we noted that the MWFs were carried in steel drums, which might present a putative source of contamination, as it is possible that metals are released from the inner surface of the steel to the fluids. In line with this, it has been postulated that the principal source of exposure in metal workers is contact with metal objects themselves at the workplace and elsewhere, thus questioning the significance of MWFs as clinically relevant exposure sources. The latter is further stressed by the lack of reaction to patch testing with MWFs that had been in use for 11.5 months in a study population of metal workers sensitized to metals.[14] Furthermore, an insight into the level of metal release from the MWFs and skin bioavailability is necessary for an accurate evaluation of the allergic potential of these haptens.

The XRF device was a poor screening instrument given the low sensitivity estimates. The advent of the XRF device has greatly improved exposure analysis in patients with ACD, particularly pertaining to metallic alloys and possibly leather products.[15] However, despite the low accuracy, it is important to emphasize that the occurrence of all three metals was either low or nonexistent in the fluids, especially regarding Co and Cr given by mean values of 0.05 (±0.01) mg/kg and 0.22 (±0.02) mg/kg, respectively. In addition, the metal concentrations found in the MWFs might be too low for the LOD of the XRF device, further explaining the poor accuracy and the high rate of false positives (10.6% for Ni). It is important to mention that XRF screening was performed on undigested organic samples comprising oil–water mixtures and other auxiliary substances, including biocides, preservatives, fragrances, and emulsifiers, thus creating a complex background scatter that might interfere with the readings of the XRF device. Furthermore, these undigested fluids might contain metal particles, which were also clearly visible to the eye in used MWFs, whereas the digested samples analyzed by GFAAS were homogeneous and particle-free. The presence of particles is suggested to interfere with the XRF measurement if higher concentrations of particles are present in the detection volume.[16]

Strengths of this study are the large MWF sample, pretreatment of the organic samples with digestion, and empirical calibration of GFAAS with standard solutions of known metal concentrations. The study was limited by a possible bias of the contributing metalworking plants; it is possible that noncontributing metalworking plants or plants from other locations have MWFs with higher concentrations of Ni, Co, and Cr, depending on the materials processed. Another important limitation includes the lack of knowledge regarding the specific materials processed on the day of MWFs collection. This study is further limited by not examining the inner surface of the steel drums as a potential source of Ni contamination.

In conclusion, considerable levels of Ni, Cr, and Co were found in some used and unused MWFs, indicating that these might represent a source of metal allergy. The XRF device is a poor screening test for these metals at these low concentrations in MWFs.

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AUTHOR CONTRIBUTIONS

Farzad Alinaghi: Data curation; formal analysis; investigation; writing-original draft; writing-review and editing. **Yolanda Hedberg:** Investigation; methodology; resources; supervision; writing-review and editing. **Claus Zachariae:** Conceptualization; supervision; writing-review and editing. **Jacob Thyssen:** Conceptualization; supervision; writing-review and editing. **Jeanne Duus Johansen:** Conceptualization; investigation; methodology; resources; supervision; validation; writing-review and editing.

CONFLICT OF INTERESTS

None declared.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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