

PhD thesis

Title: Allergy to chlorhexidine

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Allergy to chlorhexidine

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NATIONAL ALLERGY RESEARCH CENTRE



**Herlev og Gentofte
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The PhD thesis is based on the following manuscripts:

- I. Opstrup MS, Johansen JD, Bossi R, Lundov MD, Garvey LH.
Chlorhexidine in cosmetic products – a market survey.
Contact Dermatitis. 2015;72:55-58.
- II. Opstrup MS, Johansen JD, Zachariae C, Garvey LH.
Contact allergy to chlorhexidine in a tertiary dermatology clinic in Denmark.
Contact Dermatitis. 2015. Epub ahead of print.
- III. Opstrup MS, Malling HJ, Krøigaard M, Mosbech H, Skov PS, Poulsen LK, Garvey LH.
Standardized testing with chlorhexidine in perioperative allergy – a large single-centre evaluation.
Allergy. 2014;69:1390-1396.
- IV. Opstrup MS, Poulsen LK, Malling HJ, Jensen BM, Garvey LH.
Dynamics of specific IgE in chlorhexidine allergic patients with and without accidental re-exposure.
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Preface

This dissertation is based on the scientific work carried out at the National Allergy Research Centre and the Danish Anaesthesia Allergy Centre, Allergy Clinic at the Department of Dermato-Allergology at Copenhagen University Hospital Gentofte from 2012 to 2015. The project received financial funding from the Ministry of Environment, the Aage Bangs Foundation and the Beckett Foundation, and Thermo Fisher Scientific provided reagents. All are gratefully acknowledged.

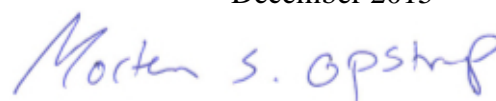
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Gentofte

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Morten Schjørring Opstrup

Abbreviations

DAAC	Danish Anaesthesia Allergy Centre
EAACI	European Academy of Allergy and Clinical Immunology
ENDA	European Network for Drug Allergy
ESCD	European Society of Contact Dermatitis
FDA	American Food and Drug Administration
HR-test	Basophil histamine release test
IDT	Intradermal test
INCI	International Nomenclature of Cosmetic Ingredients
SFAR	Société Française d'Anesthésie et de Réanimation
SPT	Skin prick test

Contents

SUMMARY IN ENGLISH	1
SUMMARY IN DANISH (DANSK RESUMÉ).....	3
BACKGROUND	5
Chlorhexidine: discovery and effectiveness	5
Use of chlorhexidine	6
Contact allergy to chlorhexidine	7
Immediate-type chlorhexidine allergy.....	9
Department of Dermato-Allergology, Copenhagen University Hospital Gentofte	11
OBJECTIVES OF THE STUDIES.....	13
METHODS AND METHODOLOGICAL CONSIDERATIONS	14
Testing for contact allergy to chlorhexidine.....	14
Testing for immediate-type allergy to chlorhexidine	14
Use of chlorhexidine in cosmetic products (study I).....	17
Contact allergy to chlorhexidine (study II)	18
Immediate-type chlorhexidine allergy (studies III and IV)	19
Statistical analysis	21
Ethical considerations	21
RESULTS AND DISCUSSION OF MAIN FINDINGS	23
Use of chlorhexidine in cosmetic products (study I).....	23
Contact allergy to chlorhexidine (study II)	25
Immediate-type chlorhexidine allergy (studies III and IV)	30
CONCLUSIONS AND PERSPECTIVES FOR FURTHER RESEARCH.....	40
REFERENCES	44
MANUSCRIPTS	51
APPENDICES	98

Summary in English

Chlorhexidine is a very effective disinfectant and thereby prevents many infections. Consequently, chlorhexidine is widely used in the healthcare setting, but it can also be used as a preservative in cosmetic products. To date, the extent of its use in cosmetic products in Denmark has never been assessed. Most people tolerate exposure to chlorhexidine well, but some develop contact allergy or immediate-type allergy. In the 1980s several studies from Denmark found that contact allergy to chlorhexidine was diagnosed in 2.0-5.4% of all patients patch tested, which are much higher prevalences than in other European countries (0.5-2.0%). It is unclear whether the prevalence is still high in Denmark. Although chlorhexidine is widely used, the products causing sensitization and allergy symptoms are currently unidentified. Immediate-type allergy to chlorhexidine has mainly been described in patients with an allergic reaction during surgery, but it can take place anywhere in the healthcare setting. Testing can be performed with the skin prick test, the intradermal test, the specific IgE (ImmunoCAP®) and the histamine release test (HR-test) (some centres may use the basophil activation test). Currently, the sensitivity and specificity for each of the tests remain unknown. Specific IgE-results decline over time, but the dynamics are poorly described.

This thesis consists of four studies. The main aims were 1) to investigate the extent of use of chlorhexidine in cosmetic products, 2) to estimate the prevalence of contact allergy to chlorhexidine in a tertiary dermatology clinic in Denmark and to investigate which products cause contact allergy to chlorhexidine and 3) to optimize the diagnostic testing for immediate-type chlorhexidine allergy.

Results of the first study showed that chlorhexidine is a commonly used preservative in cosmetic products in Denmark (3.6% of 2,251 checked cosmetic products). Chlorhexidine was mainly found in hair products but also in some creams, wet wipes, face washes, skin tonics, make-up removers and a mouth wash. The concentration of chlorhexidine was estimated in 10 selected products and was below the allowed limit of 0.3% in all.

The second study was divided in two: (i) a retrospective database study and (ii) a questionnaire study. Results of the database study showed that 1.0% of all patients patch tested with chlorhexidine at the Dermatology Clinic at Copenhagen University Hospital Gentofte from 2003 to 2013 were

sensitized. This finding indicates that the prevalence is no longer higher than in other European countries. In the questionnaire study, patients reported both healthcare products and cosmetic products as causes of the allergy. Notably, 32% of the patients reported one or more re-exposures to chlorhexidine in cosmetics or in healthcare products after the diagnosis was established.

In the third study it was found that allergy to chlorhexidine is common among patients with suspected perioperative allergic reactions: 9.6% of patients investigated for a suspected perioperative allergic reaction in the Danish Anaesthesia Allergy Centre from 2004 to 2012 were positive to chlorhexidine. Results also showed that both skin prick test and specific IgE had high estimated sensitivities and specificities, whereas both the intradermal test and the histamine release test had high specificities but lower sensitivities.

Results of the fourth study showed that levels of specific IgE vary greatly between patients and over time: specific IgE increased in the first weeks to months after the allergic reaction and subsequently decreased and eventually declined below 0.35kUA/l in most patients (the most rapid decline was four months). Re-exposure to chlorhexidine was reported by 35% and most of these caused symptoms (also in a patient with specific IgE<0.35kUA/l) and an increase in specific IgE.

In conclusion, the thesis has demonstrated that chlorhexidine is widely used not only in healthcare products but also in cosmetic products. The prevalence of contact allergy to chlorhexidine is not higher in our tertiary dermatology clinic than in other countries. Chlorhexidine in both healthcare products and in cosmetics can cause contact allergy, demonstrating the importance of thorough exposure assessment during allergy investigations. Immediate-type chlorhexidine allergy is common among patients with suspected perioperative allergic reactions and these patients should therefore always be tested with chlorhexidine. Specific IgE and skin prick test should be performed as a minimum. Levels of specific IgE can decline below 0.35kUA/l over time, but this does not necessarily indicate tolerance. Consequently, time since allergic reaction should be considered when analysing specific IgE-results, as results can become false negative over time. Re-exposures are common, highlighting that healthcare workers need to be informed about possible sources of exposure when treating a patient with chlorhexidine allergy.

Summary in Danish (Dansk resumé)

Klorhexidin er et meget virksomt desinfektionsmiddel, som forebygger mange infektioner. Det bliver således hyppigt brugt i sundhedsvæsenet, men det kan også bruges som konserveringsmiddel i kosmetik. Aktuelt er det uvist, i hvilken udstrækning klorhexidin bliver brugt i kosmetiske produkter. De fleste mennesker tolererer udsættelse for klorhexidin uden problemer, men nogle mennesker udvikler kontakt-allergi eller straks-allergi. I 1980'erne konstaterede flere danske studier højere forekomster af kontakt-allergi over for klorhexidin end i studier fra andre europæiske lande (2.0-5.4% af alle lappetestede sammenlignet med 0.5-2.0%). Det er uvist, om forekomsten i Danmark stadig er høj, ligesom det heller ikke er klarlagt, hvilke produkter, der forårsager kontakt-allergien. Straks-allergi over for klorhexidin har primært været beskrevet hos patienter med en allergisk reaktion under en operation, men reaktionerne kan finde sted hvor som helst i sundhedsvæsenet. Symptomerne ved de straks-allergiske reaktioner er ofte alvorlige såsom nældefeber eller anafylaktisk shock. Diagnosen stilles ud fra en relevant klinisk sygehistorie i kombination med resultater af priktest, intracutantest, specifik IgE (ImmunoCAP®) og histamin release test (HR-test). Det er endnu uafklaret, hvor brugbare disse tests er til at stille diagnosen (sensitivitet og specificitet er ukendte). Niveauer af specifikke IgE-antistoffer i blodet aftager over tid blandt patienter med klorhexidin-allergi, men dynamikken er uafklaret.

Denne afhandling består af fire studier, og de overordnede formål var 1) at undersøge brugen af klorhexidin i kosmetik, 2) at undersøge hyppigheden af kontakt-allergi over for klorhexidin og undersøge, hvilke produkter der forårsager allergien samt 3) at optimere diagnostikken ved straks-allergi over for klorhexidin.

Resultater af det første studie viste, at klorhexidin er hyppigt brugt som konserveringsmiddel i kosmetiske produkter i Danmark (3.6% af 2,251 gennemsete produkter). Klorhexidin blev primært fundet i hårprodukter, men også i cremer, vådservietter, ansigtsvask, skin tonics, make-up fjernere og i en mundskyllevæske. Koncentrationen af klorhexidin blev bestemt i 10 produkter, og var under den tilladte grænse på 0.3% i alle.

Det andet studie om kontakt-allergi over for klorhexidin var inddelt i to: (i) et retrospektivt database studie og (ii) en spørgeskemaundersøgelse. Resultaterne af database-studiet viste, at 1.0% af alle, der blev lappetestet med klorhexidin på Hudafdelingen på Gentofte Hospital fra 2003 til 2013 var sensibiliserede. Forekomsten er således ikke længere højere i Danmark end i andre lande. I spørgeskemaundersøgelsen rapporterede patienterne, at både produkter brugt i sundhedsvæsenet og kosmetiske produkter havde forårsaget deres allergi. Det blev desuden fundet, at 32% af patienterne havde været udsat for klorhexidin i sundhedsvæsenet eller i kosmetik, efter diagnosen blev stillet.

Resultaterne af det tredje studie om straks-allergi over for klorhexidin viste, at 9.6% af alle, der blev undersøgt for en mistænkt allergisk reaktion under en operation i Dansk Anæstesi Allergi Center fra 2004 til 2012, var allergiske over for klorhexidin. Det blev også estimeret, at specifik IgE og priktest havde høj sensitivitet og specificitet, mens både intracutantesten og histamin release testen havde høj specificitet men lav sensitivitet.

Resultaterne af det fjerde studie viste, at specifik IgE stiger i de første uger efter den allergiske reaktion, for derefter at aftage og til sidst falde under 0.35kUA/l, som er det anbefalede cut-off for en positiv test. Re-eksponering blev rapporteret af 35%, og de fleste re-eksponeringer gav symptomer (også i en patient med specifik IgE <0.35kUA/l) og en stigning i specifik IgE.

Det kan konkluderes, at klorhexidin ikke kun er hyppigt brugt i sundhedsvæsenet men også i kosmetik. Forekomsten af kontakt-allergi over for klorhexidin er ikke højere i vores tertiære hudafdeling end i andre lande. Både produkter brugt i sundhedsvæsenet og kosmetik kan forårsage kontakt-allergien. Hos patienter med en mistænkt allergisk straks-reaktion under en operation er klorhexidin en hyppig allergi, og disse patienter bør således altid undersøges for straks-allergi over for klorhexidin. Både specifik IgE og priktest har høj estimeret sensitivitet og specificitet, og undersøgelserne bør således som minimum inkludere disse to tests. Niveauer af specifik IgE aftager over tid og kan falde <0.35kUA/l, men det betyder ikke nødvendigvis, at patienten er tolerant. Re-eksponering er hyppig, og det er derfor vigtigt, at sundhedspersonale og klorhexidin-allergiske patienter er opmærksomme på, hvor klorhexidin bruges, således at re-eksponering kan undgås.

Background

Chlorhexidine: discovery and effectiveness

Chlorhexidine is a disinfectant discovered in the 1950s by Imperial Chemical Industries while researching for antimalarial drugs¹. A few years earlier - in the 1940s - the company had discovered the antimalarial drug proguanil (chlorguanide) which is structurally closely related to chlorhexidine, see figure 1.

Chlorhexidine has bacteriostatic, bactericidal, fungicidal, fungistatic and some virus killing properties². In a recent systematic review and meta-analysis including more than 5,000 patients in six studies performed between 1982 and 2010, the effectiveness of chlorhexidine was compared with that of povidone-iodine, which is another commonly used disinfectant. It was found that surgical-site infection rate was significantly lower in patients disinfected with chlorhexidine compared with those disinfected with povidone-iodine (pooled odds ratio 0.68; 95% CI [0.50-0.94], $p=0.019$)³. These findings are in line with a recent randomized controlled trial from France including 2,546 intensive care patients. In this study, it was found that the number of catheter-related infections was significantly lower when disinfecting the skin with chlorhexidine-alcohol compared with povidone iodine-alcohol (hazard ratio 0.15; 95% CI [0.05-0.41], $p=0.0002$)⁴. Taken all together, these findings show that chlorhexidine is a highly effective disinfectant.

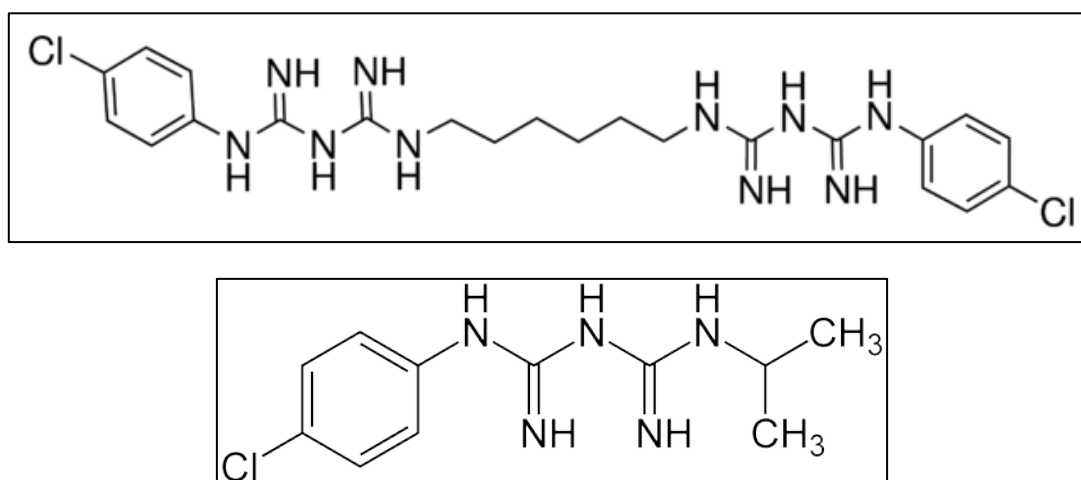


Figure 1. Molecular structure of chlorhexidine (upper) and proguanil (lower)^{5,6}.

Use of chlorhexidine

Healthcare products

As a result of its excellent antimicrobial properties, chlorhexidine has gained wide use as a disinfectant in many different products used in the healthcare setting e.g. in mouth washes, urethral gels, bandages, skin disinfectants and creams. In appendix II, there is a list of all product types containing chlorhexidine distributed from the pharmacy in the Capital Region of Denmark to the hospitals in 2013. Notably, in Denmark, and most likely also in many other countries, chlorhexidine is used not only in pharmaceutical products but also in some non-pharmaceutical products used in the healthcare setting such as some central venous catheters and skin swabs. In an effort to create a complete list of products containing chlorhexidine used in the hospitals in the Capital Region of Denmark, the Corporate Procurement (distributor of all non-pharmaceutical products to the hospitals) was contacted. In their product catalogue there are currently more than 100,000 products, and it is not possible to search for chlorhexidine in the catalogue. Reading through the material safety data sheet for each product would be the only way to check whether it contains chlorhexidine. To complicate matters even further, the declaration of chlorhexidine can be very difficult to find on the package on some chlorhexidine containing products such as the central venous catheters^{7,8}. Consequently, it is very difficult, if not impossible, to get a complete overview of products containing chlorhexidine in the healthcare setting. Additionally, use of healthcare products containing chlorhexidine is not restricted to the healthcare setting. Indeed, exposure can also take place in the home because many products are sold over-the-counter in supermarkets and pharmacies e.g. mouth washes, bandages and wound cleansers. As a result, both healthcare personnel and the chlorhexidine allergic patients have to be aware of possible sources to avoid accidental re-exposure to chlorhexidine.

Cosmetic products

Besides being a disinfectant in healthcare products, chlorhexidine can also be used as a preservative in cosmetic products. In the United States and Finland, chlorhexidine is reported to be used in many

different cosmetic products^{9,10}. Nonetheless, it is unclear how widely chlorhexidine is used in cosmetic products in other European countries such as Denmark. Chlorhexidine is allowed in a concentration of up to 0.3% in cosmetics as set by the European Cosmetics Regulations¹¹, but no studies have estimated whether the concentrations used remain below this limit.

Contact allergy to chlorhexidine

Contact allergy is a T-cell mediated allergy characterized by contact dermatitis at the skin site of contact. The pathophysiological mechanism can be divided into two phases: an induction phase, where the allergy is developed (also called sensitization phase); and an elicitation phase, where re-exposure to the allergen can cause allergic symptoms such as erythema, infiltration, oedema and vesicles¹².

Contact allergy to chlorhexidine was first described in 1962¹³. In the 1980s several studies from Denmark reported high prevalences of contact allergy to chlorhexidine: 2.0% to 5.4% of all patients patch tested were positive to chlorhexidine¹⁴⁻¹⁶. It was found that contact allergy to chlorhexidine was diagnosed primarily among men with leg eczema and leg ulcers¹⁴⁻¹⁶, and it was presumed that wound dressings containing chlorhexidine (such as Bactigras®) were the main sensitizer in these patients¹⁶. No additional studies from Denmark or other European countries have further characterized these patients. Around Europe later studies have reported lower prevalences: 0.01% in England¹⁷, 0.5% in Finland¹⁰, 1.5% in Czech Republic¹⁸ and 2.0% in Switzerland¹⁹. It remains unknown whether the prevalence in Denmark is still higher than in other European countries.

Diagnosing contact allergy to chlorhexidine

Patch testing is the gold standard for diagnosing contact allergy¹². A positive patch test is a sign of sensitization, but this may not cause allergic symptoms in all patients. Consequently, the diagnosis of allergic contact dermatitis is only given when clinical relevance is established. To assess the clinical relevance, it is necessary to perform an exposure assessment. This assessment can include a review of ingredients in products from the patient's environment, but in some cases a chemical analysis of a product is required to document the presence of the allergen in the product. Several

types of chemical analysis exist including simple spot tests, thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC)²⁰.

Chlorhexidine can be used in healthcare products and cosmetics as one of three salts: chlorhexidine diacetate (molecular weight 626 g/mol), chlorhexidine digluconate (898 g/mol) and chlorhexidine dihydrochloride (578 g/mol), see figure 2. It is currently unknown which salts are most frequently used in the products. The test regimen for patch testing for contact allergy to chlorhexidine varies from centre to centre. Many centres patch test only with one of the three salts e.g. chlorhexidine digluconate^{10,18,19}. In contrast, at the Dermatology Clinic at Copenhagen University Hospital Gentofte, where study II was performed, patch testing is done with both chlorhexidine diacetate and chlorhexidine digluconate. The rationale behind testing with more than one salt dates back to a Danish study from 1991, where it was found that some patients tested positive to one of the salts but negative to the other²¹. However, no new studies have investigated whether testing with more than one salt is indeed necessary.

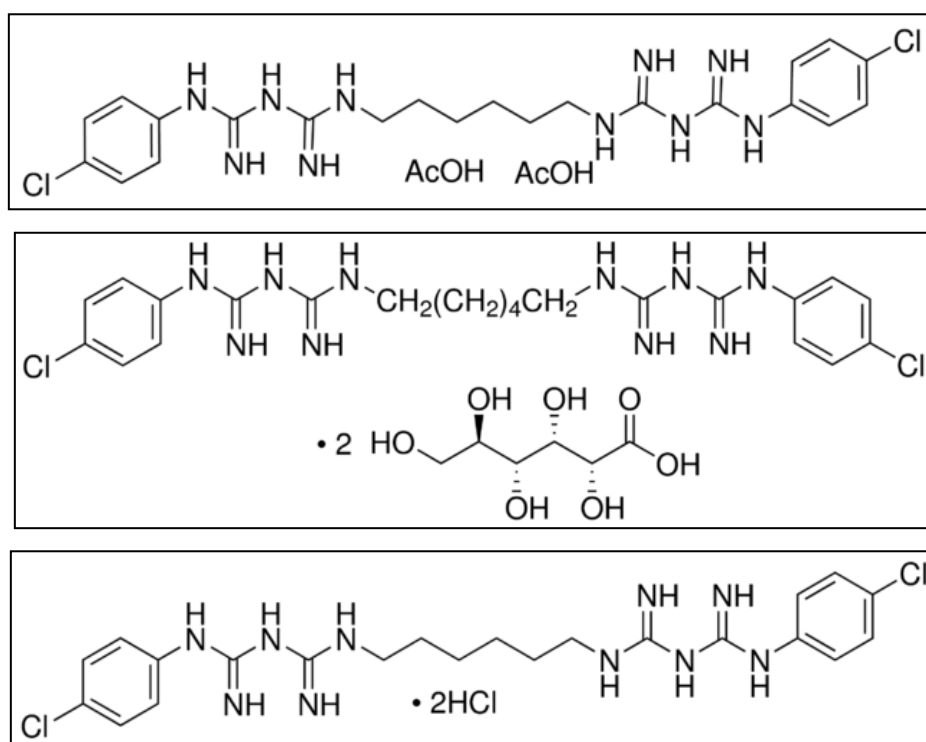


Figure 2. Molecular structure of chlorhexidine diacetate (upper figure), chlorhexidine digluconate (middle figure) and chlorhexidine dihydrochloride (lower figure)²².

Sources of sensitization in contact allergy to chlorhexidine

In Finland, it was recently found that creams containing a combination of corticosteroids and chlorhexidine (Sibicort® and Duocort®) were the most frequent sources of sensitization to chlorhexidine¹⁰. These creams are not available in Denmark and here it remains unknown which products cause the contact allergy. In addition, it remains unclear whether patients with contact allergy to chlorhexidine are aware of possible sources of chlorhexidine exposure and whether they have accidentally been exposed to chlorhexidine after the diagnosis. Accidental re-exposure has been described in a few case reports of patients with immediate-type chlorhexidine allergy^{7,8,23-26}.

Immediate-type chlorhexidine allergy

Immediate-type allergy is also divided into two phases: a sensitization phase, where exposure to an allergen leads to the production of specific IgE-antibodies which bind to the high affinity IgE-receptor on mast cells and basophil granulocytes; and an elicitation phase, where re-exposure to the allergen can result in binding to the specific IgE-antibodies. This results in cross-linking of two IgE-receptors, which leads to an intracellular cascade, ending in degranulation and release of histamine and other mediators such as leukotrienes, heparin, tryptase and prostaglandins from the mast cell and the basophil granulocyte.

Immediate-type allergy to chlorhexidine was first described in Japan in 1984 in a 9-year-old boy, who developed anaphylactic shock during surgery²⁷. Since then, several case reports followed from Japan^{28,29} and in 1984 the Japanese Ministry of Welfare recommended that the use of chlorhexidine on mucous membranes be prohibited²⁸. Additionally, it was recommended by the manufacturer that chlorhexidine digluconate not be used on wound surfaces in a concentration higher than 0.05%²⁸. These recommendations are clearly not followed in Denmark (see appendix II), and likely not in most countries around the world. Since the 1980s, many case reports and case series have followed from all over the world^{7,8,23-26,30-53}.

In 2007, it was confirmed that there is an IgE-mediated mechanism behind immediate-type chlorhexidine allergy⁵⁴.

Immediate-type chlorhexidine allergy is easily overlooked

Chlorhexidine can cause severe allergic reactions leading to urticaria and anaphylactic shock^{43,54}, but its use is 'hidden' in many of the products containing the disinfectant e.g. urethral gels, skin swabs, bandages and central venous catheters. As a consequence, chlorhexidine is easily missed as the cause of the allergic reaction. Indeed, in many cases, chlorhexidine is not suspected as the cause, resulting in more than one allergic reaction before the correct diagnosis is established^{35,37,43}.

Moreover, at the time of the diagnosis, many patients report one or more reactions in the past, which were not believed to be associated with chlorhexidine at that time, but turn out to likely be a result of exposure to chlorhexidine^{26,29,38,43,49}. Especially two products have caused many of the reactions: the urethral gel and the central venous catheter. As a result of the risk of serious allergic reactions caused by these products, the American Food and Drug Administration (FDA) warned in 1998 of the potential of serious hypersensitivity reactions caused by chlorhexidine impregnated medical devices; the Department of Health in Australia warned in 2012 of the potential for anaphylaxis caused by gels containing lignocaine and chlorhexidine; and the Medicines and Healthcare products Regulatory Agency in UK warned in 2012 of the risk of anaphylactic reactions due to chlorhexidine in all medical devices and medicinal products containing chlorhexidine⁵⁵⁻⁵⁷.

Prevalence of immediate-type chlorhexidine allergy

Immediate-type chlorhexidine allergy is rare, but the prevalence in the general population remains unknown. Most of the case reports on chlorhexidine allergy include patients who had allergic reactions during surgery, where exposure is high, and it was recently found that 5% (UK), 7% (UK) and 8.6% (Netherlands), respectively, of all patients with a suspected perioperative allergic reaction were diagnosed with chlorhexidine allergy⁵⁸⁻⁶⁰. In Denmark, the prevalence of chlorhexidine allergy among patients with a suspected perioperative allergic reaction remains unknown.

Diagnosing immediate-type chlorhexidine allergy

The diagnosis of chlorhexidine allergy is based on a relevant clinical history combined with in vivo and in vitro tests. At the Danish Anaesthesia Allergy Centre, Allergy Clinic at Copenhagen

University Hospital Gentofte, where study III and IV were performed, the following tests are included: skin prick test, intradermal test, specific IgE (ImmunoCAP ®) and histamine release test (HR-test). Currently, sensitivity and specificity for each of the tests are unknown.

It was shown in 2007 that plasma levels of specific IgE to chlorhexidine decline over time⁵⁴ and this has also been shown for ethylene oxide and penicillins⁶¹⁻⁶³. As a consequence, the manufacturer recommends testing within six months of the allergic reaction⁶⁴. However, the dynamics of specific IgE over time in patients with chlorhexidine allergy are poorly described, and it is unknown whether specific IgE will drop below the recommended cut-off of 0.35kUA/l in chlorhexidine allergic patients with previously elevated levels as has been described for the penicillins⁶³. The influence of re-exposure to chlorhexidine on levels of specific IgE in chlorhexidine allergic patients also remains unclear.

Combined contact allergy and immediate-type allergy

A few case reports have described patients diagnosed with both contact allergy and immediate-type allergy to chlorhexidine⁶⁵⁻⁶⁸. Currently, the proportion of combined allergy is unknown. Due to the severity of the immediate-type allergic reactions to chlorhexidine, it would be especially beneficial to find out how many of the patients with contact allergy to chlorhexidine also have immediate-type chlorhexidine allergy.

Department of Dermato-Allergology, Copenhagen University Hospital Gentofte

The studies in this PhD took place at the Department of Dermato-Allergology at Copenhagen University Hospital Gentofte, which consists of several clinics and centres including the National Allergy Research Centre, the Dermatology Clinic and the Danish Anaesthesia Allergy Centre, Allergy Clinic.

The National Allergy Research Centre

The National Allergy Research Centre was founded in 2001 by the Ministry of Environment and is engaged in research, surveillance, prevention and collecting information about allergic reactions to chemicals.

The Dermatology Clinic

The Dermatology Clinic is a highly specialized tertiary dermatology clinic, investigating patients with all kinds of dermatological diseases. More than 1,000 patients are investigated for contact dermatitis in the clinic each year. Besides investigating patients, research and education are important aspects of the work at the clinic.

Danish Anaesthesia Allergy Centre, Allergy Clinic

Patients with suspected perioperative allergic reactions are investigated in the Danish Anaesthesia Allergy Centre (DAAC), which was established at Copenhagen University Hospital Gentofte in 1998 and in 2010 obtained status as the Danish national reference centre approved by the Danish Board of Health. The organisational structure of the centre is based on close collaboration between allergologists and anaesthesiologists with many years of experience in investigating perioperative allergic reactions. Investigations follow a systematic individualized protocol testing all the drugs to which patients have been exposed before the allergic reaction using in vitro tests, skin tests and provocation tests. In addition, all patients are tested with substances with definite exposure in the perioperative setting such as chlorhexidine, latex, ethylene oxide and macrogols. Besides investigating patients with suspected perioperative allergic reactions, research and education are also here important aspects of the work at the centre.

Objectives of the studies

Study I

- To identify cosmetic product types containing chlorhexidine.
- To measure the concentration of chlorhexidine in selected products.

Study II

- To estimate the prevalence of chlorhexidine contact allergy in a tertiary dermatology clinic and to characterize the patients.
- To investigate whether patch testing with both chlorhexidine diacetate and chlorhexidine digluconate is necessary.
- To estimate how many patients have both immediate-type allergy and contact allergy to chlorhexidine.
- To investigate which products cause contact allergic reactions and whether patients are aware of possible sources of chlorhexidine exposure and are able to avoid these.

Study III

- To estimate the prevalence of immediate-type chlorhexidine allergy among patients with a suspected perioperative allergic reaction.
- To estimate the sensitivity and specificity for the tests used to diagnose immediate-type chlorhexidine allergy.

Study IV

- To follow the dynamics of specific IgE to chlorhexidine over time in patients with immediate-type chlorhexidine allergy with and without known re-exposure to chlorhexidine.

Methods and methodological considerations

Testing for contact allergy to chlorhexidine

Patch testing

The rationale behind patch testing is to expose patients on the skin to various possible allergens under controlled and standardized conditions to check whether this results in an allergic response. At the Dermatology Clinic at Copenhagen University Hospital Gentofte, patch testing is performed on the upper back with eight-millimetre Finn Chambers attached by Scanpor tape. Occlusion time is 48 hours and readings are done at day 2, day 3 and day 5/7, in accordance with recommendations by the European Society of Contact Dermatitis¹². If a reaction occurs on the skin site where exposure took place, it can be an allergic reaction, an irritant reaction or a doubtful reaction. The latter two are interpreted as negative reactions. Allergic reactions are scored as 1+ (weak positive reaction: erythema, infiltration and possibly papules), 2+ (strong positive reaction: erythema, infiltration, papules and vesicles) and 3+ (extreme positive reaction: intense erythema, infiltration and coalescing vesicles)¹².

Since the 1980s, chlorhexidine digluconate and chlorhexidine diacetate have been included in the supplement to the baseline series at the Dermatology Clinic at Copenhagen University Hospital Gentofte, and therefore almost all patients have been investigated for contact allergy to chlorhexidine. Until 1 September 2008 test concentrations of 1.0% aq. were used and test substances were prepared in the department. Since 1 September 2008 test substances from Chemotechnique ® and Trolab ® have been used in a concentration of 0.5%.

Testing for immediate-type allergy to chlorhexidine

At the Danish Anaesthesia Allergy Centre (DAAC), all patients are tested for allergy to chlorhexidine. The diagnosis of chlorhexidine allergy is based on a relevant clinical history

combined with test results of skin prick test, intradermal test, specific IgE (ImmunoCAP ®) and in some cases histamine release test (HR-test).

Skin testing

In skin testing (skin prick testing [SPT] and intradermal testing [IDT]) mast cells in the skin are exposed to the suspected allergen. If a patient is allergic, a wheal and flare response will appear on the skin as an indication of an IgE-mediated reaction, where the mast cells degranulate.

Performance of skin testing is recommended a minimum of six weeks after the allergic reaction⁶⁹, and at DAAC, skin testing is usually performed two to six months after the allergic reaction.

For a skin prick test, a small volume of high concentration of allergen is pricked into the epidermis. There are no blood vessels in epidermis and therefore blood should not appear when performing the skin prick test. At DAAC, the skin prick test is performed on the forearm in duplicate with chlorhexidine digluconate 5 mg/ml. This concentration has been used at DAAC for many years and is also the concentration recommended by the European Academy of Allergy and Clinical Immunology/European Network for Drug Allergy (EAACI/ENDA) Drug Allergy Interest Group^{54,70}. The reaction is read after 20 minutes and compared with a negative control with saline. Histamine 10 mg/ml serves as positive control. Positivity criterion: mean diameter of wheal ≥ 3 mm⁷¹.

For an intradermal test, a larger volume of a low concentration of allergen is injected into the dermis, where there are blood vessels, producing a bleb. As the allergen is introduced deeper into the skin, and closer to the mast cells, which are located around the blood vessels, the intradermal test is considered more sensitive than the skin prick test. The risk of systemic reactions is also considered higher when performing the intradermal test compared with the skin prick test. At DAAC, the intradermal test is performed titrated up to a concentration of a maximum of 1:10 of vial concentration, depending on the allergen. The intradermal test is performed in duplicate on the back with chlorhexidine digluconate 0.002 mg/ml. This concentration has been used for many years at DAAC and is also recommended by the EAACI/ENDA Drug Allergy Interest Group^{54,70}. At DAAC, two different procedures have been used. Until January 2011, a bleb of 3-5 mm was

induced without measuring volume. Since January 2011, in an attempt to standardize the procedure, a fixed volume of 0.02 ml has been injected. Both test procedures were performed with a 0.5 mL syringe and read after 20 minutes. A negative control with saline was used. Since 2011 we have compared the test results with the size of the bleb instead of the negative control, but these test results are not included in the studies conducted in this thesis. At DAAC we used until 2014 the positivity criterion: mean diameter of wheal \geq twice the diameter of the negative control. Since 2014 we have used the positivity criterion: increase in diameter of wheal \geq 3 mm.

Specific IgE

Detection of specific IgE-antibodies in serum can be used as a sign of sensitization. Measurement of specific IgE is especially helpful as a supplement to skin tests, where provocation models are not available e.g. in chlorhexidine allergy. Although the specific IgE immunoCAP-assay (Thermo Fisher Scientific, Uppsala, Sweden) is only available for a limited number of drugs (mainly penicillins), it has been available for chlorhexidine since January 2007⁷². Levels of specific IgE-antibodies to chlorhexidine are measured in sera from all patients investigated at DAAC on blood samples drawn usually four to six weeks after the allergic reaction. The positivity criterion is a value above 0.35kUA/l as recommended by the manufacturer⁶⁴. The principle is rather simple, see figure 3. The test is safe for the patient as there is no risk of a systemic reaction.

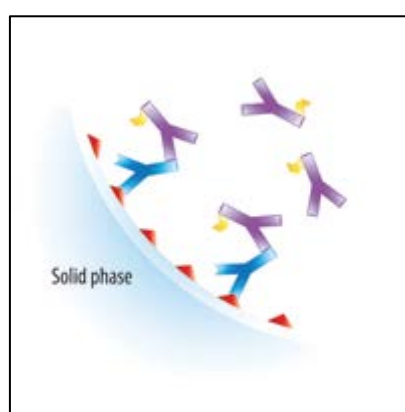


Figure 3. Test principle for the ImmunoCAP® assay. Chlorhexidine (red) is bound to the solid phase (the CAP). When serum is added from a patient with specific IgE-antibodies to chlorhexidine (blue), specific IgE will bind to chlorhexidine. After washing, enzyme-labelled antibodies against IgE are added (purple). They bind to IgE and create a change in fluorescence, which can be detected⁷³.

On the downside, specific IgE-testing is relatively expensive and sensitivity and specificity vary from allergen to allergen.

Basophil histamine release test (HR-test)

The basophil histamine release test (HR-test) (Reflab Aps, Copenhagen, Denmark) detects histamine released from patient blood after incubation with the culprit drug e.g. chlorhexidine⁷⁴.

The test is performed on fresh heparinized blood and has several strengths: it is safe for the patient because there is no risk of a systemic reaction and in principle all substances can be examined in the test. A limitation of the test is that it has to be performed within 24 hours of the blood sample being drawn due to the short-lived basophil granulocytes. In the case that the blood sample is more than 24 hours old, it is possible to measure histamine release to chlorhexidine after passive sensitization of IgE-stripped basophil granulocytes from a buffy coat blood sample (from the blood bank) with patients' sera. In 2007, it was found that this method with passively sensitized basophils had high specificity but lower sensitivity for chlorhexidine allergy⁵⁴. These findings are in line with a recent study on cefuroxime allergy, where it was found that the HR-test had a low sensitivity (22.2%) but high specificity (100%)⁷⁵. This indicates that it is useful for excluding the allergy in non-allergics but not very useful for detecting the allergy in allergics. At DAAC, most patients have histamine release test performed on fresh blood, but some are tested with passively sensitized basophil granulocytes.

Use of chlorhexidine in cosmetic products (study I)

Market survey

To obtain an overview of the extent of use of chlorhexidine as a preservative in Denmark, it was decided to conduct a market survey from February 2013 to April 2013 in Copenhagen, Denmark. In an effort to check as many products as possible, we checked for chlorhexidine in cosmetic products in 14 supermarkets, one hair dressing salon and one beauty and retail store by reading the ingredient labels. We checked for chlorhexidine by looking for its International Nomenclature of Cosmetic Ingredients (INCI)-names: chlorhexidine (CAS number 55-56-1), chlorhexidine diacetate (CAS

number 56-96-1), chlorhexidine digluconate (CAS number 18472-51-0) and chlorhexidine dihydrochloride (CAS number 3697-42-5)¹¹. We checked for content of chlorhexidine in the following product types: hair products (shampoos, conditioners, hair dyes, hair treatments, hair wax, hair sprays and hair gels), creams and ointments, body lotions, deodorants, bath soaps, hand soaps, toothpastes, aftershaves/shaving foams, wet wipes, make-up removers, face washes, skin tonics, mouth washes, face masks, lip balms and hand disinfectants. To avoid duplicates, photographs were taken of all products and product names were noted.

Concentration of chlorhexidine in products

Initially, we planned to estimate the concentration of pure chlorhexidine in all products, but because the method was very expensive, only 10 selected products were analysed. The product types with the most products containing chlorhexidine were selected for analysis. However, some product types were not analysed because of technical limitations, as analysis of complex matrix solid compounds, such as wet wipes, would require a different extraction method than that used for liquid and semi-solid samples. Analytical high-performance liquid chromatography (HPLC)-with ultraviolet (UV)-detector was used for the analysis. More details on the method can be found in manuscript I. The measurement of chlorhexidine concentration was performed by the Department of Environmental Science, Aarhus University.

Contact allergy to chlorhexidine (study II)

Study population

All patients patch tested with chlorhexidine from 2003 to 2013 at the Dermatology Clinic at Copenhagen University Hospital Gentofte were included.

Study design

Two studies were performed: (i) a retrospective database study and (ii) a questionnaire study. Regarding the database study, patient data were collected from the clinical database of contact allergy hosted in the department. Information available included age, MOAHLFA index (Male,

Occupation, Atopic Dermatitis, Leg dermatitis, Hand dermatitis, Facial dermatitis and age > 40 years) and results of patch tests.

Regarding the questionnaire study, all patients with a positive patch test to chlorhexidine in the inclusion period and who were still alive and living in Denmark received a questionnaire about their contact allergy to chlorhexidine. It was decided to ask specific questions about chlorhexidine and chlorhexidine allergy such as 'Do you know what product caused your allergy to chlorhexidine?' and 'Have you been exposed to chlorhexidine since the allergy was diagnosed?'. No control group was included as it would not have made sense for patients without the allergy to answer the questionnaire. The questionnaire consisted of 6 questions, see appendix III.

Immediate-type chlorhexidine allergy (studies III and IV)

Study population

Study III

Study III included all patients investigated for chlorhexidine allergy as part of the investigation at the Danish Anaesthesia Allergy Centre (DAAC) from July 2004 to July 2012 (n=343 patients).

Study IV

Inclusion criteria in study IV were:

- Patients diagnosed with chlorhexidine allergy in DAAC from January 1999 to March 2015
- Aged ≥ 18 years and still alive at time of inclusion

Overall, 44 patients had been diagnosed with chlorhexidine allergy in DAAC from January 1999 to March 2015, but 11 had since died and one was under the age of 18 years at time of inclusion.

Consequently, 32 patients fulfilled the inclusion criteria. The first patients were enrolled in 2013, but patients were consecutively enrolled until March 2015. Invitations included a letter with information about the study and a consent form. Patients who did not respond to the letter were contacted by telephone. Those who did not respond to the letter or telephone call were not contacted further.

In study IV, non-chlorhexidine allergic patients investigated at DAAC served as controls. They were matched with patients with regard to age and sex.

Study design

Study III

In study III, we calculated the prevalence of chlorhexidine allergy in patients with suspected perioperative allergic reactions by dividing the number of chlorhexidine allergic patients with the total number of patients investigated at DAAC during the inclusion period. We estimated the sensitivity and specificity of the tests by comparing test results with allergy status after completion of investigations at DAAC (allergy to chlorhexidine/not allergy to chlorhexidine).

To evaluate the influence of the positivity criterion for the intradermal test on the estimated sensitivity and specificity, the normal positivity criterion used at DAAC was first performed, that is (A) diameter of wheal \geq twice the diameter of the negative control, and subsequently two other positive criteria were applied: (B) diameter of wheal \geq 3 mm larger than negative control and (C) diameter of wheal \geq 8 mm independent of negative control.

Study IV

This study included retrospective blood samples as well as prospective blood samples. Blood samples were analysed for specific IgE using the ImmunoCAP-assay with a cut-off of 0.1kUA/l.

Retrospective blood samples

The following retrospective blood samples were available: a) a blood sample drawn shortly after the allergic reaction (one to three hours) leading to referral to DAAC for tryptase analysis; b) a blood sample drawn four to six week after the allergic reaction after initial contact with the patient; and c) a blood sample drawn two to four months after the allergic reaction during investigation at DAAC.

If not already available, specific IgE-analysis was done on all retrospective blood samples from patients as well as from controls on blood samples drawn at the time of investigation at DAAC. For blood samples with a specific IgE-value below 0.35kUA/l, a re-analysis was performed with the cut-off of 0.1kUA/l.

Prospective blood samples

Prospective blood samples were drawn for patients included in the study. The number of annual blood samples depended on time since allergic reaction:

- 0-2 years after allergic reaction: four annual blood samples were drawn
- 2-4 years after allergic reaction: two annual blood samples were drawn
- > 4 years after allergic reaction: one annual blood samples was drawn

Blood samples were stored at -20°C and all analyses were performed from September to December 2015.

Patient follow-up

All included patients were contacted by telephone at the end of the study and were asked whether they had been accidentally re-exposed to chlorhexidine after the diagnosis. Patients who did not respond to the telephone calls were mailed a letter telling them to contact the investigators. The time of re-exposure, product type used during re-exposure and allergy symptoms were noted.

Statistical analysis

For studies II and IV, data were processed using SPSS (SPSSTM Statistics, Chicago, IL, USA; IBM PASW Statistics) for WindowsTM, edition 22.0. The Mann-Whitney U test was used to detect differences in age in study II. The chi square test was applied for analysis of differences in the MOAHLFA-index and irritant reactions in study II. In study III, two-by-two tables were done manually after extracting data from the database. Sensitivity and specificity were calculated manually. In study IV, Microsoft Excel was also used.

For all manuscripts, the threshold for statistical significance was predefined as a p-value <0.05.

Ethical considerations

Study I did not include patients and therefore there was no need for ethical approval for this study. Before checking the cosmetic products, permission to do so was given by staff at the supermarkets, the beauty and retail store and the hair dressing salon. Studies II and III were based on retrospective

analysis of data collected during routine clinical practice at the Dermatology Clinic and at DAAC, and therefore ethical approval was not necessary.

According to the Local Human Ethics Committee, questionnaire studies do not require ethical approval. Therefore, there was no need for ethical approval of the questionnaire in study II.

The Local Human Ethics Committee approved the study protocol of study IV (Project ID H-3-2012-144). Patients were included after giving written informed consent. The Data Protection Agency approved storage of data for studies II and IV (Journal number 2007-58-0015).

Results and discussion of main findings

Use of chlorhexidine in cosmetic products (study I)

In total 2,251 cosmetic products were checked for chlorhexidine content. Of these, chlorhexidine was found in 80 products (3.6%), which is almost twice the number reported from Finland (1.9%)¹⁰. A total of 66 products came from international companies and 14 came from Danish companies, indicating that chlorhexidine is not only a preservative used in Denmark but probably also in many other countries. Chlorhexidine was mainly found in hair products (57 products), but it was also found in nine creams/ointments, four wet wipes, four face washes, three skin tonics, two make-up removers and one mouth wash, see table 1.

Product types	Products checked, n	Products with chlorhexidine, n (%)
Hair products, total	760	57 (8)
Shampoos	245	0
Conditioners	153	30 (20)
Hair treatments/masks	40	10 (25)
Hair dyes	22	13 (59)
Hair sprays	154	0
Hair wax/gels	88	1 (1)
Other leave-on hair products	58	3 (5)
Creams/ointments	324	9 (3)
Deodorants	262	0
Bath soaps	219	0
Body lotions	134	0
Hand soaps	108	0
Toothpastes	77	0
Aftershaves/shaving foams	70	0
Wet wipes	63	4 (6)
Lip balms	36	0
Make-up removers	25	2 (8)
Face washes	24	4 (17)
Skin tonics	22	3 (14)
Mouth washes	17	1 (6)
Face masks	11	0
Disinfectants	7	0
Miscellaneous	92	0
Total	2251	80 (3.6)

Table 1. Results of the market survey for chlorhexidine in cosmetic products.

The concentration of chlorhexidine was below the allowed limit of 0.3% in all 10 selected products, see table 2.

Product type	Concentration of chlorhexidine (%)
Conditioner	0.02
Hair treatment	0.01
Acne cream	0.12
Mild antiseptic ointment	0.02
Mild antiseptic ointment	0.05
Eye cream	0.15
Skin tonic	0.06
Skin tonic	0.04
Face wash	0.03
Face wash	0.05

Table 2. Concentration of chlorhexidine in 10 cosmetic products.

A limitation of the study is that not all 80 products were analysed and therefore we cannot be certain that all products contained chlorhexidine in a concentration below 0.3%.

The role of chlorhexidine in cosmetic products in contact allergy and immediate-type allergy is largely unknown. In Finland, it was found in 2011 that chlorhexidine in cosmetic products caused or worsened the symptoms in 19% of patients with a positive patch test with chlorhexidine¹⁰, indicating that chlorhexidine in cosmetic products is of clinical relevance for some patients with contact allergy.

Regarding immediate-type chlorhexidine allergy, there are no publications on chlorhexidine in cosmetic products causing allergic reactions. A possible reason for this is that concentrations used in cosmetic products are too low to cause an immediate-type allergic reaction especially considering that chlorhexidine only penetrates poorly into the deeper layers of the skin⁷⁶. Most patients with immediate-type allergy to chlorhexidine are probably sensitized in the healthcare setting, but it remains unknown whether chlorhexidine in cosmetic products may sensitize some patients. One could speculate that exposure on broken skin or a broken mucous membrane could lead to sensitization.

Contact allergy to chlorhexidine (study II)

Overall, 8,497 patients were patch tested with chlorhexidine diacetate and/or chlorhexidine digluconate at the Dermatology Clinic at Copenhagen University Hospital Gentofte from 2003 to 2013 and these patients were included retrospectively. In total 110 patch tests with chlorhexidine diacetate or chlorhexidine digluconate were positive in 82 patients (1.0% of all patients patch tested with chlorhexidine). This finding is in line with prevalences reported from other countries^{10,18,19}, but lower than the prevalences published from Denmark in the 1980s¹⁴⁻¹⁶. In figure 4 the prevalence rates for both salts can be seen. This figure shows a decrease in the number of positive patch tests over time from 1.7% in 2003 to 0.3% in 2013. Test concentrations were lowered 1 September 2008 from 1.0% to 0.5%. The overall prevalence from 1 January 2003 to 31 August 2008 was 1.4% (54/3,867). The overall prevalence from 1 September 2008 to 31 December 2013 was 0.6% (28/4,630). Overall, there was a significantly higher rate of irritant reactions in the group tested with 1.0% chlorhexidine diacetate and 1.0% chlorhexidine digluconate than in the group tested with 0.5% chlorhexidine diacetate and 0.5% chlorhexidine digluconate (3.6% [n=279]) and 0.4% [n=39]), $p < 0.00001$). In particular 1.0% chlorhexidine diacetate produced many irritant reactions (5.1% [n=199]).

This raises the question: what is the optimal test concentration? Currently this is unknown. Based on the personal experience of members of the EAACI/ENDA Drug Allergy Interest Group, testing with 1.0% is recommended by the group⁷⁰. Nonetheless, as found in a previous study as well as in this study, especially 1.0% chlorhexidine diacetate is a strong irritant²¹. In addition, in two older studies it was shown that positive patch tests are difficult to reproduce when testing with 1.0% chlorhexidine digluconate^{15,16}. These findings indicate that 1.0% is too high a test concentration. Nonetheless, it is unclear whether testing with 0.5% is reproducible or whether it causes false negative reactions. Trolab® and Chemotechnique® produce test substances in a concentration of 0.5%⁷⁷⁻⁷⁹ and this is also the test concentration used by other centres^{10,18}.

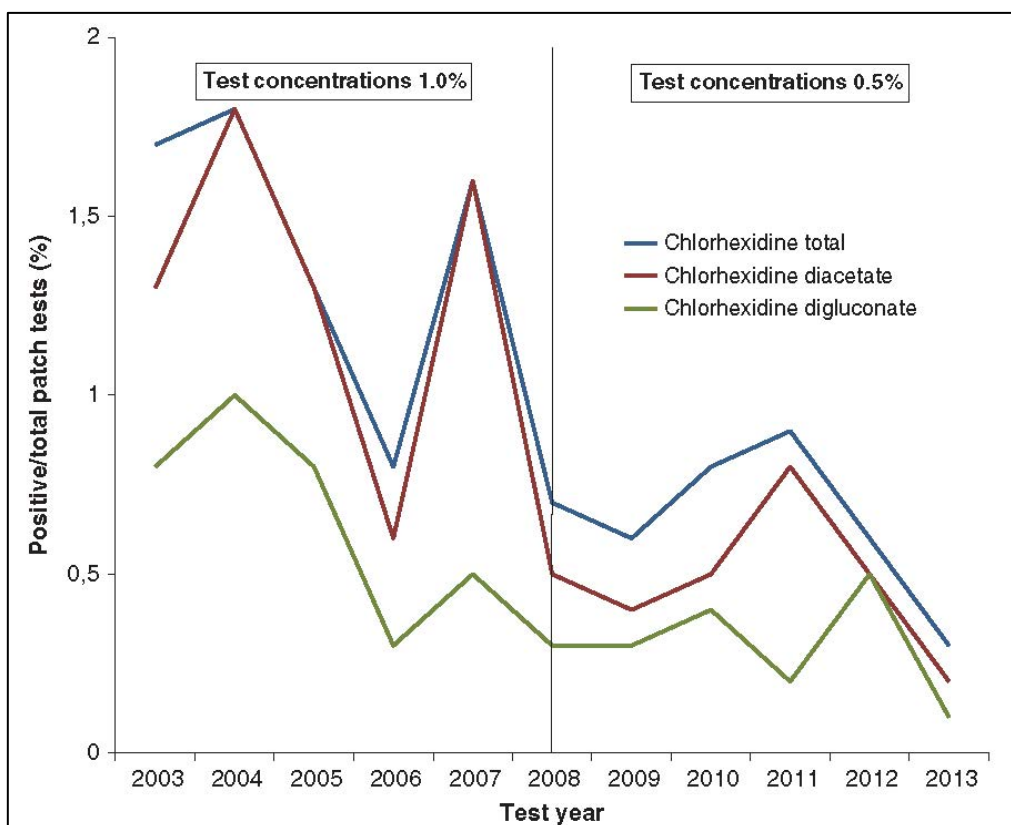


Figure 4. Prevalence of positive patch tests to chlorhexidine.

Of the 82 patients with positive patch test reactions to chlorhexidine, 28 (0.3%) had positive reactions to both chlorhexidine salts, 43 (0.5%) had a positive reaction to chlorhexidine diacetate only and 11 (0.1%) had a positive reaction to chlorhexidine digluconate only, see figure 5.

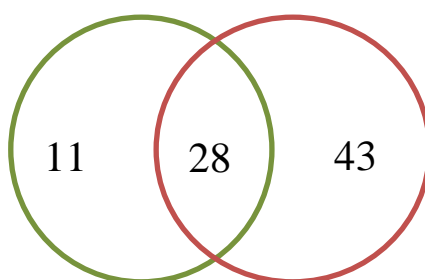


Figure 5. Number of positive patch tests with chlorhexidine digluconate (green), diacetate (red) or both salts (middle).

In a review of the 42 products used in the hospitals in the Capital Region of Denmark (appendix II), 27 contained chlorhexidine digluconate, six contained chlorhexidine diacetate and three contained chlorhexidine dihydrochloride (the salt could not be identified in six products). In a review of the 80

cosmetic products containing chlorhexidine (study I), 42 products contained chlorhexidine digluconate, 33 contained chlorhexidine dihydrochloride and none contained chlorhexidine diacetate (the salt could not be identified in five products). Due to different molecular weights of chlorhexidine diacetate (626 g/mol) and chlorhexidine digluconate (898 g/mol), there are 43% more chlorhexidine molecules in test substances containing chlorhexidine diacetate than in those containing chlorhexidine digluconate ($898/626=1.43$). Although chlorhexidine digluconate is more frequently used in both healthcare products and cosmetic products, more than half of the patients were positive only to the chlorhexidine diacetate salt. It could be speculated that more patients had positive reactions to chlorhexidine diacetate simply because there was a higher number of chlorhexidine molecules in these test substances. However, 11 of the 82 patients tested positive to chlorhexidine digluconate only, making it uncertain whether sensitization is caused by chlorhexidine itself or by the salts consisting of chlorhexidine and diacetate or digluconate. Until this has been further investigated, testing with both salts is recommended. It remains unknown why different salts are used in different products and although chlorhexidine dihydrochloride is widely used in cosmetic products in Denmark, it is not included in the diagnostic testing in our clinic and probably not in any other clinic.

Of the 82 patients with a positive patch test reaction to chlorhexidine, 29 were skin prick tested and 3 (10%) were positive, indicating a combined contact allergy and immediate-type allergy. A limitation of this study is that specific IgE to chlorhexidine was not measured and therefore we cannot be absolutely certain that these patients have immediate-type chlorhexidine allergy.

Nonetheless, it seems that some patients with contact allergy to chlorhexidine also have immediate-type chlorhexidine allergy and this finding is in line with case reports on patients with combined contact allergy and immediate-type allergy to chlorhexidine⁶⁵⁻⁶⁸. Therefore it is recommended also to test for immediate-type allergy in patients with contact allergy to chlorhexidine.

The findings for the MOAHLFA-index can be found in table 3.

Total tested	% (n) positive (n = 82)	% (n) negative (n = 8415)	p-value*
Male sex	58.5 (48)	32.5 (2735)	<0.00001
Occupational	15.9 (13)	19.8 (1664)	0.37
Atopic dermatitis	12.2 (10)	16.8 (1417)	0.26
Hand dermatitis	41.5 (34)	38.7 (3258)	0.61
Leg dermatitis	18.3 (15)	4.7 (392)	<0.00001
Face dermatitis	15.9 (13)	24.1 (2029)	0.08
Age above 40 years	74.4 (61)	66.0 (5554)	0.11
Significant values are in bold.			
*Pearson chi-square.			

Table 3. MOAHLFA index for patients with positive and negative patch test results to chlorhexidine (a positive reaction to patch test with chlorhexidine diacetate, chlorhexidine digluconate or both).

A positive patch test with chlorhexidine was significantly associated with male sex and leg dermatitis. Although the number of patients aged above 40 years was not significantly higher in the patch test positive group, median age was significantly higher in the patch-test positive group (55 years [IQR 38-65 years]) than the patch test negative group (47 years [IQR 34-60 years]), $p=0.01$. These findings are in line with Danish studies from the 1980s¹⁴⁻¹⁶. Back then, it was believed that dressings containing 0.5% chlorhexidine diacetate caused many reactions. Indeed, these dressings are still used in Denmark, and 5 out of 47 patients in the questionnaire (results shown in table 4) reported exposure to chlorhexidine in dressings. Therefore it seems that chlorhexidine in dressings may sensitize some patients.

Of the 82 patients with a positive patch test to chlorhexidine, 66 were still alive and living in Denmark and were mailed the questionnaire. The response rate was 71% (47/66). The MOAHLFA-index and test results were comparable to the whole group of patch test positive patients, see manuscript II for more details.

Of the 47 patients who responded, 19 (40%) reported a known cause of the allergy, see table 4.

Both products used in the healthcare setting and cosmetic products were reported. There are several possible reasons for the 28 patients not reporting a known cause. First, a positive patch test to chlorhexidine is a sign of sensitization, but this may not result in allergic symptoms in all patients. Second, some patients may not remember an exposure which took place a long time ago. Third,

some patients may have been exposed to chlorhexidine in a product containing chlorhexidine without knowing that it contained chlorhexidine. Indeed, the latter may very well be the case in some patients because only 38% knew that chlorhexidine can be used in cosmetic products while 83% knew that it can be used in hospitals and by dentists. Although exposure assessment is an important part of allergy investigation, data saved in the database from the time of the original investigation were largely incomplete, and could therefore not be used as a supplement in this study.

	Number (%)	Reported products (n)
Patients reporting a known cause	19 patients (40)	Cream (7); hair product (5); make-up or make-up remover (1); wet wipe or other skin disinfectant (5); mouth wash (2); wound dressing (5); product used at the hospital or by a doctor (6); product used by a dentist (3); other (2)*
Patients reporting re-exposure after the diagnosis was established	15 patients (32) (of these, 13 patients reported symptoms)	Cream (5); hair product (2); wet wipe or other skin disinfectant (7); mouth wash (3); wound dressing (4); product used at the hospital or by a doctor (6); product used by a dentist (3); other (1)*
In total, 22 patients (47%) reported a known cause and/or symptoms at re-exposure (some patients reported both a known cause and symptoms at re-exposure).		
*Many reported more than one cause/exposure.		

Table 4. Questionnaire results regarding cause of the allergy and possible re-exposure.

Overall, 15 patients (32%) reported a known re-exposure to chlorhexidine in either healthcare products or cosmetic products after diagnosis was established, see table 4. Of the 15 patients, 13 reported symptoms at exposure: rash and/or skin itching (n=12), general discomfort (n=4) and breathlessness (n=2) were reported. As shown above, on the one hand, many patients were not aware of sources of chlorhexidine, and the number of re-exposures may be underestimated. On the other hand, it may be that the responders were those who were most troubled by the contact allergy, creating a selection bias. Nevertheless, the high number of re-exposures highlights how difficult it is to avoid chlorhexidine and how important it is to inform the patient about possible routes of exposure. Finally, it is important that healthcare personnel are not the ones causing the re-exposure

by accidentally administering a product containing chlorhexidine. Chlorhexidine content is not always easy to identify on medical devices^{7,8}. It is therefore recommended that healthcare personnel develop a strategy to identify chlorhexidine-containing products in their local hospital to reduce the risk of accidental re-exposure in patients with chlorhexidine allergy.

Immediate-type chlorhexidine allergy (studies III and IV)

Study III

A total of 343 patients were investigated for chlorhexidine allergy at the Danish Anaesthesia Allergy Centre (DAAC) from July 2004 to July 2012. To obtain a homogenous group, only patients who had all four tests for chlorhexidine allergy performed (skin prick test, intradermal test, specific IgE and histamine release test) were retrospectively included (n=228, 214 adults/14 children, 141F/87M, mean age 49 years).

Traditionally, the drug provocation test is considered the gold standard for diagnosing drug allergy. Nonetheless, there is currently no internationally accepted provocation model for chlorhexidine allergy⁸⁰. An ideal provocation model should mimic the original exposure at the time of the allergic reaction. During surgery, patients are exposed to chlorhexidine in varying concentrations through numerous products that come in contact with the skin and mucous membranes (skin disinfectants, skin swabs, urethral gels, central venous catheters) and this situation is impossible to reproduce for test purposes. The second best option would be to challenge in only one of the locations. A provocation model on intact skin would be simple, but chlorhexidine only poorly penetrates intact skin⁷⁶ and therefore this is not useful. Another option would be to challenge on the oral mucosa, which would also be simple. However, exposure on oral mucosa only very rarely causes allergic reactions⁸¹, and it may very well be that some of the true chlorhexidine allergic patients would not be exposed in sufficient amounts to elicit an allergic reaction as long as the oral mucosa is intact. Therefore, this provocation model could very well lead to false-negative results. An intravenous provocation would probably be an excellent way to detect the allergy, but this model would require extensive ethical and technical consideration before attempting. A urethral provocation

model would maybe also be useful but difficult to justify for test purposes. A conjunctival provocation model could perhaps produce symptoms in some chlorhexidine allergic patients, but this is far from an optimal provocation model because it would not at all mimic the original exposure. All taken together, it is difficult, if not impossible, to create a realistic provocation model for chlorhexidine.

With the lack of a gold standard (provocation model), it is not possible to calculate exact sensitivities and specificities. However, to solve this dilemma and for the purpose of this study, we defined chlorhexidine allergy as one or more relevant clinical reactions combined with a minimum of two positive tests, and we used this definition to estimate the sensitivity and specificity. This definition has previously been applied for Rocuronium, another drug for which a provocation model is currently not available⁸². To estimate sensitivity and specificity for each test, the result of the test in question was omitted from the diagnostic calculation and results of the remaining three tests were used to define the patient as allergic or non-allergic.

Overall, 32 patients had a minimum of one positive test for chlorhexidine, see figure 6. Of these, 22 patients (9.6%) met the definition of chlorhexidine allergy (20 adults/2 children, 4F/18M, mean age 57 years). Although these results show that chlorhexidine allergy is common among patients with perioperative allergic reactions, it has to be remembered that around 3,600,000 surgeries were performed in Denmark from July 2004 to July 2012. Consequently, chlorhexidine caused allergic reactions in around 1:150,000 (3,400,000/22) surgeries, indicating that it is a rare allergy in the general population. All 22 patients were specific IgE-positive, 21 were skin prick test-positive, 15 were intradermal test-positive and 12 were histamine release test-positive. Ten patients were positive only in one of the tests and all these test results were just above the cut-off value, and five of the ten patients had another verified allergy. In these ten patients, a provocation model would really be useful as it is uncertain whether these tests are false positive or true positive.

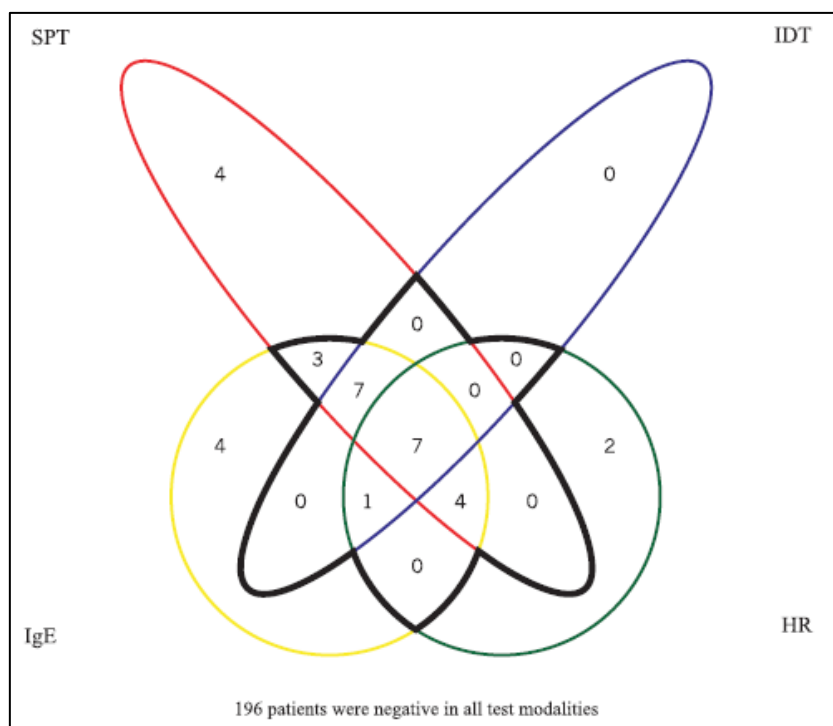


Figure 6. Number of patients with one or more positive test for chlorhexidine. The patients within the black line met the definition of chlorhexidine allergy.

Using our definition of chlorhexidine allergy, the estimated sensitivity and specificity were high for both the skin prick test (sensitivity 95% and specificity 97%) and the specific IgE (sensitivity 100% and specificity 97%). The estimated sensitivity was low, but specificity was high for both the intradermal test (sensitivity 68% and specificity 100%) and the histamine release test (sensitivity 55% and specificity 99%), see table 5.

	Allergy*	No allergy	Estimated sensitivity in %	Estimated specificity in %
IgE positive	19/19	7/209	100	97
HR positive	12/22	2/206	55	99
SPT positive	18/19	7/209	95	97
IDT	15/22	0/206	68	100

HR, histamine release test; SPT, skin prick test; IDT, intradermal test.
 *Allergy was defined as a minimum of two positive tests omitting the test in question to avoid comparing the test with itself; thus, the number of patients with allergy varies from test to test.

Table 5. Test results compared with allergy status to chlorhexidine.

We were surprised by the low sensitivity for the intradermal test. In the intradermal test, the allergen is injected into the dermis, which is vascularised. In contrast, in the skin prick test the allergen is pricked into the epidermis, which is more superficial and without vascularisation. Intuitively, the intradermal test should therefore be more sensitive than the skin prick, and this has also been reported in several publications⁸³⁻⁸⁵. It could be that the test concentration was too low (0.002 mg/ml) and therefore the chlorhexidine allergic patients did not react. However, increasing the concentration is not possible because higher concentrations produce irritant reactions⁸⁶. It could also be that the positivity criterion caused the low sensitivity. Currently, there is no international consensus on the criterion for a positive intradermal test. Therefore, we investigated whether applying other positivity criteria could increase the estimated sensitivity and specificity for the intradermal test. The French Society of Anaesthesiologists (Société Française d'Anesthésie et de Réanimation [SFAR]) recommends (A) the positivity criterion of a wheal diameter of a minimum twice the size of the induced bleb^{71,83,87} and this criterion was used in previous calculations with the modification that we compared with a negative control instead of its own bleb. The EAACI/ENDA Drug Allergy Interest Group recommends (B) an increase in wheal of a minimum of 3 mm associated with a flare after 15-20 minutes⁸⁴. A third commonly used positivity criterion is (C) a wheal diameter of 8 mm independent of negative control^{88,89}.

	Positivity criterion A	Positivity criterion B	Positivity criterion C
Estimated sensitivity (%)	68	86	91
Estimated specificity (%)	100	100	95
Positivity criterion A, diameter of wheal \geq twice the diameter of negative control; positivity criterion B, diameter of wheal \geq 3 mm larger than negative control; positivity criterion C, diameter of wheal \geq 8 mm independent of negative control.			

Table 6. Estimated sensitivity and specificity for the intradermal test when applying different positivity criteria.

As can be seen in table 6, although specificity was a little lower for positivity criterion C (diameter of wheal of a minimum of 8 mm independent of negative control), both this positivity criterion and positivity criterion B (an increase in wheal of a minimum of 3 mm) resulted in higher estimated

sensitivity. These findings suggest that either of these should be used in the future. However, there is one important limitation: the test protocol changed in the study period. Until January 2011, a bleb of 3-5 mm was induced without knowing the induced volume. Since January 2011, a fixed volume of 0.02 mL has been induced. Therefore, it is up for discussion whether data from the two test periods can be pooled. Nonetheless, based on the results from this study, positivity criterion B (an increase in wheal of a minimum of 3 mm) has been used at DAAC since 2014.

Study IV

Informed consent was obtained from 23 of the 32 patients fulfilling the inclusion criteria: a total of seven patients were not interested in participating in the study and two patients did not respond to the letter or telephone call. Of the 23 included patients in this study, 15 were also included in study III. Overall, 114 blood samples from the patients (55 retrospective and 59 prospective) and 23 from controls (retrospective) were included in the study.

Graphical representation of all specific IgE-results is not meaningful because of highly variable initial values and dynamics, but all results can be found in appendix 1 in manuscript IV.

Specific IgE at time of reaction and in subsequent weeks/months (retrospective samples)

A total of eight patients had a specific IgE-result available from time of reaction and values showed great variation (0.24kUA/l to 66.7kUA/l). In six of the eight blood samples specific IgE was above 0.35kUA/l. Previous studies on chlorhexidine, ethylene oxide and neuromuscular blocking agents have also shown that specific IgE can be elevated at the time of reaction^{54,62,90}. However, the test should be repeated after a few weeks/months as specific IgE is likely to increase. Indeed, specific IgE increased over the subsequent weeks/months in seven of eight patients; in the last patient blood samples were only available from days 0, 5 and 9 and here an increase was not observed. Specific IgE was >0.35kUA/l in 22 of 23 patients at the time of investigation at DAAC including all eight patients with a specific IgE available at time of reaction.

In all 23 controls, specific IgE was below 0.35kUA/l at the time of investigation at DAAC and in 18 of these below 0.1kUA/l (data not shown). The five controls with a detectable specific IgE above 0.1kUA/l had values up to 0.24kUA/l.

Dynamics of specific IgE over time in patients with no known re-exposure (retrospective and prospective samples)

One patient with many blood samples available and no known re-exposure was selected to illustrate the dynamics of specific IgE over time, see figure 7.

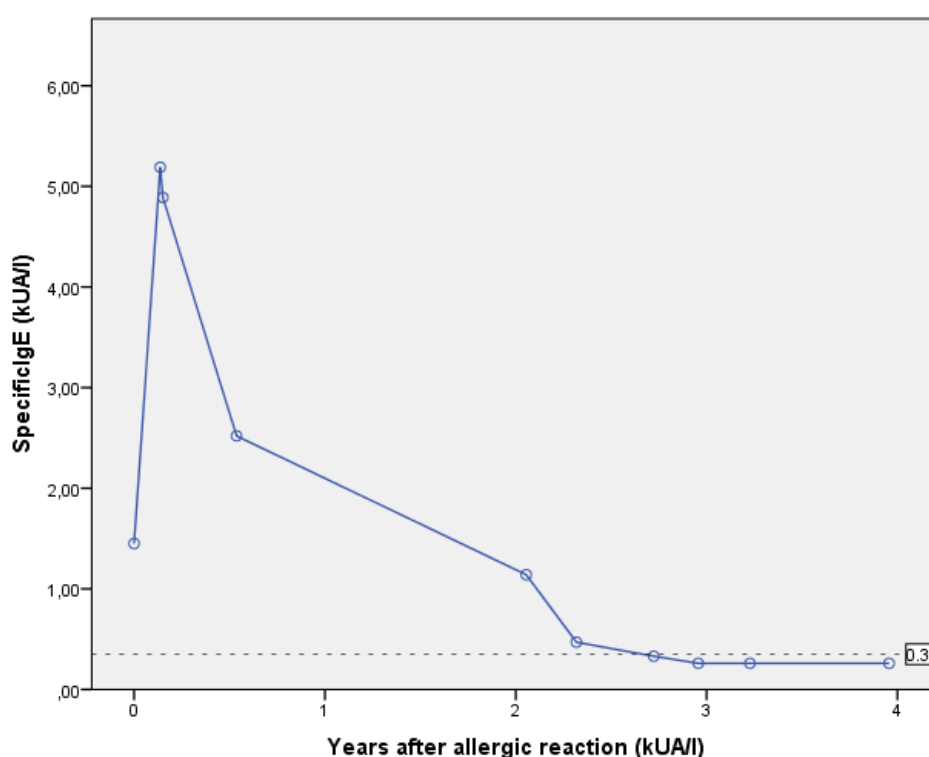


Figure 7. Specific IgE over time in a chlorhexidine allergic patient with no known re-exposure (patient 16 in appendix 1 in manuscript IV). T=0 is time of allergic reaction.

It is illustrated in figure 7 that specific IgE increased in the first months after the allergic reaction but then subsequently declined and eventually fell below 0.35kUA/l. Lower values of specific IgE were measured in blood samples drawn at the end of the study compared with values measured during investigations at DAAC in 21 of 23 patients. Notably, 17 of 23 patients had a specific IgE-value below 0.35kUA/l measured during the study, and a value below 0.1kUA/l was measured in seven of these. These findings indicate that specific IgE can indeed decline below 0.35kUA/l and

also 0.1kUA/l in chlorhexidine allergic patients with previously elevated levels. Shortest interval from allergic reaction to a value below 0.35kUA/l was four months (patient 11 in appendix 1 in manuscript IV) and shortest interval to a value below 0.1kUA/l was 64 months (also patient 11). These findings indicate that in some patients, it may be that testing within six months after the allergic reaction, as recommended by the manufacturer, is too late. The rate of decline seemed to vary greatly between patients and in four patients, specific IgE remained above 0.35kUA/l for many years without a known re-exposure (patients 7, 8, 10 and 13 in appendix 1 in manuscript IV). Consequently, it is not possible to predict how long specific IgE will remain elevated in a chlorhexidine allergic patient. This emphasizes that the diagnosis of chlorhexidine allergy should not be based solely on specific IgE but testing should also include skin prick test as a minimum. However, to complicate matters, the dynamics of skin test results to chlorhexidine over time remain unknown and therefore it is unknown whether the sensitivity of the skin prick test is still high when testing is performed long after the allergic reaction.

Dynamics of specific IgE in patients with known re-exposure (retrospective and prospective samples)

Overall, 20 of 23 patients responded to the telephone call or letter at the end of the study to identify accidental re-exposures. Of these, seven patients (35%) reported nine accidental re-exposures in the healthcare setting. Seven re-exposures were symptomatic and two were asymptomatic. Three re-exposures took place during surgery and they all resulted in anaphylactic shock and in two patients a subsequent increase in specific IgE was observed, see figure 8A and 8B (there was no blood sample available close to the reaction in the third patient). Two re-exposures were caused by chlorhexidine in urethral gels. This resulted in local symptoms in one patient (patient 15 in appendix 1 in manuscript IV) with no increase in specific IgE (0.24kUA/l one year before re-exposure and 0.21kUA/l two months after re-exposure), whereas the other patient reported that re-exposure was asymptomatic, but specific IgE had increased in a subsequent blood sample, see figure 8C. Four re-exposures were caused by chlorhexidine in skin swabs. One patient reported asymptomatic re-exposure and another general discomfort and redness of the skin, but specific IgE

was not measured in close relation to these re-exposures. A third patient reported local symptoms only and specific IgE was $<0.1\text{kUA/l}$ one year after this exposure, see figure 8A. In the fourth patient, exposure resulted in anaphylactic shock and an increase in specific IgE was observed, see figure 8B.

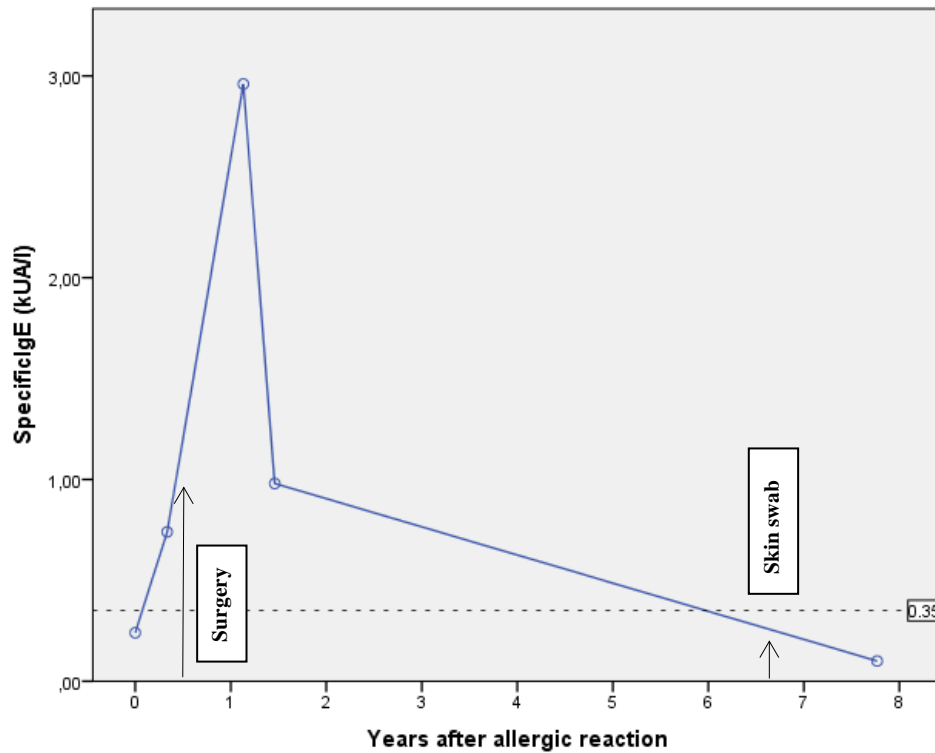


Figure 8A. Specific IgE in patient re-exposed twice (patient 5 in appendix 1 in manuscript IV). T=0 is time of original reaction. Black arrow indicates symptomatic re-exposure.

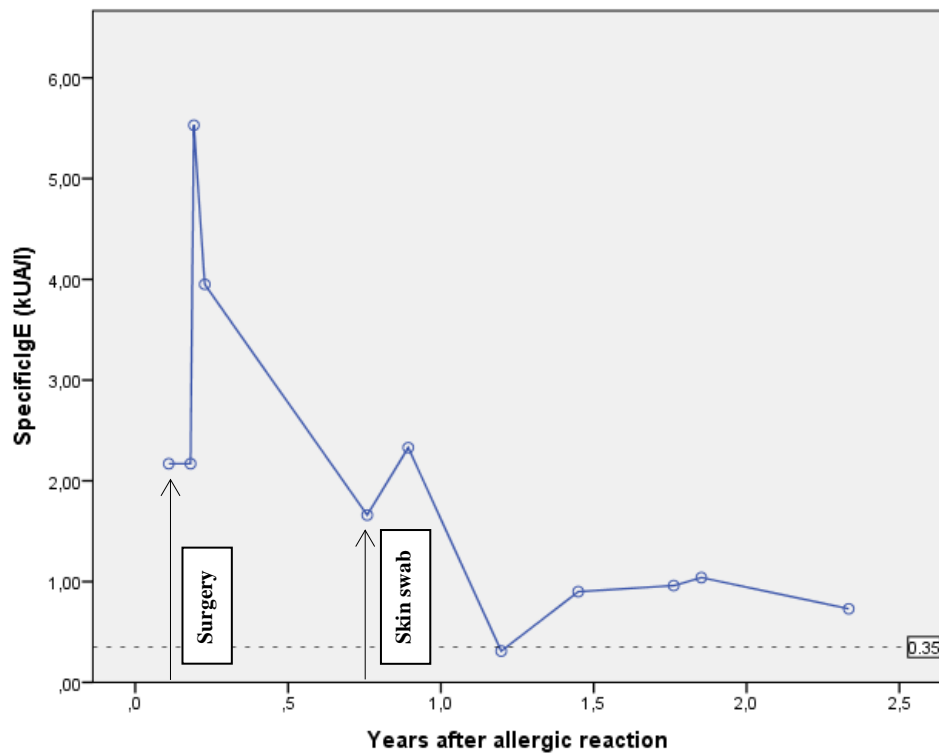


Figure 8B. Specific IgE in patient re-exposed twice (patient 18 in appendix 1 in manuscript IV). T=0 is time of original reaction. Black arrow indicates symptomatic re-exposure.

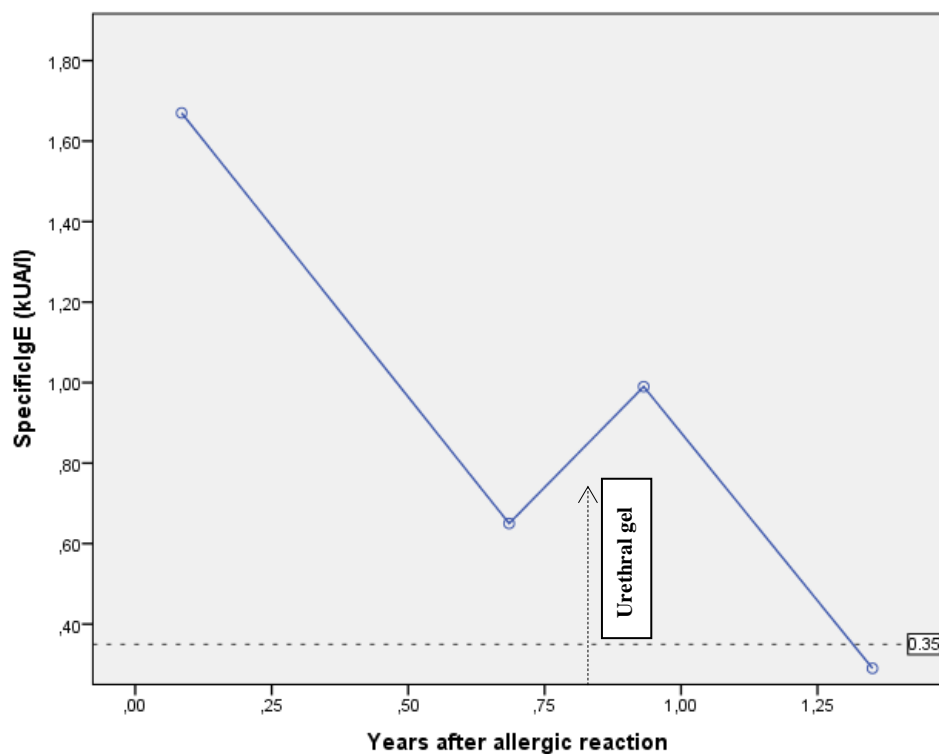


Figure 8C. Specific IgE in patient re-exposed once (patient 19 in appendix 1 in manuscript IV). T=0 is time of original reaction. Dotted arrow indicates asymptomatic re-exposure.

Altogether, most re-exposures caused symptoms and subsequent increase in specific IgE.

Nonetheless, some re-exposures caused symptoms only with no increase in specific IgE while other re-exposures were asymptomatic but resulted in an increase in specific IgE. It seems that patients can develop symptoms even when specific IgE is below 0.35kUA/l and this is in line with penicillin allergy where it has been shown that some patients with specific IgE below 0.35kUA/l still develop symptoms on subsequent drug provocation⁶³.

The effect of chlorhexidine in cosmetic products on levels of specific IgE is unknown. As previously described, chlorhexidine penetrates intact skin poorly⁷⁶ but it may be that exposure on a broken skin barrier or mucous membrane influences the levels of specific IgE. Indeed, in the current study, specific IgE increased at times where patients denied re-exposure and perhaps unknown exposure to chlorhexidine in cosmetic products or in healthcare products could play a role.

Conclusions and perspectives for further research

Use of chlorhexidine

In study I it was shown that chlorhexidine is not only widely used in the healthcare setting but also in cosmetic products: chlorhexidine was found in 3.6% of the checked cosmetic products in Denmark. It was found mainly in hair products but also in some creams/ointments, wet wipes, face washes, skin tonics, make-up removers and in a mouth wash. The concentrations used were below the allowed limit of 0.3% in 10 checked products. In terms of contact allergy, chlorhexidine in cosmetic products is of clinical relevance, see study II, but it is unknown whether this is also the case for immediate-type chlorhexidine allergy. Future research could focus on investigating whether exposure on broken skin or mucous membranes can lead to sensitization. These studies could for instance include experimental animal studies.

Contact allergy to chlorhexidine

Although high prevalences of contact allergy to chlorhexidine were reported from Denmark in the 1980s, the prevalence found in study II is similar to that reported from other European countries: 1.0% of patients patch tested with chlorhexidine at the tertiary Dermatology Clinic at Copenhagen University Hospital Gentofte from 2003 to 2013 tested positive. Over the 11-year period, a decline in the number of positive tests was observed. This is probably due to a change in test concentrations from 1.0% to 0.5% in 2008, but it could also be due to fewer patients being sensitized. Further research should aim at finding the optimal test concentration. These studies could for instance include repeated open application testing (ROAT) of solutions of chlorhexidine as this better mimics the original exposure than the patch test. Male sex, leg eczema and higher age were more predominant in those with the contact allergy than in those without. It is uncertain whether sensitization is caused by chlorhexidine itself or by the salts consisting of chlorhexidine and diacetate or digluconate. This should be investigated in further studies, but until this is done, testing with both salts is recommended. Although chlorhexidine dihydrochloride is used in cosmetic products, it has never been used in patch testing. Future studies should investigate whether this salt

causes sensitization/symptoms for instance by including the salt in patch testing for chlorhexidine contact allergy.

Three patients were both patch-test positive and skin prick test positive, indicating a combined immediate-type allergy and contact allergy. A few case reports have also described patients with both contact allergy and immediate-type chlorhexidine allergy. Therefore it seems beneficial to test for immediate-type allergy in patients with contact allergy to chlorhexidine. Future studies should focus on investigating the mechanism behind this combined allergy. These studies could for instance include experimental animal studies.

In the questionnaire study, both products used in the healthcare setting and cosmetic products were reported as causes of the contact allergy. More than half of the patients were not aware of the use of chlorhexidine in cosmetic products, but most of the patients were aware of its use in the healthcare setting. Notably, 15 patients (32%) were re-exposed after diagnosis and 13 of these reported symptoms at re-exposure.

Immediate-type allergy to chlorhexidine

In study III it was shown that immediate-type chlorhexidine allergy is common among patients with perioperative allergic reactions in Denmark: overall, 9.6% (n=22) of all patients tested at the Danish Anaesthesia Allergy Centre from July 2004 to July 2012 tested positive to chlorhexidine. Therefore, all patients with a suspected perioperative allergic reaction should be tested for chlorhexidine allergy. With the lack of a provocation model, chlorhexidine allergy was defined as a relevant clinical history in combination with a minimum of two positive tests. Future studies should investigate whether this definition is an acceptable way of diagnosing drug allergy. These studies could for instance evaluate the definition for drugs where a provocation model is available.

Alternatively future studies could aim at developing a useful provocation model for chlorhexidine allergy. The skin prick test and specific IgE were found to have the highest estimated combined sensitivities and specificities, and these tests should be performed as a minimum when investigating patients for immediate-type chlorhexidine allergy. Notably, the intradermal test had a low sensitivity but a high specificity. The low sensitivity was probably a result of the applied positivity

criterion (wheal diameter \geq twice the diameter of negative control). When applying other positivity criteria (wheal \geq 3 mm larger than negative control; wheal diameter of 8 mm independent of negative control) the sensitivity increased markedly. As a consequence of the results found in this study, the positivity criterion used at DAAC was changed in 2014 to wheal \geq 3 mm larger than the induced bleb. Future studies should focus on investigating the optimal positivity criterion for the intradermal test. These studies could follow the same method as in this study for drugs with an available provocation model. Although chlorhexidine diacetate is the salt most frequently causing contact allergy, skin prick testing is currently only performed with chlorhexidine digluconate. Future studies should investigate the role of chlorhexidine diacetate in immediate-type chlorhexidine allergy as well as cross-reactivity with other molecules such as proguanil. These studies could for instance include inhibition studies in the ImmunoCAP-assay or stimulation studies in histamine release testing. Alternatively skin testing with these molecules could be performed. In study IV it was shown that specific IgE varies greatly over time and between patients. At the time of reaction most patients had a specific IgE-value above 0.35kUA/l but some only became positive after a few weeks/months. After an initial increase, specific IgE gradually declined to values below 0.35kUA/l and even 0.1kUA/l on lack of exposure. This emphasizes that time elapsed from the allergic reaction should always be considered when analysing specific IgE-results. The fastest decline below 0.35kUA/l was four months, indicating that in some patients testing within six months after the allergic reaction, as recommended by manufacturer, may be too late. Dynamics of skin test results to chlorhexidine over time are unknown and it is therefore unknown whether the skin prick test still has high sensitivity when performed long after the allergic reaction. This should be the focus of future studies and these could for instance include the same method used in this study. Further studies could also investigate the role of other immunoglobulins e.g. IgA, which is found on mucous membranes where exposure to chlorhexidine often takes place. Re-exposure to chlorhexidine in the healthcare setting is common (35% in this study). Most re-exposures caused symptoms (also in a patient with specific IgE<0.35kUA/l) and an increase in specific IgE.

In conclusion, this thesis concerning contact allergy and immediate-type allergy to chlorhexidine contributes to the research area with the following observations:

- Chlorhexidine is widely used not only in the healthcare setting but also as a preservative in cosmetic products in Denmark. Re-exposures are common both in patients with contact allergy and in patients with immediate-type allergy to chlorhexidine. This highlights that healthcare workers need to be well informed about sources of exposure when treating a patient with chlorhexidine allergy.
- High prevalences of contact allergy to chlorhexidine were reported from Denmark in the 1980s, but we found that it is not higher at our tertiary dermatology clinic than in other European countries (1% of all tested). Contact allergy to chlorhexidine can be caused by many different healthcare products and cosmetic products, demonstrating the importance of thorough exposure assessment during allergy investigations.
- Immediate-type chlorhexidine allergy is common among patients with suspected perioperative allergic reactions (9.6%) and testing for chlorhexidine should therefore be performed routinely during allergy investigations of these patients. Specific IgE and skin prick test both have high estimated sensitivities and specificities and these tests should therefore be performed as a minimum. Levels of specific IgE can decline below 0.35kUA/l over time, but this does not necessarily indicate tolerance. Consequently, time since allergic reaction should be considered when analysing specific IgE-results, as results can become false negative over time.

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Manuscripts

Manuscript I: **Opstrup MS**, Johansen JD, Bossi R, Lundov MD, Garvey LH.

Chlorhexidine in cosmetic products – a market survey.

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Chlorhexidine in cosmetic products – a market survey

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Summary

Background. Chlorhexidine may cause type I and type IV allergy. Some chlorhexidine-allergic individuals have been exposed in the healthcare setting as patients or healthcare workers, but for others the source of sensitization is unknown. Chlorhexidine may be used as a preservative or an antimicrobial agent in cosmetic products at a concentration up to 0.3%, as set by the European Cosmetics Directive (now Regulations).

Objectives. To identify cosmetic product types containing chlorhexidine, and to measure the concentration of chlorhexidine in selected products.

Methods. Between February 2013 and April 2013, we checked for chlorhexidine in cosmetic products in 14 supermarkets, one hairdressing salon and one beauty and retail store in Copenhagen, Denmark by reading the ingredient labels. The chlorhexidine concentration was measured in 10 selected products by high-performance liquid chromatography (HPLC) with an ultraviolet (UV) detector.

Results. Chlorhexidine was found in 80 of 2251 checked products (3.6%) in the following categories: hair products (57/760), creams (9/324), face washes (4/24), wet wipes (4/63), skin tonics (3/22), make-up removers (2/25), and mouth washes (1/17). Chlorhexidine concentrations were 0.01–0.15%.

Conclusions. We found chlorhexidine in various cosmetic product types, predominantly aimed at females, and in hair products. The measured chlorhexidine concentrations were all within the permitted limit. The relevance for allergic sensitization should be further explored.

Key words: allergy; chlorhexidine; cosmetic products; exposure concentration; market survey.

Chlorhexidine is a very effective and commonly used disinfectant (1, 2), and, although the allergenic potential is often overlooked, it may cause type I and type IV allergy

(3, 4). For both types of allergy, the number of publications in the literature is rising (5). Some chlorhexidine-allergic individuals have no known previous exposure to chlorhexidine in the healthcare setting or elsewhere, and among these the route of sensitization is unknown.

Chlorhexidine is widely used as a disinfectant on skin and mucous membranes in the healthcare setting (5, 6), and here chlorhexidine may sensitize both patients (3) and, rarely, healthcare workers (7, 8). Outside the healthcare setting, chlorhexidine may be used in cosmetic products as a preservative or antimicrobial agent. In the United States, chlorhexidine is reported to be used in a wide range of cosmetic products (5). In Europe, a recent study from

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Finland reported that the Helsinki Asthma and Allergy Association had found chlorhexidine in 153 of 8012 cosmetic products (1.9%) from 1999 to 2008 (3). In the same study, it was found that cosmetic products caused or worsened the allergic symptoms in 7 of 36 patients (19%) with type IV chlorhexidine allergy (3). These findings indicate that, in Finland, chlorhexidine is indeed used in cosmetic products and is of clinical relevance. However, the extent of use of chlorhexidine in cosmetic products in other European countries is not known.

Chlorhexidine is allowed in cosmetic products at a concentration up to 0.3%, as set by the European Cosmetics Directive (now Regulations) (9). We could not find any studies in the literature that had estimated the concentration of chlorhexidine in cosmetic products.

The aim of this study was to identify cosmetic product types containing chlorhexidine that are available to Danish consumers, and to measure the concentration of chlorhexidine in selected products.

Methods

The study was undertaken in February 2013 to April 2013 in Copenhagen, Denmark. We checked for chlorhexidine in cosmetic products in 14 supermarkets, one hairdressing salon and one beauty and retail store by reading the ingredient labels. We checked for chlorhexidine looking for its INCI names: chlorhexidine (CAS no. 55-56-1), chlorhexidine diacetate (CAS no. 56-95-1), chlorhexidine digluconate (CAS no. 18472-51-0), and chlorhexidine dihydrochloride (CAS no. 3697-42-5) (9). The following cosmetic product types were checked: hair products (shampoos, conditioners, hair treatments, hair dyes, hair sprays, and hair wax/gels), creams/ointments, deodorants, bath soaps, body lotions, hand soaps, toothpastes, aftershaves/shaving foams, wet wipes, lip balms, make-up removers, face washes, skin tonics, mouth washes, face masks, and hand disinfectants. All products were photographed and product names were noted, to avoid duplication. All products containing chlorhexidine were purchased, except for some expensive hair products ($n = 10$).

Analysis – chlorhexidine concentration

The concentration of pure chlorhexidine was measured in 10 selected products. The product types with the most products containing chlorhexidine were selected for analysis, but some product types were not analysed because of technical limitations, as analysis of complex matrix solid compounds, for example wet wipes, would require a different extraction method than the one used for liquid and semi-solid samples.

Chemicals used for analysis. Ammonium acetate, acetic acid (analytical grade) and acetonitrile [high-performance liquid chromatography (HPLC) grade] were purchased from Merck (Darmstadt, Germany); chlorhexidine (CAS no. 55-56-1) (99% purity) was purchased from Sigma Aldrich (Seelze, Germany).

Extraction and analysis. Liquid samples were diluted 200-fold (1 ml of product in 200 ml of acetate buffer) with 20 mM ammonium acetate/acetonitrile (80:20, vol/vol) containing 0.1% glacial acetic acid. Semi-solid samples (1 g) were extracted with 20 ml of the same mixture by sonication for 60 min. The extract was filtered through a paper filter (AP25; Millipore, Billerica, MA, USA), and an aliquot was transferred to an HPLC vial.

A stock solution of pure chlorhexidine (1 mg/ml) was prepared in methanol, and working solutions for the HPLC analysis were prepared in 20 mM ammonium acetate/acetonitrile (80:20, vol/vol) containing 0.1% glacial acetic acid.

Analytical HPLC was performed on an Agilent 1100 Series (Agilent, Palo Alto, CA, USA) equipped with a variable wavelength detector operated at a detection wavelength (λ) of 258 nm. A Supelco Discovery RP-amide C16 column (15 cm \times 2.1 mm; particle size, 5 μ m; Supelco, Bellefonte, PA, USA) with a pre-column was used for analysis. Chlorhexidine was eluted at a flow rate of 0.5 ml/min with a programmed gradient: solvent A was 20 mM ammonium acetate containing 0.1% glacial acetic acid, and solvent B was acetonitrile. Chlorhexidine concentrations in samples were calculated by using a mean response factor of three standard concentration levels (5, 10 and 20 μ g/ml).

The precision of the method was calculated by extracting three different samples (one conditioner and two ointments) five times each, giving relative standard deviations of 2.3%, 4.0%, and 13.1%, respectively.

Results

A total of 80 of 2251 cosmetic products (3.6%) contained chlorhexidine (Table 1). Chlorhexidine was found in 57 hair products: 30 conditioners, 13 hair dyes, 10 hair treatments, and four hair styling products. Chlorhexidine was also found in nine creams/ointments, four face washes, four wet wipes, three skin tonics, two make-up removers, and one mouth wash. Of the 80 products, 66 were from international companies and 14 were from Danish companies.

The concentration of chlorhexidine in 10 selected products was between 0.01% and 0.15% (Table 2).

Table 1. Results of the market survey of chlorhexidine in cosmetic products

Product types	Products checked, n	Products with chlorhexidine, n (%)
Hair products, total	760	57 (8)
Shampoos	245	0
Conditioners	153	30 (20)
Hair treatments/masks	40	10 (25)
Hair dyes	22	13 (59)
Hair sprays	154	0
Hair wax/gels	88	1 (1)
Other leave-on hair products	58	3 (5)
Creams/ointments	324	9 (3)
Deodorants	262	0
Bath soaps	219	0
Body lotions	134	0
Hand soaps	108	0
Toothpastes	77	0
Aftershaves/shaving foams	70	0
Wet wipes	63	4 (6)
Lip balms	36	0
Make-up removers	25	2 (8)
Face washes	24	4 (17)
Skin tonics	22	3 (14)
Mouth washes	17	1 (6)
Face masks	11	0
Disinfectants	7	0
Miscellaneous	92	0
Total	2251	80 (3.6)

Table 2. Concentration of chlorhexidine in 10 cosmetic products

Product type	Concentration of chlorhexidine (%)
Conditioner	0.02
Hair treatment	0.01
Acne cream	0.12
Mild antiseptic ointment	0.02
Mild antiseptic ointment	0.05
Eye cream	0.15
Skin tonic	0.06
Skin tonic	0.04
Face wash	0.03
Face wash	0.05

Discussion

It is well known that chlorhexidine is widely used as a disinfectant in the healthcare setting. Less well known is the fact that it may be used in cosmetic products. In this large market survey investigating the extent of use of chlorhexidine in cosmetic products in Denmark, we found chlorhexidine in 80 of 2251 products (3.6%),

which is almost twice the number reported from Finland (1.9%) (3). We identified chlorhexidine primarily in hair products, but also in creams, wet wipes, face washes, skin tonics, make-up removers, and a mouth wash. Chlorhexidine was mainly found in products from international companies and in products aimed at the female consumer.

The concentrations of chlorhexidine in 10 selected products were all well below the allowed limit of 0.3% set by the European Cosmetics Directive (9). A limitation of this study is that we only analysed chlorhexidine concentrations in 10 of 80 products. Therefore, we cannot be certain that all 80 products had a concentration of chlorhexidine below the permitted limit.

Adverse effects of chlorhexidine are rare (1), but chlorhexidine may cause both type I and type IV allergy. Type I allergic reactions to chlorhexidine are often severe, and may lead to anaphylactic shock or even cardiac arrest. The allergy is predominantly found in males aged > 50 years, and the allergic reactions often occur during urological procedures or surgery (4). In Denmark, a recent study of patients investigated for suspected perioperative allergy found that chlorhexidine was the allergen in 9.6% of the cases (10). Chlorhexidine is widely used in the perioperative setting, and patients are often exposed via several different routes at the same time.

Some chlorhexidine-allergic patients are likely to have been sensitized via exposure to chlorhexidine in the healthcare setting prior to the allergic reaction, but the sensitization potential of chlorhexidine in cosmetic products is still unknown. Moreover, chlorhexidine may cause allergy among healthcare workers (7, 8), and the route of sensitization in this group is considered to be via occupational exposure.

Regarding type IV allergy to chlorhexidine, older studies from Denmark have shown a prevalence of positive patch test reactions to chlorhexidine of 2.3–5.4% (11–13). The reported prevalence in more recent studies varies from country to country: Germany, 0.4% (14); Finland, 0.5% (3, 15); Czech Republic, 1.5% (16); and Switzerland, 2.0% (17). Although the reported prevalence seems to be higher in Denmark, the studies are very difficult to compare, owing to different test concentrations and study populations; for example, the Danish studies used a test concentration of 1.0% chlorhexidine digluconate, whereas other studies used 0.5% chlorhexidine digluconate. Additionally, the Danish studies are older, from the 1980s, and recent studies are not available for comparison. In Finland, the number of positive patch test reactions to chlorhexidine was reported to decrease from 1.2% in 1995–1996 to 0.5% in 2000–2002 (15). In a recent Danish study, the preservative methylisothiazolinone (MI) was found in 3.3% of 1795 cosmetic products

(18). Although the numbers of cosmetic products containing chlorhexidine and MI are almost the same (3.6% and 3.3%, respectively), it seems that MI is causing more cases of allergy than chlorhexidine (18).

Age and sex distributions have rarely been reported in studies on type IV chlorhexidine allergy. In two studies, the median ages of patients with positive patch test reactions to chlorhexidine were 57 and 58 years (3, 14). In three studies, positive patch test reactions to chlorhexidine were found in equal numbers of females and males, indicating a relative over-distribution of males, as more females are patch tested (3, 11, 14). Overall, patients with

positive patch test reactions to chlorhexidine have been only poorly characterized. Therefore, there is not enough evidence to suggest an association between cosmetic products containing chlorhexidine and type IV allergy to chlorhexidine. However, it was reported from Finland that chlorhexidine in cosmetic products caused or worsened the symptoms in 19% of patients with positive patch test reactions to chlorhexidine (3). Future studies should therefore focus on characterizing patients with type IV chlorhexidine allergy and identifying the risk of allergic sensitization to chlorhexidine from cosmetic products and other exposures.

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Manuscript II: Opstrup MS, Johansen JD, Zachariae C, Garvey LH.

Contact allergy to chlorhexidine in a tertiary dermatology clinic in Denmark.

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Contact allergy to chlorhexidine in a tertiary dermatology clinic in Denmark

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Summary

Background. Chlorhexidine is a widely used disinfectant in the healthcare setting and in cosmetic products. A high prevalence of chlorhexidine contact allergy was reported in Denmark in the 1980s (2.0–5.4% of patients patch tested). It is unknown whether the prevalence is still high, which products cause the contact allergy, and whether accidental re-exposure occurs in some patients.

Objectives. To estimate the prevalence of chlorhexidine contact allergy in a tertiary dermatology clinic in Denmark; to investigate whether patch testing with both chlorhexidine diacetate and chlorhexidine digluconate is necessary; to investigate how many patients have combined immediate-type allergy and contact allergy; and to identify which products cause chlorhexidine contact allergy, and whether patients are accidentally re-exposed.

Methods. This was a retrospective study including all patients patch tested with chlorhexidine during 2003–2013 at the Department of Dermato-Allergology at Copenhagen University Hospital Gentofte (n = 8497). All patients with a positive patch test reaction to chlorhexidine were sent a questionnaire comprising questions about the cause of the allergy and re-exposure.

Results. Overall, 1.0% (n = 82) of all patients patch tested with chlorhexidine were positive. A decrease in the prevalence was observed over time, most likely because of lowering of the test concentration from 1.0 to 0.5% in 2008. Of the 82 patients, 28 (0.3%) had positive test reactions to both chlorhexidine salts, 43 (0.5%) had a positive test reaction only to chlorhexidine diacetate, and 11 (0.1%) had a positive test reaction to chlorhexidine digluconate. Three patients were both patch test-positive and prick test-positive. A known cause of the allergy was reported by 19 patients (40%) in the questionnaire: the products used in the healthcare setting were mainly reported, but some reported cosmetic products. Accidental re-exposure was reported by 15 patients (32%), of whom 13 reported symptoms.

Conclusions. The prevalence of chlorhexidine contact allergy does not seem to be higher in Denmark than in other European countries. Patch testing with both chlorhexidine diacetate and chlorhexidine digluconate may be beneficial. Testing for immediate-type allergy in patients with a positive patch test reaction to chlorhexidine is recommended. Chlorhexidine-containing products used in the healthcare setting and in cosmetics are potential causes of sensitization and allergy. Re-exposure is common, highlighting the fact that patients and healthcare personnel need to be well informed about possible sources of exposure.

Key words: chlorhexidine; chlorhexidine allergy; chlorhexidine contact allergy; chlorhexidine contact dermatitis; chlorhexidine diacetate; chlorhexidine digluconate; contact allergy; contact dermatitis.

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Chlorhexidine is superior to other disinfectants and has few side-effects (1, 2). It is widely used in the hospital setting to disinfect skin and mucous membranes (3, 4). Outside the hospital setting, chlorhexidine is also widely used, for example by dentists, in cosmetic products as a preservative, and in products sold over the counter in pharmacies (5, 6).

The focus on contact allergy to chlorhexidine has increased within the last decade, and accordingly the number of publications has risen (7). One reason for this is the increased awareness of the 'hidden' use of chlorhexidine in many products, which can result in chlorhexidine not being suspected as an allergen. Most chlorhexidine-allergic patients have dermatitis caused by contact allergy at the skin site of chlorhexidine exposure; it is estimated that contact allergy to chlorhexidine is diagnosed in 0.01–5.4% of all patch tested patients (5, 8–14). The studies with the highest prevalence rates (2.0–5.4%) are from Denmark, and were conducted in the 1980s (10–12). No newer studies concerning the prevalence in Denmark have been performed, and it remains unknown whether the prevalence is still higher than in other European countries. Chlorhexidine can also cause immediate-type allergy leading to severe reactions such as urticaria, anaphylactic shock, or even cardiac arrest (15, 16). A few case reports have described patients with combined immediate-type allergy and contact allergy, but, so far, the proportion of combined allergy is unknown (17–19).

Chlorhexidine can be used as three different salts: chlorhexidine diacetate [molecular weight (MW) 626 g/mol], chlorhexidine digluconate (MW 898 g/mol), and chlorhexidine dihydrochloride (MW 578 g/mol) (20). According to the European Cosmetics Regulation, all three salts are allowed in cosmetic products (21), but it remains unknown why different salts are used in different products. In 2013, we checked for chlorhexidine content in 2251 cosmetic products in Denmark by reading the ingredient label, and found chlorhexidine in 80 of the products (3.6%) (6). Back then, we did not check which salts were used, but, in a retrospective review of the photographs, we found that 42 products contained chlorhexidine digluconate, 33 contained chlorhexidine dihydrochloride, but none contained chlorhexidine diacetate (the salt used could not be identified in five products). In the same year, we identified 42 different products used in hospitals in the Capital Region of Denmark (3). In a retrospective review of these products, we could see that 27 contained chlorhexidine digluconate, six contained chlorhexidine diacetate, and three contained chlorhexidine dihydrochloride (the salt used could not be identified in six products). In our tertiary dermatology clinic at

Copenhagen University Hospital Gentofte, chlorhexidine diacetate and chlorhexidine digluconate are used for patch testing. In contrast, other centres patch test only with one of the salts. In a study from 1991, some patients had a positive test result with one salt and a negative test result with the other salt, but no newer studies have investigated whether testing with more than one salt is indeed necessary (22).

In Danish studies from the 1980s, it was found that contact allergy to chlorhexidine was primarily diagnosed among men with leg eczema or ulcers (10–12), but there are no newer studies from Denmark or other countries characterizing the patients. In a recent study from Finland, two chlorhexidine-containing corticosteroid creams were suggested to be the principal sources of chlorhexidine contact sensitization (5). In Denmark, however, these products are not available, and it is unknown which chlorhexidine-containing products cause sensitization. Despite the widespread use of chlorhexidine, it is also unknown whether patients are aware of possible sources of exposure.

The aims of this study were (i) to determine the prevalence of contact allergy to chlorhexidine in a tertiary dermatology clinic and to characterize the patients, (ii) to investigate whether patch testing with both chlorhexidine diacetate and chlorhexidine digluconate is necessary, (iii) to estimate how many patients have both immediate-type allergy and contact allergy to chlorhexidine, and (iv) to investigate which products cause allergic reactions, and whether patients are aware of possible sources of chlorhexidine exposure and are able to avoid these.

Materials and Methods

Study population

This was a retrospective study of all patients patch tested with chlorhexidine at the Department of Dermato-Allergology at Copenhagen University Hospital Gentofte during an 11-year-period: 1 January 2003 to 31 December 2013 ($n = 8497$). Chlorhexidine diacetate and chlorhexidine digluconate were routinely applied as a supplement to the baseline series for the entire period: from 1 January 2003 to 1 September 2008 in concentrations of 1.0% aq., and from 1 September 2008 to 31 December 2013 in concentrations of 0.5% aq. The chlorhexidine test substances were prepared in the department from 2003 to 1 September 2008; from 1 September 2008 to 15 March 2009, test substances from Chemotechnique were used; from 15 March 2009 until 31 December 2013, chlorhexidine diacetate 0.5% aq. from Chemotechnique (Vellinge, Sweden) and chlorhexidine digluconate 0.5% aq. from Trolab (Barsbüttel,

Germany) were used. Patient data were collected from the clinical database of contact allergy hosted in the department. Information available from the database included age, MOAHLFA index (Male, Occupation, Atopic dermatitis, Leg dermatitis, Hand dermatitis, Facial dermatitis, and Age > 40 years), and patch test results.

Patch testing

Eight-millimetre Finn Chambers® were used, and patch tests were applied to the upper back. The occlusion time was 48 hr, and reading was performed at D2, D3, and D5/D7, in accordance with ICDRG recommendations. Reactions scored as 1+, 2+ and 3+ were interpreted as positive. Irritant reactions and doubtful reactions were interpreted as negative. In cases of retesting, patch test data obtained at the first visit were used in the analysis. In cases of the same patch test severity on more than one reading, the day of the first reading was used in the analysis.

Prick testing

Prick testing with chlorhexidine was undertaken in some patients with a positive patch test reaction to chlorhexidine. The test was performed on the forearm with chlorhexidine digluconate 0.5% aq. The reaction was read after 20 min. Normal saline and histamine 10 mg/ml served as negative and positive controls, respectively. The test was only considered to be valid when the positive control was positive and the negative control was negative. The criterion for positivity was a mean wheal diameter of ≥ 3 mm.

Statistics

The data were processed with SPSS (SPSS™ Statistics, Chicago, IL, USA; IBM PASW Statistics) for Windows™, edition 22.0. The median and interquartile range (IQR) were used to determine the difference in median age for the populations with and without contact allergy to chlorhexidine. The Mann–Whitney *U*-test was applied for analysis of differences in age between the two groups. The chi-square test was applied for analysis of the MOAHLFA index and irritant reactions. The threshold for statistical significance was predefined as a *p*-value of <0.05.

Questionnaire

In the autumn of 2014, a questionnaire was sent to all patients who had shown a positive reaction to chlorhexidine diacetate and/or chlorhexidine digluconate from 1

January 2003 to 31 December 2013. A reminder was sent to those who had not responded after 3 weeks. The questionnaire comprised six questions (Table A1). First, we asked patients whether they were aware of the cause of the allergy and of possible re-exposures after the allergy was diagnosed. Second, we asked whether patients were aware of the use of chlorhexidine in cosmetic products and in the healthcare setting. Third, we asked whether the allergy to chlorhexidine caused limitations in their everyday life.

Results

A total of 8497 contact dermatitis patients (5714 women and 2783 men) patch tested with chlorhexidine diacetate and/or chlorhexidine digluconate during 1 January 2003 to 31 December 2013 were retrospectively included in the study. Overall, 110 patch tests with chlorhexidine diacetate or chlorhexidine digluconate gave positive results in 82 patients (1.0%). The median age was significantly higher in patch test-positive patients than in patch test-negative patients [55 years (IQR 38–65 years) versus 47 years (IQR 34–60 years), *p* = 0.01]. Of the 82 patients with positive patch test reactions, 28 (0.3%) had positive reactions to both chlorhexidine salts, 43 (0.5%) had a positive reaction only to chlorhexidine diacetate, and 11 (0.1%) had a positive reaction only to chlorhexidine digluconate.

The prevalence rates of sensitization to chlorhexidine are shown in Fig. 1. Overall, a decrease in prevalence was observed over time for both chlorhexidine salts. In 2003, 1.7% of patch tests were positive for one or both salts. In contrast, this figure was 0.3% in 2013. From 1 January 2003 to 31 August 2008, when the test concentrations were 1.0%, the overall prevalence was 1.4% (54/3867); from 1 September 2008 to 31 December 2013, when the test concentrations were lowered to 0.5%, the overall prevalence was 0.6% (28/4630).

Of the positive reactions to chlorhexidine diacetate, 55% (39/71) were weakly positive (1+), 42% (30/71) were strongly positive (2+), and 3% (2/71) were extremely positive (3+). Of the positive reactions to chlorhexidine digluconate, 64% (25/39) were weakly positive, 36% (14/39) were strongly positive, and none was extremely positive (Table 1). In total, 16% (18/110) of the patch tests were positive at day 2, 51% (56/110) were positive at day 3, and 33% (36/110) were positive at days 5–7.

There was a significantly higher rate of irritant reactions when testing was performed with 1.0% chlorhexidine diacetate than when it was performed with 1.0% chlorhexidine digluconate [5.1% (*n* = 199) and

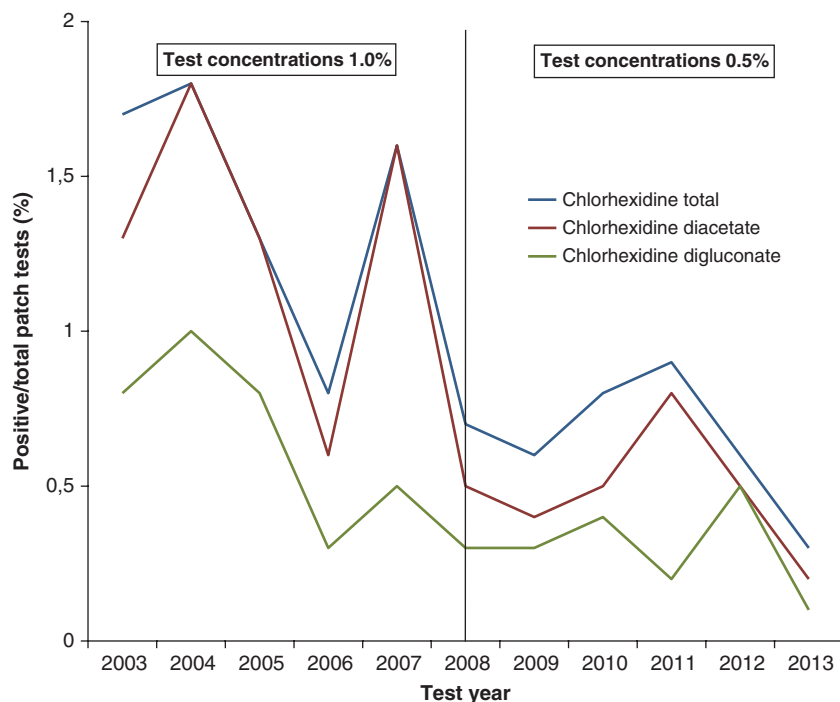


Fig. 1. Prevalence of positive patch test reactions to chlorhexidine 2003–2013.

Table 1. Severity of positive patch test results and year of testing

Test concentration (%)	Year of testing	Chlorhexidine digluconate (n)			Chlorhexidine diacetate (n)		
		1+	2+	3+	1+	2+	3+
1.0	2003	5	1	0	6	4	0
	2004	2	5	0	7	5	1
	2005	3	3	0	7	3	0
	2006	1	1	0	2	2	0
	2007	3	0	0	5	5	0
	1 January to 31 August 2008	1	1	0	2	0	0
	1 September to 31 December 2008	0	0	0	0	1	0
0.5	2009	1	1	0	1	2	0
	2010	2	1	0	2	2	0
	2011	2	0	0	5	1	1
	2012	4	1	0	1	4	0
	2013	1	0	0	1	1	0

2.1% ($n = 80$), respectively, $p < 0.00001$]. In contrast, there was no statistically significance difference in the rate of irritant reactions when testing was performed with 0.5% chlorhexidine diacetate and when it was performed with 0.5% chlorhexidine digluconate [0.5% ($n = 24$) and 0.3% ($n = 15$), respectively, $p = 0.14$]. Overall, there was a significantly higher rate of irritant reactions in the group tested with 1.0% chlorhexidine diacetate and 1.0% chlorhexidine digluconate than in the group tested with 0.5% chlorhexidine diacetate and 0.5% chlorhexidine

Table 2. MOAHLFA index for patients with positive and negative patch tests results for chlorhexidine (a positive reaction to either chlorhexidine diacetate, chlorhexidine digluconate, or both)

Total tested	% (n) positive (n = 82)	% (n) negative (n = 8415)	p-value*
Male sex	58.5 (48)	32.5 (2735)	<0.00001
Occupational	15.9 (13)	19.8 (1664)	0.37
Atopic dermatitis	12.2 (10)	16.8 (1417)	0.26
Hand dermatitis	41.5 (34)	38.7 (3258)	0.61
Leg dermatitis	18.3 (15)	4.7 (392)	<0.00001
Face dermatitis	15.9 (13)	24.1 (2029)	0.08
Age above 40 years	74.4 (61)	66.0 (5554)	0.11

Significant values are in bold.

*Pearson chi-square.

digluconate [3.6% ($n = 279$) and 0.4% ($n = 39$), respectively, $p < 0.00001$].

The findings for the MOAHLFA index are shown in Table 2. Chlorhexidine allergy was significantly associated with male sex and leg dermatitis.

Prick testing

Of the 82 patients with a positive patch test reaction to chlorhexidine, 29 were prick tested with chlorhexidine digluconate, and 3 were positive. Of the 3 patients, 1 had a strongly positive (2+) patch test reaction to 1.0% chlorhexidine digluconate and an extremely positive (3+) digluconate [3.6% ($n = 279$) and 0.4% ($n = 39$), respectively, $p < 0.00001$].

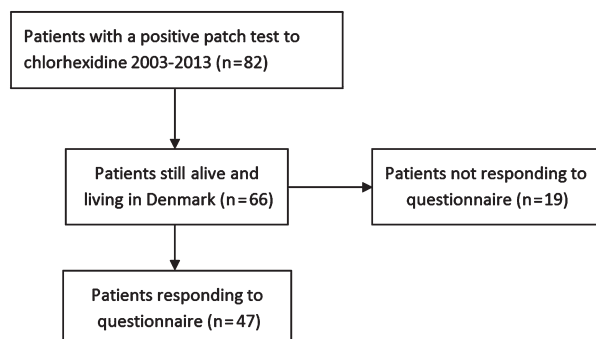


Fig. 2. Flowchart of response to questionnaire.

patch test reaction to 1.0% chlorhexidine diacetate; the 2 remaining patients had strongly positive (2+) patch test reactions to 1.0% and 0.5% chlorhexidine diacetate, respectively.

Questionnaire

Of the 82 patients with a positive patch test reaction to chlorhexidine, 66 were still alive and living in Denmark, and were mailed the questionnaire (see flowchart in Fig. 2). A total of 71% ($n = 47$) responded. Of these, during the initial investigation, 53% ($n = 25$) were positive only for chlorhexidine diacetate, 13% ($n = 6$) were positive only for chlorhexidine digluconate, and 34% ($n = 16$) were positive for both salts. A total of 62% ($n = 29$) had a weakly positive patch test reaction (1+), 36% ($n = 17$) had a strongly positive patch test reaction (2+), and 2% ($n = 1$) had an extremely positive patch test reaction (3+). Overall, 60% ($n = 29$) were tested before 1 September 2008 with concentrations of 1.0%, and 40% ($n = 19$) were tested after 1 September 2008 with a concentration of 0.5%. The MOAHLFA index among those who responded was comparable to that of the whole group of patch test-positive patients [male sex 53% ($n = 25$); occupational 19% ($n = 9$); atopic dermatitis 19% ($n = 9$); hand dermatitis 45% ($n = 21$); leg dermatitis 11% ($n = 5$); face dermatitis 15% ($n = 7$); age > 40 years 74.5% ($n = 35$)].

A total of 19 patients (40%) reported knowing the cause of the allergy (Table 3); of these, 4 reported more than one product as the cause. Mainly healthcare products, but also cosmetic products, were reported as causes. Regarding exposure after the diagnosis was established, 15 patients (32%) reported a known re-exposure; also here, mainly healthcare products but also cosmetic products were reported. Of the 15 patients reporting re-exposure, 13 reported symptoms: most reported a

Table 3. Questionnaire results regarding cause of the allergy and possible re-exposure

	Number (%)	Reported products (n)
Patients reporting a known cause	19 patients (40)	Cream (7); hair product (5); make-up or make-up remover (1); wet wipe or other skin disinfectant (5); mouth wash (2); wound dressing (5); product used at the hospital or by a doctor (6); product used by a dentist (3); other (2)*
Patients reporting re-exposure after the diagnosis was established	15 patients (32) (of these, 13 patients reported symptoms)	Cream (5); hair product (2); wet wipe or other skin disinfectant (7); mouth wash (3); wound dressing (4); product used at the hospital or by a doctor (6); product used by a dentist (3); other (1)*

In total, 22 patients (47%) reported a known cause and/or symptoms at re-exposure (some patients reported both a known cause and symptoms at re-exposure).

*Many reported more than one cause/exposure.

rash and/or skin itching ($n = 12$), but some also reported general discomfort ($n = 4$) or breathlessness ($n = 2$). Concerning the timing between exposure and symptoms, 6 of 13 reported symptoms within an hour, 3 of 13 reported symptoms between 1 and 24 hr after exposure, and 2 of 13 reported symptoms more than 24 hr later (2 did not report the timing). Of the 6 patients reporting symptoms within an hour, 5 were tested with a prick test during the initial investigations, and they were all negative.

Regarding the patients' knowledge about possible exposure to chlorhexidine in cosmetic products and in the healthcare setting, only 38% ($n = 18$) were aware of its use in cosmetics, but 45% ($n = 21$) checked for chlorhexidine in cosmetic products before using them. In contrast to these findings, 83% ($n = 39$) were aware of the use of chlorhexidine in hospitals and by dentists, and the same number of patients reported informing about the allergy when in contact with hospitals or a dentist.

Regarding possible work-related exposure, 15% ($n = 7$) had been in contact with chlorhexidine at work: 1 nurse was exposed from skin swabs and a hand disinfectant; 1 nurse assistant and 1 painter reported exposure, but did not specify from what product; 1 cleaner was exposed from a soap; 1 blacksmith was exposed from a hand cleaner; 1 machine operator was exposed from an ointment; and 1 patient was exposed from different products when working in two different jobs – from a hair product when working as a hairdresser, and from cleaning swabs

when working in a kindergarten. The 2 latter patients had to quit their jobs because of the allergy.

Regarding the burden of disease, 21% ($n = 9$) reported that the allergy caused limitations in their everyday life.

Discussion

In this retrospective study evaluating patients diagnosed with contact allergy to chlorhexidine in a Danish tertiary dermatology clinic, we found that 1.0% of all patients patch tested from 2003 to 2013 had a positive patch test reaction to chlorhexidine. This finding is in line with the prevalence found in studies from other European countries, but is lower than the prevalence in previous studies from Denmark in the 1980s (5, 8–13). Most of the reactions were weakly positive, which is in line with a recent study from Finland, but is in contrast to an older study from our clinic, where most of the reactions were strongly/extremely positive (5, 11). Notably, in our centre, there was a marked decrease in the prevalence, from 1.7% in 2003 to 0.3% in 2013. This is probably because we lowered the test concentration from 1.0 to 0.5% in 2008. Currently, the optimal test concentration remains unknown, and different centres use different concentrations: The European Network for Drug Allergy recently suggested a test concentration of 1% (23), but some centres use 0.5% (5, 9). In this study, we found that 0.5% chlorhexidine diacetate and 0.5% chlorhexidine digluconate caused fewer irritant reactions than 1.0% chlorhexidine diacetate and 1.0% chlorhexidine digluconate. These findings are in line with an older study from our clinic, which also found that, especially, 1.0% chlorhexidine diacetate is a strong irritant (22). Additionally, two older studies found that it was difficult to reproduce the test results when patch testing (10, 12). Taken together, these findings suggest that 1.0% is too high as a test concentration. Nonetheless, no studies have investigated whether testing with 0.5% is reproducible or whether it causes false-negative results. Therefore, future studies should focus on finding the optimal concentration for patch testing with chlorhexidine; such studies could, for instance, include use testing or patch testing with titrated concentrations, and repeated open applications of solutions of chlorhexidine.

In our questionnaire study, 40% of respondents reported a potential cause of their allergy – products used in the healthcare setting were mainly reported, but some reported cosmetic products. The remaining patients (60%) did not report a known cause of their allergy, and there could be several reasons for this. First, a positive patch test reaction to chlorhexidine indicates sensitization, but this may not lead to allergic symptoms

in all patients. Second, some patients may not remember an exposure a long time ago. Third, some patients may have been using a product causing the symptoms without knowing this. Indeed, the last of these could very well be the case in some patients, because 62% of the patients in the questionnaire were unaware of the use of chlorhexidine as a preservative in cosmetic products, and 17% were unaware of the use in the healthcare setting. Exposure assessment is an important part of allergy investigation, but the data saved in the database from the time of the original investigation were incomplete, and could therefore not be included as a supplement in this study.

Notably, 32% of patients reported re-exposure to chlorhexidine after the diagnosis was established, and almost all reported symptoms. This highlights how difficult it is to avoid chlorhexidine, and how important it is to give information about possible exposure routes when a patient is diagnosed with chlorhexidine allergy.

In our clinic, patch testing is performed with both chlorhexidine diacetate and chlorhexidine digluconate. Although chlorhexidine digluconate is more frequently used in both the healthcare setting and in cosmetic products in Denmark, more than half of the patients were positive only for chlorhexidine diacetate. Therefore, this salt is especially important when testing for chlorhexidine contact allergy is performed. Nonetheless, some patients were positive only for chlorhexidine digluconate, and testing should therefore include both salts. Chlorhexidine dihydrochloride is widely used in Denmark, mainly in cosmetic products, but it is not included in diagnostic testing in our centre, or, to our knowledge, in any other centres. Future studies should investigate the possibility of chlorhexidine dihydrochloride causing contact sensitization/allergy.

In this study, 3 patients with a positive patch test reaction to chlorhexidine were also prick test-positive for chlorhexidine digluconate, indicating combined immediate-type allergy and contact allergy. Therefore, we recommend testing for immediate-type allergy in patients with a positive patch test reaction to chlorhexidine. Investigations should include prick testing with chlorhexidine digluconate 0.5% and measurement of specific IgE (23, 24). A limitation of this study is that measurement of specific IgE was not performed, but this is now included in our routine testing for immediate-type allergy. In immediate-type chlorhexidine allergy, testing only with chlorhexidine digluconate is recommended (23). As found in this study, many of the patients with a positive patch test reaction to chlorhexidine were positive only for chlorhexidine diacetate. Therefore, the

role of this salt should be further investigated in the group of patients suspected of having immediate-type chlorhexidine allergy.

Three characteristics separated the group of patients with a positive patch test reaction to chlorhexidine from the group with negative patch test results for chlorhexidine: higher age, male sex, and leg eczema. These characteristics are in line with findings in Danish studies from the 1980s (10–12). At that point, dressings containing chlorhexidine diacetate 0.5% were believed to be the cause of the contact allergy. Today, the same dressings are still used in Denmark (3), and 5 of the 47 patients in the questionnaire reported exposure to chlorhexidine in dressings. Therefore, it seems that chlorhexidine-containing dressings can sensitize some patients. However, 4 of the 5 patients also reported other exposures in the healthcare setting.

In conclusion, 1.0% of patients tested in our tertiary dermatology clinic had a positive patch test reaction to chlorhexidine in 2003–2013. The prevalence decreased over the 11-year-period, probably because of a lower test concentration (0.5% versus 1.0%) in the second half of the period. Male sex, leg eczema and higher age were more predominant in the patch test-positive group than in the patch test-negative group. It may be beneficial to patch test with both chlorhexidine diacetate and chlorhexidine digluconate, but the optimal test concentration remains to be established. Three patients were both patch test-positive and prick test-positive; we recommend testing for immediate-type allergy in patch test-positive patients. Finally, in most patients, the allergy was caused by chlorhexidine used in the healthcare setting, but, for some, cosmetic products were responsible. Moreover, 32% were re-exposed after the diagnosis, highlighting the fact that patients and healthcare personnel need to be well informed about possible sources of exposure.

Appendix

Table A1. Questionnaire about chlorhexidine contact allergy

1a.	Do you know what product caused your allergy to chlorhexidine?
	Yes; No
1b.	If yes in 1a: What product caused the allergy?
	Cream; hair product; make-up or make-up remover; wet wipe or other skin disinfectant; mouth wash; wound bandage; product used at the hospital or by a doctor; product used by a dentist; other: _____

Table A1. Continued

2a.	Have you been exposed to chlorhexidine since the allergy was diagnosed?
	Yes; No; Don't know
2b.	If yes in 2a: What product was it?
	Cream; hair product; make-up or make-up remover; wet wipe or other skin disinfectant; mouth wash; wound bandage; product used at the hospital or by a doctor; product used by a dentist; other: _____
2c.	If yes in 2a: What symptoms did you experience?
	Rash; itching skin; urticaria; breathing difficulties; feeling unwell; fainting; other: _____
2d.	If yes in 2a: When did you experience the symptoms?
	0–60 min; 1–24 hr; more than 24 hr
3a.	Have you been exposed to chlorhexidine in your current job or in a previous job?
	Yes; No; Don't know
3b.	If yes in question 3a: What was your job?

3c.	If yes in question 3a: What product contained chlorhexidine?

3d.	If yes in question 3a: Did you have to quit your job?
	Yes; No
4a.	Do you know that chlorhexidine can be used in some cosmetic products?
	Yes; No
4b.	Do you check whether there is chlorhexidine in a cosmetic product before you use it?
	Yes; No
5a.	Do you know that chlorhexidine is used at hospitals and by dentists?
	Yes; No
5b.	Do you mention your allergy when you are at a hospital or at your dentist?
	Yes; No
6a.	Does the allergy cause limitations in your everyday life?
	Yes; No
6b.	If yes in 6a: In what way does the allergy limit you?

Translated from Danish.

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Standardized testing with chlorhexidine in perioperative allergy – a large single-centre evaluation.

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ORIGINAL ARTICLE

ANAPHYLAXIS

Standardized testing with chlorhexidine in perioperative allergy – a large single-centre evaluation

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Keywords

anaphylaxis; drug allergy; IgE; immunologic tests.

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Abstract

Background: Perioperative allergic reactions to chlorhexidine are often severe and easily overlooked. Although rare, the prevalence remains unknown. Correct diagnosis is crucial, but no validated provocation model exists, and other diagnostic tests have never been evaluated. The aims were to estimate (i) the prevalence of chlorhexidine allergy in perioperative allergy and (ii) the specificity and sensitivity for diagnostic tests for chlorhexidine allergy.

Methods: We included all patients investigated for suspected perioperative allergic reactions in the Danish Anaesthesia Allergy Centre during 2004–2012. The following tests were performed: specific IgE (Immunocap[®]; Phadia AB, Sweden), histamine release test (HR) (RefLab ApS, Denmark), skin prick test (SPT) and intradermal test (IDT). Positivity criteria were as follows: specific IgE >0.35 kUA/l; HR class 1–12; SPT mean wheal diameter ≥3 mm; IDT mean wheal diameter ≥ twice the diameter of negative control. Chlorhexidine allergy was *post hoc* defined as a relevant clinical reaction to chlorhexidine combined with two or more positive tests. Based on this definition, sensitivity and specificity were estimated for each test.

Results: In total, 22 of 228 patients (9.6%) met the definition of allergy to chlorhexidine. Estimated sensitivity and specificity were as follows: specific IgE (sensitivity 100% and specificity 97%), HR (sensitivity 55% and specificity 99%), SPT (sensitivity 95% and specificity 97%) and IDT (sensitivity 68% and specificity 100%).

Conclusions: In patients investigated for suspected perioperative allergic reactions, 9.6% were diagnosed with allergy to chlorhexidine. Using our definition of chlorhexidine allergy, the highest combined estimated sensitivity and specificity was found for specific IgE and SPT.

Chlorhexidine is one of the most effective disinfectants (1, 2). In Denmark and many other countries, it is widely used in both healthcare and private households, and exposure to chlorhexidine is almost inevitable (2–6).

In the last decade, focus on immediate-type chlorhexidine allergy has increased. Several recent case series indicate that allergic reactions to chlorhexidine may lead to anaphylactic

shock or even cardiac arrest (3–5, 7). The allergy is easily overlooked because chlorhexidine is often not suspected as the allergen (5, 8) or might be administered by mistake in patients with known allergy (9–11). The American Food and Drug Administration (FDA) has previously issued a warning concerning the potential of serious allergic reactions to chlorhexidine-impregnated medical devices (12). The risk appears to be increased in the perioperative setting (4, 5, 13–16), in urological procedures (7, 17, 18) and in patients having a central venous catheter inserted (3, 9, 10). Despite the widespread exposure, allergy to chlorhexidine is considered rare, but the prevalence is still unknown.

Abbreviations

DAAC, Danish Anaesthesia Allergy Centre; HR, histamine release test; IDT, intradermal test; SPT, skin prick test.

The Danish Anaesthesia Allergy Centre (DAAC) is the Danish national reference centre for the investigation of perioperative allergic reactions, and patients have systematically been investigated since 1999. Investigation for chlorhexidine allergy is performed routinely in all patients; thus, considerable experience has been gathered in this field.

No validated provocation model is available for chlorhexidine. In DAAC, the diagnosis of chlorhexidine allergy is based on one or more relevant clinical reactions to chlorhexidine in combination with results of the following tests: specific IgE, histamine release test (HR), skin prick test (SPT) and intradermal test (IDT). In the absence of a validated provocation model, it is obviously crucial to ensure maximum specificity and sensitivity in the tests performed. In DAAC, we aim especially for high sensitivity to ensure a low number of false-negative conclusions, and this is achieved by combining several test modalities for the same allergen. However, diagnostic tests in chlorhexidine allergy have never previously been evaluated.

Thus, the aims of this study were to estimate (i) the prevalence of chlorhexidine allergy among patients with suspected perioperative allergic reactions and (ii) the sensitivity and specificity for the diagnostic tests used in chlorhexidine allergy.

Methods

Study population

This retrospective single-centre study involved all patients investigated in DAAC July 2004–July 2012 ($n = 343$ patients). Perioperative allergic symptoms ranged in severity from rash to anaphylactic shock, and in some cases cardiac arrest. The severity of allergic reactions was classified using a previously described classification (19): class 1: mild reactions with generalized cutaneous signs, self-limiting; class 2: moderate, multi-organ involvement, may be self-limiting; class 3: severe, life-threatening, usually multi-organ involvement, requires specific treatment; class 4: cardiac arrest. All patients underwent a systematic individualized investigation protocol for all drugs they had been exposed to before the allergic reaction using the following criteria: (i) all drugs given intravenously within 1 h of the reaction were tested; (ii) all drugs given orally, subcutaneously, epidurally, intrathecally or intramuscularly within 2 h of the reaction were tested. Furthermore, all patients were tested for allergy to chlorhexidine, latex and ethylene oxide, as all were exposed to these compounds. The most frequently tested drugs were opioids, anaesthetic drugs including local anaesthetics, neuromuscular blocking agents and antibiotics. A calculation of Δ Tryptase was carried out by subtracting baseline tryptase from tryptase level in a blood sample drawn 1–4 h after the perioperative allergic reaction. Investigations usually took place 2–4 months after the allergic reaction.

Diagnostic tests in chlorhexidine allergy

The following tests were performed:

- 1 *Specific IgE for chlorhexidine* (Immunocap[®]; Phadia AB, Uppsala, Sweden). Criterion for positivity: specific IgE > 0.35 kUA/l.
- 2 *Histamine release test* (RefLab ApS, Copenhagen, Denmark). Criterion for positivity: HR class 1–12. For logistic reasons, HR tests were usually performed 2 weeks–3 months later than specific IgE analysis.
- 3 *Skin prick test* on the forearm in duplicate with chlorhexidine digluconate 5 mg/ml (4, 20). The reaction was read after 20 min and compared with a negative control with saline. Histamine 10 mg/ml served as a positive control. Criterion for positivity: mean diameter of wheal ≥ 3 mm (21).
- 4 *Intradermal test* on the back in duplicate with chlorhexidine digluconate 0.002 mg/ml (4, 20). In DAAC, two different procedures have been used. Until January 2011, a bleb of 3–5 mm was induced with a 0.5-ml syringe without measuring volume. Since January 2011, in an attempt to standardize the procedure, a fixed volume of 0.02 ml has been injected with a 0.5-ml syringe. Both test procedures were read after 20 min and compared with a negative control with saline. Criterion for positivity: mean diameter of wheal \geq twice the diameter of negative control (21).

To assess the influence of the positivity criterion for IDT on the estimated sensitivity and specificity, two other commonly used positivity criteria were subsequently applied. Thus, the following three positivity criteria for IDT were evaluated at 20 min: (A) diameter of wheal \geq twice the diameter of negative control (19, 21, 22), (B) diameter of wheal ≥ 3 mm larger than negative control (23) and (C) diameter of wheal ≥ 8 mm independent of size of negative control (24, 25).

To obtain a homogenous group, only patients with all four tests performed were included ($n = 228$ patients) (see Fig. 1).

It is not possible to calculate exact sensitivity and specificity in the absence of a provocation model ('Gold Standard'). However, to solve this dilemma, we *post hoc* defined chlorhexidine allergy as one or more relevant clinical reactions to chlorhexidine in combination with a minimum of two positive tests, and we used this definition to calculate estimated sensitivity and specificity.

To estimate sensitivity and specificity for each test, the result of the test in question was omitted from the diagnostic calculation and results of the remaining three tests were compared with the allergy status for chlorhexidine (allergy/no allergy).

Results

A total of 228 patients investigated in DAAC July 2004–July 2012 were included (214 adults/14 children, 141 F/87 M, mean age 49 years).

Thirty-two patients had one or more positive tests for chlorhexidine (see Fig. 2). Demographic data and details of reaction class, serum tryptase and specific investigation results for all 32 patients can be seen in Table 1. Of these, 22

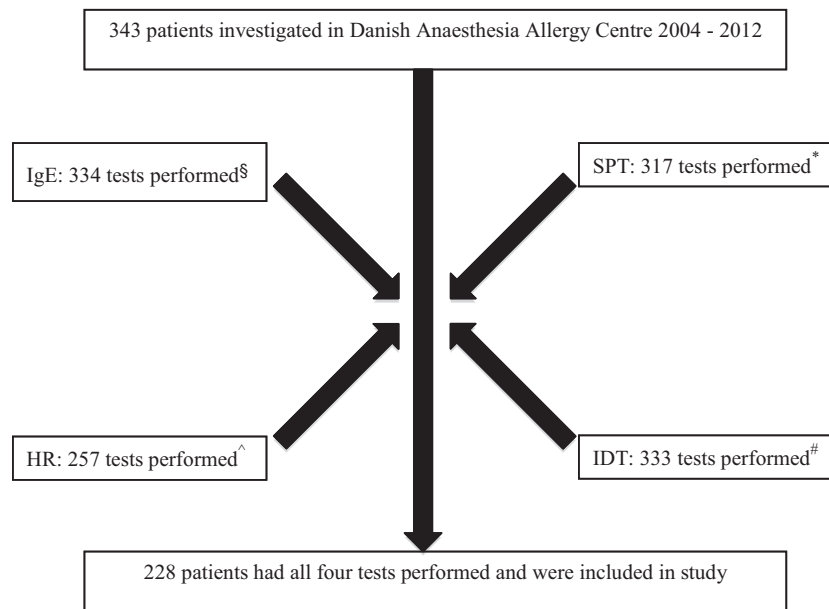


Figure 1 Flowchart of inclusion of patients tested with chlorhexidine. [§]Specific IgE became commercially available in 2007, but most patients from 2004–2007 had stored serum analysed. [^]HR = histamine release test was only performed routinely since

2007. ^{*}SPT = skin prick test results were excluded in cases of dermographism or inadequate positive controls. [#]IDT = intradermal test was not performed in all children.

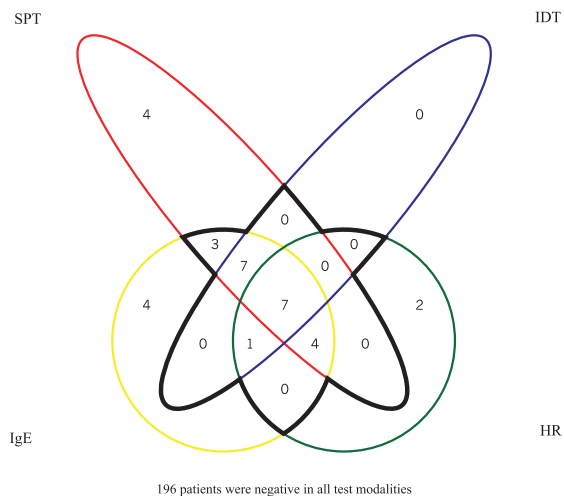


Figure 2 Number of patients with ≥ 1 positive test in the four test modalities ($n = 32$). The patients within the black line met the definition of chlorhexidine allergy (≥ 2 positive tests) ($n = 22$). SPT, skin prick test; IDT, intradermal test; HR, histamine release test.

patients (9.6%) met the definition of chlorhexidine allergy (20 adults/two children, 4 F/18 M, mean age 57 years). One patient had been included in previous study (4). All 22 patients diagnosed with chlorhexidine allergy had positive specific IgE, 21 had positive SPT, 15 had positive IDT, 12 had positive HR, and two had an additional verified allergy. Ten patients had one positive test only (nine adults/one child, 5 F/5 M, mean age 46 years). As can be seen in Table 1, the

majority of these test results were just above the cut-off values, and five of the ten patients had another verified allergy.

The remaining 196 patients were negative in all tests for chlorhexidine allergy.

Specific IgE

Overall, 26 had a positive specific IgE to chlorhexidine. Of these, specific IgE was the only positive test in four patients with a maximum value of 0.50 kUA/l. Specific IgE results for the remaining 22 patients, who all met the definition of chlorhexidine allergy, were class 1 (0.35–0.70 kUA/l) in seven patients, class 2 (0.71–3.5 kUA/l) in nine patients, class 3 (3.51–17.5) in five patients and class 4 (17.6–50) in one patient. For concordance between specific IgE results and allergy status to chlorhexidine, see Table 2.

Histamine release test

In total, 14 patients had a positive HR. Of these, HR was the only positive test in two patients: HR class 2 and class 4, respectively. Histamine release test results for the remaining 12 patients were class 2 ($n = 1$), class 10 ($n = 1$), class 12 ($n = 9$), and one patient had a positive test without further classification. For concordance between HR and allergy status to chlorhexidine, see Table 2.

Skin prick test

Overall, 25 patients had a positive SPT. Of these, SPT was the only positive test in four patients, and the wheal diameter

Table 1 Test results for patients with one or more positive tests for chlorhexidine allergy ($n = 32$). Chlorhexidine allergy was diagnosed on the basis of a minimum of two positive tests ($n = 22$)

Sex F/M	Age years	Δ Tryptase* $\mu\text{g/l}$	Reaction class 1–4†	SPT mm	IDT‡ mm	Specific IgE kUA/l	HR class	Other allergen(s)
1 Positive test ($n = 10$)								
F	27	ND	3	3	4/4	0	0	No
F	41	1.0	2	3.5	8/8	0	0	No
M	16	5.6	1	0	7/6.5	0.45	0	No
M	21	−1.7	2	4	5/4	0	0	No
M	69	4.0	3	0	4/3.5	0.43	0	No
F	57	−0.1	2	0	4/4.5	0	4	Latex
F	62	12.7	3	0	6/5	0.50	0	Pantoprazole
F	75	1.5	1	0	4/2.5	0	2	Latex
M	31	17.8	3	0	3.5/4	0.46	0	Cefuroxime and ethylene oxide
M	64	77.6	3	3	4.5/5.5	0	0	Propofol
2 Positive tests ($n = 3$)								
M	48	4.8	3	4.5	7/12	1.49	0	No
M	71	26.7	3	7	7.5/10.5	0.68	0	No
M	81	6.5	2	3	5.5/5	0.83	0	No
3 Positive tests ($n = 12$)								
F	28	1.5	3	5	6.5/14	2.96	0	No
M	14	2.6	1	4.5	6.5/23	1.34	0	No
M	16	ND	2	14.5	3.5/15	0.44	0	No
M	32	−0.9	3	5.5	3/12	2.45	0	No
M	33	4.6	2	7	6.5/15.5	3.76	0	No
M	52	8.1	2	0	6/12.5	4.90	12	No
M	57	24.5	3	3.5	5/8	0.61	Pos§	No
M	59	ND	2	3	4.5/9.5	0.52	0	No
M	68	0.6	2	8.5	5/10	0.51	0	No
M	73	27.1	3	5	5.5/10	3.07	12	No
M	79	40.7	3	5.5	5.5/5.5	8.53	2	No
F	74	ND	3	4.5	8/8	0.65	12	Latex
4 Positive tests ($n = 7$)								
F	48	1.1	1	4.5	3/10	5.63	12	No
F	51	ND	3	6	6.5/13.5	1.40	12	No
M	67	24.6	3	5	3.5/8	1.35	10	No
M	68	ND	3	9	5.5/12	11.90	12	No
M	68	19.4	3	4.5	4.5/9	33.70	12	No
M	86	15.3	3	10.5	5/11	0.67	12	No
M	70	14.9	3	6	6.5/13.5	1.45	12	Ampicillin

SPT, skin prick test; IDT, intradermal test; HR, histamine release test; ND, not done.

*Tryptase measured 1–4 h after reaction minus baseline tryptase.

†Class 1: mild reactions with generalized cutaneous signs, self-limiting; class 2: moderate, multi-organ involvement, may be self-limiting; class 3: severe, life-threatening, usually multi-organ involvement, requires specific treatment; class 4: cardiac arrest.

‡IDT: diameter of negative control measured in mm/diameter of chlorhexidine digluconate 0.002 mg/ml measured in mm.

§The HR result was not further classified.

was ≤ 4 mm in all four. The wheal diameter in the remaining 21 patients was 3.0–4.0 mm ($n = 3$), 4.1–5.0 mm ($n = 8$), 5.1–7.0 mm ($n = 6$), 7.1–10.0 mm ($n = 2$) and 10.1–15.0 mm ($n = 2$). For concordance between SPT results and allergy status to chlorhexidine, see Table 2.

Intradermal test

In total, 15 patients had a positive intradermal test, and they all met the definition of chlorhexidine allergy. The wheal diameters were in the range of 8–23 mm, and the negative

Table 2 Test results compared with allergy status to chlorhexidine

	Allergy*	No allergy	Estimated sensitivity in %	Estimated specificity in %
IgE positive	19/19	7/209	100	97
HR positive	12/22	2/206	55	99
SPT positive	18/19	7/209	95	97
IDT	15/22	0/206	68	100

HR, histamine release test; SPT, skin prick test; IDT, intradermal test.

*Allergy was defined as a minimum of two positive tests omitting the test in question to avoid comparing the test with itself; thus, the number of patients with allergy varies from test to test.

controls were 3–7 mm. For concordance between IDT and allergy status to chlorhexidine, see Table 2.

Subsequently, two other positivity criteria for the IDT were applied. Thus, the following three criteria were evaluated: (A) diameter of wheal \geq twice the diameter of negative control (as used above), (B) diameter of wheal ≥ 3 mm larger than negative control and (C) diameter of wheal ≥ 8 mm independent of negative control.

We found that criteria B and C had a higher concordance with other tests for chlorhexidine allergy than criterion A. Of the 22 patients with chlorhexidine allergy, 20, 19 and 15 patients had a positive IDT when applying positivity criteria C, B and A, respectively. However, when applying positivity criterion C, the number of positive tests in the remaining 206 chlorhexidine-negative patients also increased resulting in a slightly lower estimated specificity (see Table 3).

All the above-mentioned calculations were also made including all patients investigated in DAAC July 2004–July 2012 (i.e. also patients with less than all four diagnostic tests; $n = 343$ patients) with comparable findings.

Discussion

At the present time, a validated provocation model is not available in chlorhexidine allergy. Although a provocation model would be useful, such a model would by definition have to reach sensitivity and specificity of 100%. This gives rise to both technical and ethical issues. Most chlorhexidine-allergic patients experience allergic reactions during surgery, where there may be multiple exposures (e.g. urethra,

venepuncture, skin incision) in differing concentrations. Furthermore, patients have often had severe reactions (shock or even cardiac arrest) leading to anxiety in the patient and leaving the allergist with ethical/safety considerations before attempting provocation.

In the absence of a validated provocation model, we *post hoc* defined chlorhexidine allergy using what we consider the best possible alternative: one or more relevant clinical reactions to chlorhexidine in combination with a minimum of two positive diagnostic tests. This definition was previously applied for Rocuronium (26), another drug for which a provocation model is not available.

We found that 22 of 228 patients (9.6%) with suspected perioperative allergic reactions met this definition of chlorhexidine allergy. Among these, the highest concordance between tests was found between specific IgE and SPT, which were positive in 22 of 22 and 21 of 22 patients, respectively. Three patients were positive in specific IgE and skin prick test only; the remaining 19 patients had a minimum of three positive tests. Chlorhexidine-allergic patients were predominantly male and older (82% M; mean age 57 years) than the chlorhexidine-negative group (33% M; mean age 48 years). This male predominance has previously been observed (4, 5, 17).

In the ten patients who had only one positive test and thus considered not allergic to chlorhexidine, test results were all just above the cut-off values. This emphasizes the limitations of exact cut-off values but also highlights an important limitation of this study: we can never be absolutely sure that we have diagnosed all patients correctly with the lack of a provocation model.

In this study, the estimated sensitivity and specificity were both high for SPT (sensitivity 95% and specificity 97%) and specific IgE (sensitivity 100% and specificity 97%). Specific IgE analysis is recommended within 6 months of the reaction by manufacturer (27). In our setting, specific IgE is always measured within this timeframe. It has been shown that specific IgE to chlorhexidine declines over time with a lack of exposure (4, 28), and this has also been shown for ethylene oxide (29, 30) and penicillins (31). However, the timing of this decline remains unclear, and it is presumed that specific IgE may eventually become negative. The latter seems likely, as a Finnish study only found six of 14 patients with a positive specific IgE on average 29 months after the allergic reaction (32).

For other allergens, SPT and IDT are reported not to change significantly over time (33, 34), but this has not been systematically investigated in chlorhexidine allergy. Future studies should focus on the changes in specific IgE, SPT and IDT results over time.

We found that estimated sensitivity was low, but estimated specificity was very high for both HR (sensitivity 55% and specificity 99%) and IDT (sensitivity 68% and specificity 100%). We were surprised by the low-estimated sensitivity for the IDT, as IDT is generally considered more sensitive than SPT (19, 23, 35). A too low test concentration seems unlikely to be responsible for the low-estimated sensitivity as higher concentrations produce false-positive IDT results (15).

Table 3 Estimated sensitivity and specificity for the intradermal test when applying different positivity criteria

	Positivity criterion A	Positivity criterion B	Positivity criterion C
Estimated sensitivity (%)	68	86	91
Estimated specificity (%)	100	100	95

Positivity criterion A, diameter of wheal \geq twice the diameter of negative control; positivity criterion B, diameter of wheal ≥ 3 mm larger than negative control; positivity criterion C, diameter of wheal ≥ 8 mm independent of negative control.

We have used the concentration of 0.002 mg/ml for many years, and this concentration has recently been endorsed by the European Network for Drug Allergy (ENDA) (20). More importantly, there is no international consensus on the criterion for a positive IDT and several different criteria exist. The French Society of Anaesthesiologists [Société Française d'Anesthésie et de Réanimation (SFAR)] has many years of experience in investigating perioperative allergy. They recommend the positivity criterion of a wheal diameter minimum twice the diameter of the induced bleb after 20 min (19, 21, 22). The ENDA group recommends an increase in wheal diameter of at least 3 mm associated with a flare after 15–20 min (23). A third commonly used positive criterion is a wheal diameter of minimum 8 mm independent of negative control (24, 25). In this study, we compared the three above-mentioned criteria with the modification that we used a negative control with saline instead of comparing with the induced bleb. This was due to the fact that most of our retrospective data had been collected in this way, but since 2011, we have used the practice of comparing IDT wheal diameter at 20 min with the induced bleb, rather than the negative control. We found that the two latter criteria gave the highest combined estimated specificity and sensitivity, that is, diameter of wheal ≥ 3 mm larger than negative control and wheal of a minimum of 8 mm independent of negative control. This study suggests that either of these criteria should be used in the future, but additional studies should investigate the positivity criteria for IDT for other allergens, where a provocation model is available.

In conclusion, in this large single-centre study, we defined chlorhexidine allergy as one or more relevant allergic reactions in combination with a minimum of two positive diag-

nostic tests. Using this definition, we identified 22 patients with chlorhexidine allergy of 228 patients (9.6%) systematically investigated for suspected perioperative allergic reactions. We recommend that all patients with perioperative allergic reactions are tested with chlorhexidine due to widespread and often hidden exposure.

We found the highest estimated combined sensitivity and specificity for the most widely available tests, specific IgE and skin prick test. We recommend the use of these tests as a minimum when investigating chlorhexidine allergy.

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Author contributions

M.S. Opstrup and L.H. Garvey took initiative to the article and drafted the first manuscript. H.-J. Malling, M. Krøigaard, H. Mosbech, P.S. Skov and L.K. Poulsen provided critical comments about the study design and the manuscript. All authors approved the final version.

Conflicts of interest

H.J. Malling has received research grants to the Allergy Clinic, Gentofte Hospital, Denmark, from the National Institute of Health, EU-commission, Danish Toyota Fund and Tryg Foundation, and is both a consultant and a speaker for ALK-Abellø, Denmark and Stallergenes, France. He is also a member of the Danish National Medico-legal Council. The other authors of the paper declare no conflicts of interest.

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Dynamics of specific IgE in chlorhexidine allergic patients with and without
accidental re-exposure

In prep.

Dynamics of specific IgE in chlorhexidine allergic patients with and without accidental re-exposure

Running head: specific IgE in chlorhexidine allergy

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Abstract

Background

Chlorhexidine is an effective disinfectant, but it can cause severe allergic reactions. Specific IgE to chlorhexidine (ImmunoCAP®) has high estimated sensitivity and specificity when measured within six months of reaction, but the dynamics of specific IgE is poorly described and it is unknown whether it will decline to values $<0.35\text{kUA/l}$ in patients with previously elevated levels. It is also unclear whether re-exposures influence levels of specific IgE.

Objective

To investigate the dynamics of specific IgE in chlorhexidine allergic patients with and without re-exposure.

Methods

All patients diagnosed with chlorhexidine allergy in the Danish Anaesthesia Allergy Centre from January 1999 to March 2015 were invited to participate. The study included retrospective blood samples from time of reaction and time of investigation and prospective blood samples drawn for this study.

Results

Overall, 23 patients were included. Specific IgE within hours of reaction was $>0.35\text{kUA/l}$ in six of eight patients. During allergy investigations, usually two to four months later, specific IgE was $>0.35\text{kUA/l}$ in 22 of 23 patients including all eight with a value from time of reaction. In the following months/years specific IgE declined to values $<0.35\text{kUA/l}$ in 17 of 23 patients (most rapid decline four months). Re-exposure in healthcare setting was reported by seven patients (35%). Most re-exposures caused symptoms and an increase in specific IgE. Two patients with specific IgE $<0.35\text{kUA/l}$ reacted upon re-exposure.

Conclusions & Clinical relevance

Time from reaction should be considered when analysing specific IgE results. Specific IgE is $>0.35\text{kUA/l}$ in most patients within hours of reaction but should be repeated after a few

weeks/months if negative. The optimal testing time seems to be >1 month and < 4 months. A value <0.35kUA/l does not necessarily exclude allergy or indicates tolerance in previously sensitized patients. Re-exposures are common and can cause an increase in specific IgE.

Introduction

Chlorhexidine is a highly effective disinfectant^{1,2} and is consequently widely used in the healthcare setting in many countries³. Although most people tolerate chlorhexidine well, it can cause severe allergic reactions such as urticaria, anaphylactic shock or even cardiac arrest^{4,5}. In 2007, an IgE-mediated mechanism behind chlorhexidine allergy was confirmed⁴.

The Danish Anaesthesia Allergy Centre (DAAC) is the national reference centre for investigation of patients with perioperative allergic reactions. During surgery, exposure to chlorhexidine can be extensive and consequently chlorhexidine allergic patients have a high risk of reacting in this setting. In DAAC, all referred patients are tested with chlorhexidine as part of the investigations and approximately 10% are diagnosed with chlorhexidine allergy⁶. Two recent studies from the UK showed that 5% and 7% of patients with suspected perioperative allergic reactions, respectively, tested positive to chlorhexidine^{7,8}.

In DAAC, the diagnosis of chlorhexidine allergy is based on a relevant clinical history in combination with results of skin prick test, intradermal test, specific IgE (ImmunoCAP®, Thermo Fisher Scientific, Uppsala, Sweden) and in some cases histamine release test (HR-test) (Reflab Aps, Copenhagen Denmark).

Based on data from patients investigated in DAAC, it was recently estimated that both sensitivity and specificity of specific IgE to chlorhexidine are high (100% and 97%, respectively)⁶. However, in that study samples were collected within six months as recommended by the manufacturer⁹. It has been shown before that plasma levels of specific IgE seem to decrease over time for chlorhexidine⁴ and for other allergens e.g. ethylene oxide and penicillins¹⁰⁻¹², but the dynamics of specific IgE to chlorhexidine over longer time periods have never been investigated in detail. Currently, it is unknown whether specific IgE will eventually drop below the recommended cut-off of 0.35kUA/l on lack of exposure in patients with previously elevated levels, as described for

penicillins¹². It is also unclear whether re-exposure to chlorhexidine influences specific IgE-values in patients with previously elevated levels.

The aim of this study was to follow the dynamics of specific IgE to chlorhexidine over time in patients with chlorhexidine allergy with and without known re-exposure to chlorhexidine.

Materials and methods

The local Human Ethics Research Committee approved the study protocol (project ID H-3-2012-144).

Patients

Inclusion criteria:

- Patients diagnosed with chlorhexidine allergy in the Danish Anaesthesia Allergy Centre (DAAC) from January 1999 to March 2015
- Aged ≥ 18 years and still alive at time of inclusion

Overall 44 patients were diagnosed with chlorhexidine allergy in DAAC from January 1999 to March 2015. Of these, 11 had died and one was below 18 years at time of inclusion. Consequently, 32 patients fulfilled the inclusion criteria and were invited to participate in the study. The first patients were enrolled in 2013, but patients diagnosed until March 2015 were also invited to participate and were consecutively enrolled. Invitations included a letter with information about the project and a consent form. Those not responding to the invitation within a few months were contacted by telephone. Patients neither responding to the letter nor telephone call were not further contacted.

Controls

Non-chlorhexidine allergic patients investigated for a suspected perioperative allergic reaction in DAAC from January 1999 to March 2015 were included as controls. Controls were matched with patients with respect to age and sex.

Study design

Data on age, sex, reaction class and type of surgery performed were collected for patients and controls from the clinical database hosted in the department and from the case files.

This study included retrospective blood samples from time of reaction and time of investigation in DAAC as well as prospective blood samples drawn for the purpose of this study. Blood samples were analysed for specific IgE (Immunocap®, Thermo Fisher Scientific, Uppsala, Sweden) with a cut-off of 0.1kUA/l and total IgE.

Retrospective blood samples

Available retrospective blood samples included: a) a blood sample for tryptase analysis drawn within one to three hours at time of reaction leading to referral to DAAC, b) a blood sample drawn after initial contact to patient four to six weeks later and c) a blood sample drawn at time of investigation in DAAC, usually two to four months after the allergic reaction.

If not already available, specific IgE was measured on all patients' retrospective blood samples and controls' blood samples drawn at time of investigation in DAAC. In case specific IgE was <0.35kUA/l, a re-analysis was performed using a cut-off of 0.1kUA/l.

Prospective blood samples

Blood samples were prospectively drawn for patients included in the study and number of blood samples depended on time since the original allergic reaction, see table 1.

Time after the allergic reaction	0-2 years	2-4 years	More than 4 years
Number of annual blood samples	4	2	1

Table 1. Schematic representation of number of prospective blood samples drawn for the purpose of this study.

Blood samples were kept at -20°C and all analyses were performed in September to December 2015.

Patient follow-up

At the end of the study in 2015 all included patients were contacted by telephone and asked whether they had been accidentally re-exposed to chlorhexidine after the diagnosis. Those not responding to the telephone calls were mailed a letter asking them to contact the investigators. The time of re-exposure, the product used during re-exposure and allergy symptoms were noted.

Statistics

The data were processed with SPSS (SPSS_{TM} Statistics, Chicago, IL, USA; IBM PASW Statistics) for Windows_{TM}, edition 22.0 and Microsoft Excel.

Results

Informed consent was obtained from 23 out of the 32 patients who fulfilled the inclusion criteria. Reasons for declining: not interested in participating (n=7); no response to letter nor telephone calls (n=2). Of the 23 included patients, 15 had been included in a previous study evaluating the tests in chlorhexidine allergy⁶. Demographic data and details on reaction class and type of surgery performed at time allergic reaction for the 23 patients can be seen in table 2. Most patients reacted during urological (n=10) or gastrointestinal surgery (n=6). Of the 23 patients, 18 reactions (78%) were graded as class 3 (anaphylaxis) and five reactions (22%) were graded as class 1 or 2.

Patient number	Age years	Sex F/M	Reaction class* 1-4	Surgical specialty
1	66	M	3	Urology
2	54	M	3	Ear-Nose-Throat
3	37	M	4	Gastrointestinal
4	40	M	3	Urology
5	27	F	3	Gastrointestinal
6	50	F	3	Gastrointestinal
7	86	M	3	Urology
8	67	M	3	Gastrointestinal
9	68	M	3	Urology
10	47	F	1	Plastic surgery
11	16	M	2	Ear-Nose-Throat
12	32	M	3	Orthopaedic
13	73	M	3	Gastrointestinal
14	32	M	2	Orthopaedic
15	68	M	2	Urology
16	70	M	3	Urology
17	59	M	2	Urology
18	48	M	3	Back surgery
19	53	M	3	Urology
20	76	M	3	Neurosurgery
21	69	M	3	Urology
22	64	M	3	Urology
23	67	M	3	Gastrointestinal

Table 2. Demographic data and information about the allergic reaction leading to referral to Danish Anaesthesia Allergy Centre. ND=Not done. * Class 1: mild reactions with generalized cutaneous signs, self-limiting. Class 2: moderate multi-organ involvement, may be self-limiting. Class 3: severe life-threatening, usually multi-organ involvement, requires specific treatment. Class 4: cardiac arrest.

A total of 114 blood samples (retrospective and prospective) from patients and 23 from controls were included in the study. Due to highly variable initial values and variable dynamics, graphical representation of all results is not meaningful, but all specific IgE-values can be found in appendix 1.

Specific IgE at time of reaction and dynamics in subsequent weeks/months (retrospective blood samples)

Overall eight patients had specific IgE-results available from a blood sample drawn within one to three hours of the initial reaction and values showed great variation ranging from 0.24kUA/l to 66.7kUA/l. Specific IgE was >0.35kUA/l in six of the eight patients. One patient had a specific IgE of 0.19kUA/l measured three months before the allergic reaction but there was no blood sample available at time of reaction in this patient (patient 19). In seven of eight patients, specific IgE increased over the subsequent weeks/months after the allergic reaction; in the last patient, only samples from days 0, 5 and 9 were available and here an increase was not observed (patient 23 in appendix 1).

Using the recommended cut-off of 0.35kUA/l, specific IgE was elevated in 22 of 23 patients at time of investigation in DAAC a median of 10 weeks after the allergic reaction including all eight patients with a blood sample available from time of reaction (data shown in appendix 1). In contrast, specific IgE was <0.35kUA/l in all 23 controls and <0.1kUA/l in 18 of these (data not shown). The five controls with a detectable specific IgE >0.1kUA/l but <0.35kUA/l had values of 0.12, 0.13, 0.15, 0.18 and 0.24kUA/l, respectively. Total IgE for these five controls was in the range of 239 to 1252kUA/l. For patients, median total IgE at time of investigation in DAAC was 113kU/l (interquartile range 53.1-181kU/l) and only one of the 114 patient blood samples had a total IgE value above 1,000ku/l (1,008kU/l, patient 22).

Dynamics of specific IgE over time in patients with no known re-exposure (retrospective and prospective blood samples)

To illustrate the dynamics of the time after the reaction, we have selected one patient with many blood samples available and no known re-exposure (figure 1).

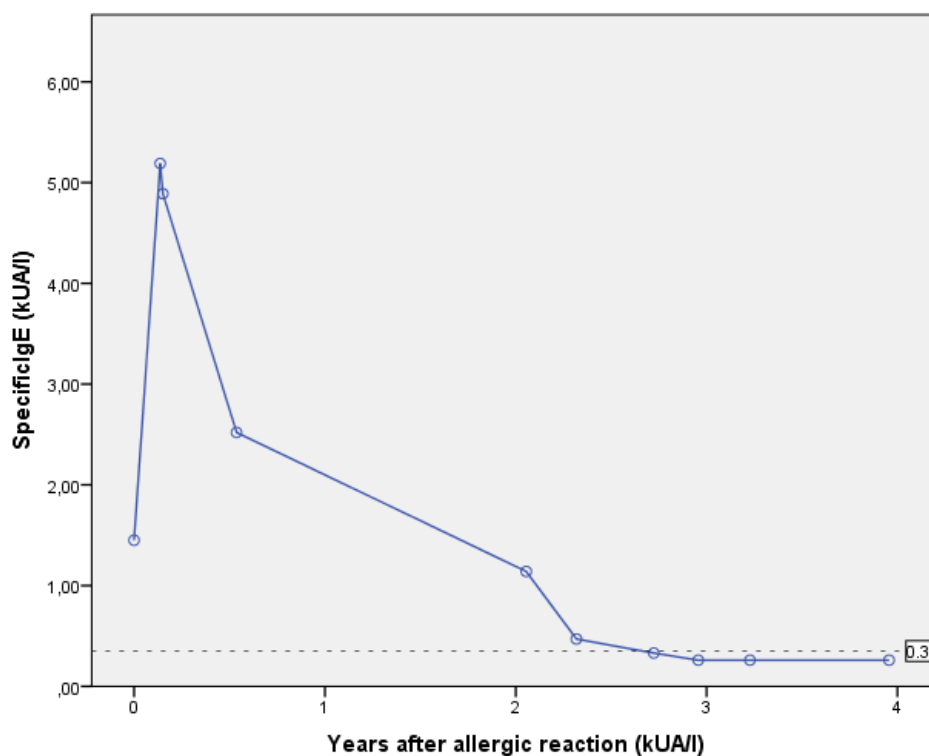


Figure 1. Specific IgE over time in a chlorhexidine allergic patient with no known re-exposure (patient 16 in appendix 1).

Figure 1 shows that specific IgE initially increased in the first months but then declined and eventually fell $<0.35\text{kUA/l}$ after two to three years in this patient. In 21 of 23 patients, lower values were measured in blood samples drawn at the end of this study compared with the values measured during the initial investigations. Indeed, 17 of 23 patients had a specific IgE-value $<0.35\text{kUA/l}$ measured at some point during the study and seven of these also had values $<0.1\text{kUA/l}$ measured. Shortest interval from allergic reaction to a measured specific IgE-value $<0.35\text{kUA/l}$ was four months (patient 11) and shortest interval to a value $<0.1\text{kUA/l}$ was 64 months (patient 11). The rate of decline seemed to vary greatly between patients and it was impossible to estimate the half-life of specific IgE due to heterogeneity of the data. Although values declined to $<0.35\text{kUA/l}$, specific IgE remained above this value for many years without a known re-exposure in four patients (patient 7, 8, 10 and 13).

Dynamics of specific IgE over time in patients with known re-exposure (retrospective and prospective blood samples)

During follow-up to identify accidental re-exposures, 20 of 23 patients responded to the telephone call or letter (patients not responding were patient 1, 9 and 12). Of these, seven patients (35%) reported a total of nine accidental re-exposures - all in the healthcare setting. Seven exposures resulted in symptoms but two were asymptomatic.

Re-exposures during surgery

Overall three patients were re-exposed to chlorhexidine during surgery and all three developed anaphylactic shock (patient 3, 5 and 18). Specific IgE-values were available from blood samples drawn in close relation to the re-exposures for two patients as illustrated in figure 2a and 2b. Both re-exposures resulted in an increase in specific IgE. There was no blood sample available for the last patient in the time around the reaction.

Re-exposures in urethral gels

Overall two patients were re-exposed to chlorhexidine in a urethral gel: one reported local itching and swelling although specific IgE was 0.24kUA/l one year earlier and 0.21kUA/l two months after the exposure (patient 15); one reported that re-exposure did not result in symptoms but specific IgE was raised in the subsequent blood sample (patient 19) (see figure 2C).

Re-exposures in skin swabs

Four patients reported re-exposure to chlorhexidine in skin swabs: one patient developed anaphylactic shock and subsequently an increase in specific IgE was observed (patient 18, figure 2B); one patient developed local symptoms only and specific IgE was <0.1kUA/l one year later (patient 5, figure 2A); one patient reported general discomfort and redness of skin (patient 23); one patient reported that exposure did not cause symptoms (patient 2). The two latter patients did not have any blood samples available in close relation to the exposures and therefore it is not possible to evaluate the influence on specific IgE-levels.

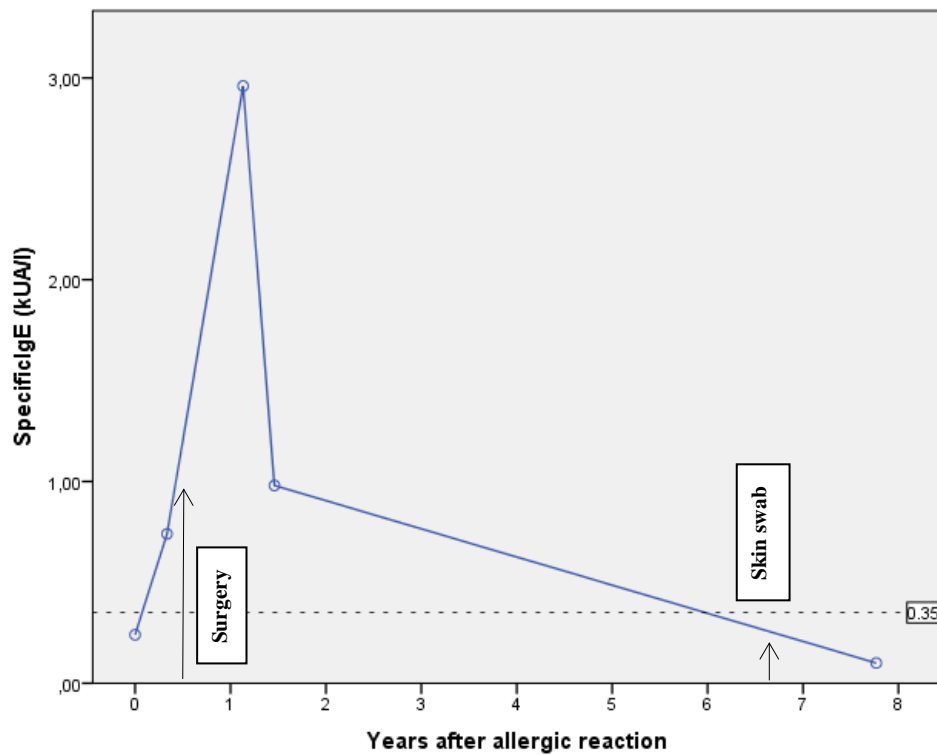


Figure 2A. Specific IgE in patient re-exposed twice (patient 5). T=0 is time of original reaction. Black arrow indicates symptomatic re-exposure.

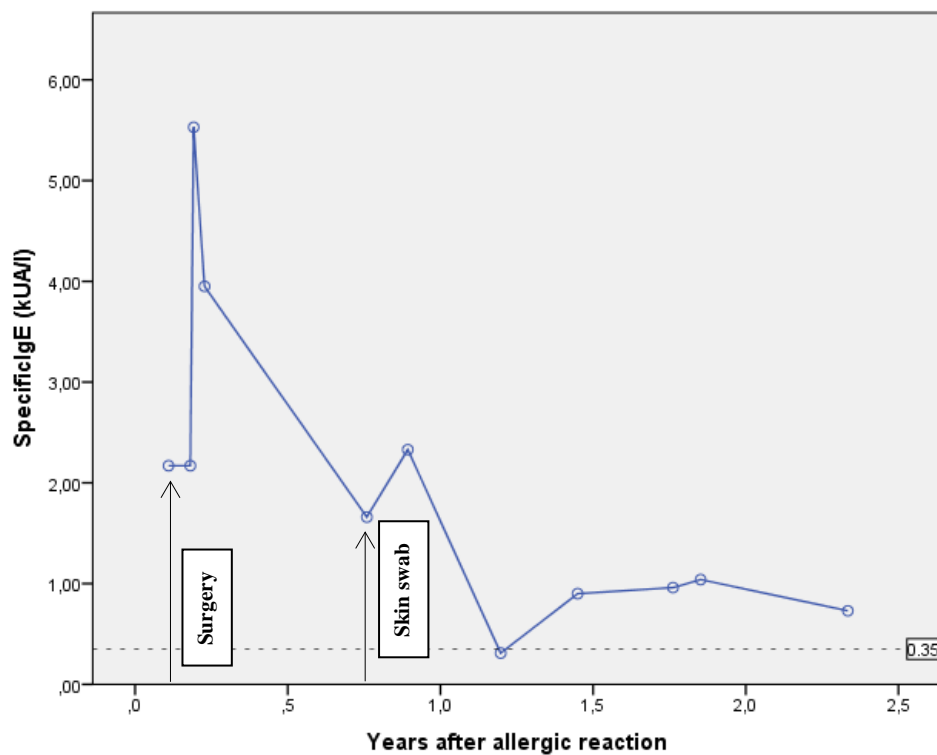


Figure 2B. Specific IgE in patient re-exposed twice (patient 18). T=0 is time of original reaction. Black arrow indicates symptomatic re-exposure.

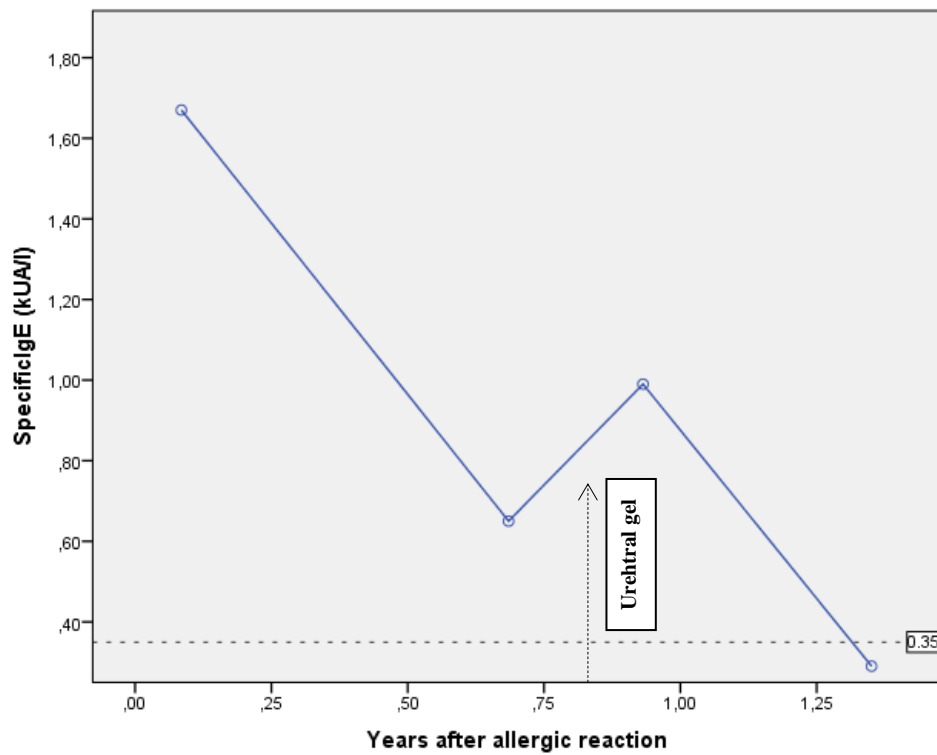


Figure 2C. Specific IgE in patient re-exposed once (patient 19). T=0 is time of original reaction. Dotted arrow indicates asymptomatic re-exposure.

Discussion

The focus of this study was on investigating the dynamics of specific IgE to chlorhexidine over time in patients with chlorhexidine allergy with and without re-exposure to chlorhexidine. Overall, 23 patients were included in the study, and eight of these had a specific IgE results available from within hours of the initial reaction. Of these eight patients, six had a specific IgE-value above the recommended cut-off of 0.35kUA/l, whereas two had a value <0.35kUA/l. At time of investigation in DAAC, two to four months after the initial reaction, specific IgE was >0.35kUA/l in 22 of 23 patients including all eight with a blood sample available from time of reaction. It has been shown previously by our group in 2007 that specific IgE could be elevated at time of initial reaction and this has been confirmed for other allergens e.g. ethylene oxide and neuromuscular blocking agents^{11,13}. However, if negative at time of reaction, the test should be repeated after a few weeks/months as specific IgE is likely to increase.

In seven of the eight patients specific IgE did in fact increase in the first weeks to months after the allergic reaction, and after this increase, specific IgE gradually declined and eventually fell below the recommended cut-off of 0.35kUA/l in 17 of 23 patients and below 0.1kUA/l in seven of these 17 patients. The time taken for values to decline below 0.35kUA/l and 0.1kUA/l varied considerably between patients with most rapid decline below 0.35kUA/l at four months after the allergic reaction and the most rapid decline below 0.1kUA/l at 64 months. This may indicate that measuring specific IgE to chlorhexidine within six months after the allergic reaction, as recommended by manufacturer, is too late in some patients. Four patients still had a specific IgE above 0.35kUA/l many years after the allergic reaction, although they reported never to have been re-exposed. Consequently, it is not possible to predict how long specific IgE to chlorhexidine will remain elevated in a patient, and this is in line with previous findings on penicillin allergy¹². In an earlier manuscript, we recommended that testing for chlorhexidine should include a clinical history

in combination with specific IgE and skin prick test as a minimum⁶. However, as shown above, specific IgE results can decline below the recommended cut-off of 0.35kUA/l in chlorhexidine allergic patients, indicating that specific IgE is not a sensitive test when the allergic reaction took place long before investigations. Currently, the dynamics of the skin prick test results to chlorhexidine are unknown and consequently it is unknown whether this test is useful when investigations take place long after the allergic reaction. The dynamics in skin prick test results over time should be the focus of future studies.

It has previously been speculated that a lower cut-off of specific IgE to chlorhexidine could be used to increase the diagnostic accuracy^{14,15}. In this study, we found that most patients with a specific IgE value <0.35kUA/l (either at time of reaction or a long time after the reaction), specific IgE was still >0.1kUA/l. However, in the patients with the longest interval between initial reaction and inclusion in this study, specific IgE values did decrease to <0.1kUA/l. It could be speculated that in all patients without re-exposures to chlorhexidine the values will eventually decline to <0.1kUA/l, which was the level measured in most non-chlorhexidine allergic controls. Five non-allergic controls had a specific IgE above 0.1kUA/l (up to 0.24kUA/l). It has previously been shown for Rocuronium that total IgE over 1,500kU/l could interfere with results in the ImmunoCAP system¹⁶. However, all five controls with a specific IgE above 0.1kU/l had a total IgE below 1,500kUA/l and therefore high levels of total IgE cannot explain the specific IgE-values. Consequently, it is difficult to assess the relevance of specific IgE values between 0.1kUA/l and 0.35kUA/l in patients due to variability in initial values and time to decline <0.35kUA/l.

Chlorhexidine is widely used in the healthcare setting. As a consequence, there is a high risk of re-exposure, and several case reports have described patients with immediate-type chlorhexidine allergy, who were accidentally re-exposed^{5,17,18}. In the current study, seven patients (35%) reported a total of nine re-exposures in the healthcare setting. Three re-exposures took place during surgery

causing anaphylactic shock and a subsequent increase in specific IgE in two of the patients (no blood sample was available close to reaction time in the third). Two re-exposures were caused by urethral gels resulting in local symptoms in one patient with no increase in specific IgE, which was below 0.35kUA/l both before and after the reaction. In contrast, exposure was asymptomatic in the other patient but led to an increase in specific IgE. Four re-exposures were caused by skin swabs. One patient reported asymptomatic re-exposure through a skin swab. In another patient only local symptoms occurred, a third patient reported general discomfort and redness of the skin, whereas exposure resulted in anaphylactic shock and an increase in specific IgE in a fourth patient.

All taken together, it seems that most re-exposures to chlorhexidine cause symptoms and an increase in specific IgE. However, some re-exposures resulted in symptoms only with no increase in specific IgE, while others were asymptomatic but caused an increase in specific IgE. Patients can still develop symptoms when specific IgE is below 0.35kUA/l and therefore does a value below 0.35kUA/l not indicate necessarily tolerance in chlorhexidine allergic patients. This finding is in line with penicillin allergy, where it has recently been shown that some patients with specific IgE below 0.35kUA/l still are allergic whereas others tolerate the drug on subsequent drug provocation¹². One could speculate that in the time leading up to surgery patients are repeatedly exposed to chlorhexidine in close proximity e.g. through blood taking and investigations prior to surgery. Each of these exposures resulting in an increase in specific IgE, making a serious reaction more likely to occur upon the extensive exposure that takes place during surgery and anaesthesia. Indeed, this is a clinical picture that we often encounter in our centre.

Besides being used as a disinfectant in the healthcare setting, chlorhexidine can also be used as a preservative in cosmetic products¹⁹. Although chlorhexidine in cosmetic products has been reported to cause contact allergy^{20,21}, it very rarely, if ever, causes immediate-type allergic reactions. To our knowledge, no case reports have ever been published of patients with immediate-type reactions

caused by chlorhexidine in cosmetics. It is well known that chlorhexidine does not penetrate intact skin²², but it is unclear whether an exposure in cosmetic products on broken skin or a mucous membrane may cause an increase in specific IgE. Indeed, for some patients included in this study, specific IgE did increase at times where patients denied re-exposure and perhaps unknown exposure in cosmetics or in the healthcare setting could play a role.

In conclusion, most patients with chlorhexidine allergy have a specific IgE above 0.35kUA/l at the time of reaction, but some only become positive a few weeks/months later. Specific IgE increases in the weeks/months after initial allergic reaction, but subsequently values gradually decline and eventually fall below 0.35kUA/l and even 0.1kUA/l on lack of exposure. This highlights the importance of taking time elapsed from the allergic reaction into consideration when analysing specific IgE results. The initial value of specific IgE and rate of decline vary greatly between patients with the most rapid decline below 0.35kUA/l at 4 months. This may indicate that testing within six months after the allergic reaction, as recommended by manufacturer, is too late in some patients, resulting in false negative specific IgE values. A specific IgE value below 0.35kUA/l in a chlorhexidine allergic patient with previously elevated values does not necessarily indicate tolerance. Re-exposures are common, in this study 35% of patients reported re-exposure in the healthcare setting. This highlights that healthcare workers need to be well-informed about possible sources of exposure when treating a patient with chlorhexidine allergy. Most re-exposures caused symptoms and an increase in specific IgE. The high rate of re-exposures to chlorhexidine illustrates the widespread use in the healthcare setting. This emphasizes the need to consider chlorhexidine as an allergen in all allergic reactions in the healthcare setting.

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Conflicts of interest

All authors declare no conflicts of interest.

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Appendix 1.
Table of all included patients' test results.

<i>Patient number</i>	<i>Specific IgE (kUA/l)</i>	<i>Total IgE (kUA/l)</i>	<i>Time from allergic reaction (days)</i>	<i>Re-exposures</i>
1	1.60	ND	71	Unknown
	<0.1	17.1	4370	
	<0.1	15.0	4784	
2	0.49	ND	201	1. Asymptomatic re-exposure in skin swab around day 600
	0.89	69.1	3542	
	<0.1	12.3	3954	
3	3.99	167	83	1. Severe allergic reaction during surgery around day 900
	1.68	ND	159	
	0.41	60.3	2927	
	0.27	238	3339	
	0.38	150	3612	
4	<0.35*	373	127	No known re-exposure
	0.11	729	163	
	<0.1	350	2976	
5 Data presented graphically in figure 2A.	0.24	30.3	0	1. Anaphylactic shock during surgery caused by chlorhexidine around day 180 2. Local redness and itching caused by skin swab around day 2500
	0.74	53.1	122	
	2.96	122	413	
	0.98	109	533	
	<0.1	24.8	2837	
6	1.14	4.63	44	No known re-exposure
	0.89	5.93	89	
	<0.1	8.69	2584	
7	0.29	18.8	0	No known re-exposure
	0.67	36.1	103	
	0.66	46.5	154	
	4.95	155	2556	
8	11.9	769	248	No known re-exposure
	11.7	873	266	
	0.8	253	2761	
	0.69	171	3131	
9	33.7	803	58	Unknown
	22.7	366	149	
	<0.1	9.98	2346	
	0.2	184	2837	
10	5.63	151	185	No known re-exposure
	4.01	101	245	
	0.73	91.4	2081	
	0.59	56.7	2487	
11	0.44	4.7	71	No known re-exposure

	0.18	5.99	121	
	<0.1	5.03	1927	
	<0.1	3.55	2360	
12	2.45	158	22	Unknown
	1.49	86.2	63	
	0.31	78.7	1799	
	0.89	84.2	2206	
13	3.07	34.0	0	No known re-exposure
	7.59	93.2	57	
	1.24	43.7	1423	
	1.03	40.5	1624	
	1.34	47.7	2080	
14	3.76	87.7	84	No known re-exposure
	3.29	80.5	96	
	0.19	169	1429	
	0.15	164	1831	
15	0.51	462	0	1. Re-exposure in urethral gel causing local itching around day 1570
	0.83	527	28	
	0.25	453	998	
	0.24	398	1148	
	0.21	275	1631	
16 Data presented graphically in figure 1.	1.45	47.1	0	No known re-exposure
	5.19	182	50	
	4.89	181	55	
	2.52	119	196	
	1.14	130	750	
	0.47	75.8	846	
	0.33	100	994	
	0.26	93.4	1079	
	0.26	84.5	1178	
	0.26	77.2	1444	
17	0.51	91.2	37	No known re-exposure
	0.52	113	105	
	0.44	95.0	160	
	0.37	99.4	677	
	0.57	91.3	779	
	0.47	115	890	
	0.37	159	923	
	0.34	101	1099	
	0.37	73.6	1335	
18 Data presented graphically in figure 2B.	2.17	ND	40	1. Anaphylactic shock caused by chlorhexidine during surgery at day 40 2. Anaphylactic shock caused by chlorhexidine in skin swab at day 277
	2.17	81.4	66	
	5.53	95.4	70	
	3.95	73.7	83	
	1.66	ND	277	
	2.33	84.5	326	
	0.31	283	437	

	0.9	48.5	529	
	0.96	74.0	643	
	1.04	76.0	676	
	0.73	61.3	852	
19 Data presented graphically in figure 2C.	0.19	62.6	-92**	1. Asymptomatic re-exposure in urethral gel during surgery at day 299
	1.67	45.4	31	
	0.65	59.9	250	
	0.99	83.1	340	
	0.29	47.1	493	
20	0.74	46.0	190	No known re-exposure
	0.65	47.1	193	
	0.58	34.4	196	
	0.34	65.0	473	
	0.22	81.1	608	
	0.15	62.0	694	
	0.23	121	951	
21	5.29	ND	0	No known re-exposure
	8.7	506	138	
	0.16	546	173	
22	66.7	516	0	No known re-exposure
	163	656	16	
	98.6	1008	26	
	126	756	109	
	42.7	558	195	
	25.7	328	355	
23	14.5	158	0	1. General discomfort and redness of skin caused by skin swab around day 240
	16.8	161	5	
	12.1	169	9	

*Not enough serum to re-analyse sample. ** A blood sample drawn 92 days before the allergic reaction was available in this patient.

Appendices

Appendix I: Brockow K.

Dilemmas of allergy diagnosis in perioperative anaphylaxis.

Allergy. 2014;69:1265-1266.

Appendix II: Opstrup MS, Johansen JD, Garvey LH.

Chlorhexidine allergy: sources of exposure in the health-care setting.

Br J Anaesth. 2015;114:704-705.

Appendix III: Questionnaire used in study II.

EDITORIAL

Dilemmas of allergy diagnosis in perioperative anaphylaxis

DOI:10.1111/all.12485

Drugs are the most common elicitors of fatal anaphylaxis in adults (1). The majority of these events as well as a significant proportion of less severe anaphylactic reactions is caused by drugs and substances associated with general anaesthesia (1, 2). Neuromuscular blocking agents (NMBA), latex, antibiotics, induction agents and opiates are the most common substances incriminated in perioperative anaphylaxis (2–5). Because of highly efficient avoidance strategies, the prevalence of anaphylaxis to latex declined during the last years and is now considerably less than would be suggested by the presence of specific IgE (sIgE) or positive skin tests (6). Instead, increasingly other elicitors of anaphylaxis have been reported in the perioperative setting (2, 7, 8).

Previous reactors carry a highly increased risk for renewed reactions and thus require an allergy workup to find the best strategy in order to avoid future episodes associated with anaesthesia (6, 9). However, allergy diagnosis in this area still faces several obstacles:

First, it remains difficult to determine the exact mechanism of these reactions, which would be important for the development of reliable test protocols. Skin tests, the most important test method for drug anaphylaxis, may at higher concentrations be positive even in tolerant controls, particularly when testing NMBAs, which has been interpreted as a sign for a nonallergic mechanism [e.g. direct histamine release (HR)] (9, 10). On the other hand, large skin test reactions are typical for previous reactors and sIgE to quaternary ammonium substances, such as the NMBA suxamethonium, has been demonstrated (9). Thus, it is believed that about 60–70% of immediate hypersensitivity reactions to anaesthetics are, in fact, mediated by IgE (9). In some European countries, the prevalence of NMBA anaphylaxis was also associated with the availability and use of pholcodine, a potentially sensitizing antitussive agent with similar structure to morphine and NMBAs, which was able to induce sIgE antibodies cross-reacting with suxamethonium (11). Unfortunately, however, the sensitivity of the presence of sIgE to drugs, such as suxamethonium and pholcodine for the prediction of anaphylaxis, appears to be rather low and its specificity is not well determined.

Second, reports on culprit drugs and substances responsible for perioperative anaphylaxis as determined by skin test positivity differ between different regions in Europe, which may lead to confusion (2, 3, 11). This may be the result of differences in anaesthetic procedures and used substances, genetic backgrounds as well as of skin test methods. Whereas skin tests are performed and interpreted in Europe predominantly according to the standards of the European Network on Drug Allergy (ENDA), the Anesthetic/Allergologic Network in France has interpreted their skin test results and

determined nonreactive drug concentrations by their own standard, probably leading to different results (9, 12).

Third and most important, uncertainty prevails as the gold standard of allergy diagnosis, the provocation test, is not performed for the majority of perioperative anaphylactic reactions (13). It is normally not justified to induce general anaesthesia including intubation for test purposes and/or the risk for the patient having experienced a severe reaction is often judged to be too high for a drug provocation test. When tolerability of subsequent anaesthesia is used as read-out, allergy diagnosis appears to be highly efficient and reaction rates are very low (9). Nevertheless, the influence of confounders, such as choice of anaesthesia using different less-reactive compounds or premedication, cannot be estimated. Without provocation test, the causal relationship as well as sensitivity and specificity for skin tests, sIgEs and cellular tests cannot be reliably determined.

Fourth, hidden and less well-known substances may cause perioperative anaphylaxis, and education of allergists about these allergic compounds is warranted. Chlorhexidine, blue dyes, macrogol, carboxymethylcellulose and ethylene dioxide have increasingly been reported as elicitors of anaphylaxis, with chlorhexidine being a most relevant in this setting (7–9).

Perioperative anaphylaxis to chlorhexidine is often severe and easily overlooked (3). Chlorhexidine is a widely used disinfectant in the hospital as well as in the private health care. In the perioperative setting, the risk for severe reactions appears to be highly increased (14). The mechanism appears to be IgE mediated, and determination of sIgE, skin tests [skin prick test (SPT) and intradermal test (IDT)] as well as cellular tests, such as the basophil HR test, has been used to confirm sensitization to this compound in patients with suspected chlorhexidine allergy (14).

In the study by Opstrup et al (7) in this issue of Allergy, of 343 patients with perioperative anaphylaxis evaluated by the Danish Anaesthesia Allergy Center (DAAC) during the last years, 32 patients had at least one positive allergy test (of those mentioned above) for chlorhexidine. Without a validated provocation test, confirmation of the diagnosis 'chlorhexidine allergy' is difficult and based on an evaluative interpretation of the history together with available test results. To follow a scientific approach, a model of at least two different positive diagnostic tests in combination with a compatible history has been chosen. This definition has previously also been applied for rocuronium allergy.

All tests were performed within a 6 months timeframe after the reaction, as longer intervals may lead to declined diagnostic test reactivity. This model works best, if the tests would be equally reliable. In total, 22 patients fulfilled the criteria of ≥ 2 positive diagnostic tests. The highest

concordance between the tests was between sIgE and the SPT, which were positive in 22 of 22 and 21 of 22 patients. The authors recommend a combination of both tests as the minimum when diagnosing chlorhexidine allergy. HR and IDT showed a lower estimated sensitivity, but excellent specificity of 99–100%. In the ten patients with only one positive diagnostic test, results were reported to be just above the cut-off value rendering the causal association questionable, but not impossible.

The present study demonstrates how difficult it is to confirm allergy in the perioperative setting without a provocation test as gold standard (13). Sensitivity and specificity can only be estimated against an available ('silver') standard, for example two concordant positive test results. Other centres should confirm the results and conclusions drawn in this study using the same methodology, best in a multicentre study.

It also shows that there are situations in allergology, where a black-and-white picture cannot be drawn by available tests. Here, we have to revert to our virtues: a critical interpretation of available results in combination with an accurate record of events including information on drug administration. Critical interpretation may become more important than primary test

results. Expertise on clinical symptoms, differential diagnoses and test characteristics are required for this task. In the complex field of perioperative anaphylaxis without a gold standard of diagnosis available, investigation in major knowledgeable allergy expert centres and close cooperation between allergists (evaluation and critical interpretation of test results) and anaesthetists (record of events, drugs given and differential diagnoses), for example, as practiced in the DAAC and centres in France is highly recommended. This should lead to a better recognition of causative drugs and should avoid serious consequences of diagnostic error.

Author contribution

Knut Brockow is the sole contributor.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Declaration of interest

None declared.

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Chlorhexidine allergy: sources of exposure in the health-care setting

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Editor—Chlorhexidine is being used increasingly in the health-care setting to avoid nosocomial infections.¹ Allergic reactions to chlorhexidine are often severe, leading to urticaria, anaphylactic shock, and even cardiac arrest.^{2–3} The serious reactions have been reported to be preceded by milder reactions; therefore, allergy should be suspected when facing symptoms such as localized swelling or systemic rashes after exposure to chlorhexidine.^{1–3–4} Although considered a rare allergen, chlorhexidine is increasingly recognized as a cause of perioperative allergy in many countries, such as England and Denmark.^{3–5} Indeed, in Denmark it was recently reported that chlorhexidine caused 9.6% of all perioperative allergic reactions.⁵ As a result of the low frequency of the allergy, health-care professionals rarely have experience in preventing patients from exposure to chlorhexidine and are not aware of which products contain chlorhexidine. As a result, chlorhexidine-allergic patients can have serious reactions on accidental re-exposure after the diagnosis has been established.^{6–7} Therefore, we decided to conduct a study investigating which products contain chlorhexidine in hospitals in Copenhagen, Denmark.

In April 2013, we contacted the Hospital Pharmacy in the Capital Region of Denmark, which is the pharmaceutical supplier for all hospitals in the Copenhagen area. We used Anatomical Therapeutic Codes (ATC) to search for products containing chlorhexidine in the pharmacy's product catalogue. The ATC codes are used for classification of drugs and are controlled by

the World Health Organization. We searched for the following nine ATC codes, each of which represents a single indication or use of chlorhexidine: A01AB03, B05CA02, D08AC02, D09AA12, R02AA05, S01AX09, S02AA09, S03AA04, and D08AC52. This provided us with a list of all 42 chlorhexidine-containing products supplied to the hospitals by the pharmacy. Table 1 gives an overview of the product types, application sites, and declared concentrations used in the products.

As seen in Table 1, chlorhexidine was found in several products used perioperatively, such as skin disinfectants and urethral gels. The products mostly reported to cause allergic reactions are the urethral gels containing chlorhexidine, such as Instillagel® (Farco-Pharma GmbH, Cologne, Germany).^{3–8} However, there are other potential sources of exposure to chlorhexidine perioperatively, such as central venous catheters and skin-cleansing wipes. Chlorhexidine in these products is easily overlooked, but it may cause severe allergic reactions.^{6–7} These products are classified as medical utensils and are not distributed via the Hospital Pharmacy, and thus, they were not identified during our search. The Corporate Procurement in the Capital Region of Denmark distributes all non-pharmacological products, such as medical utensils, to the hospitals. In their product catalogue, there are currently more than 100 000 products, but a list of ingredients used in these products is not available. The only way of knowing whether a product contains chlorhexidine is to check the material safety data sheet of each product, which is very time

Table 1 Products containing chlorhexidine and their declared concentrations. *The pharmacy also supplied 20 and 85%, requiring further dilution before use

Application site	Product type	Declared concentration (%)
Mouth	Dental gel (n=2)	1–2
	Mouthwash (n=4)	0.1–0.2
	Thrush treatment (n=1)	0.1
	Tablet for pharyngitis (n=1)	5 mg per tablet
Urinary tract	Bladder irrigation (n=1)	0.02
	Urethral gel in combination with lidocaine (n=3)	0.05
Vagina	Cream (n=1)	1
Skin	Skin disinfectant (n=19)	0.05–4*
	Ointment, gel, or cream (n=3)	0.05–1
	Powder (n=1)	1
	Scrub (n=4)	4
	Dressing (n=1)	0.5
Eyes	Eye drops (n=1)	0.02

consuming. As described in a previous study, the declaration of chlorhexidine on central venous catheters can be difficult to find on the package; therefore, these products require special attention before inserting in a chlorhexidine-allergic patient.⁶ To complicate matters further, chlorhexidine is also used outside hospitals in pharmacies, by dentists, and as a preservative in cosmetic products. In Denmark in 2013, it was shown that 3.6% of more than 2000 cosmetic products contained chlorhexidine.⁹ Overall, this illustrates how difficult, if not impossible, it is to obtain a complete overview of chlorhexidine use in and outside the health-care setting.

After a high number of reactions to chlorhexidine reported in Japan, the Japanese Ministry of Welfare recommended in 1984 that the use of chlorhexidine should be prohibited on mucous membranes because of the risk of anaphylactic shock.¹⁰ As illustrated by Table 1, however, this recommendation is clearly not followed in other countries. With the increasing focus on reduction of hospital-acquired infections by use of disinfectants such as chlorhexidine, perhaps now is the time to re-evaluate the optimal concentrations of chlorhexidine needed to prevent infections.

In conclusion, we have found that chlorhexidine is used in many products in various concentrations in the health-care setting in Denmark. In line with the extensive use, chlorhexidine is increasingly recognized as a cause of perioperative allergic reactions.^{3–5} We hope that this small study can help health-care professionals to identify chlorhexidine-containing products. In the perioperative setting, exposure to chlorhexidine may be massive and from several different products simultaneously. We therefore recommend that anaesthetic personnel develop a strategy to identify chlorhexidine-containing products in their local hospital, thereby reducing the risk of accidental re-exposure and additional reactions among chlorhexidine-allergic patients.

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Declaration of interest

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Appendix

Table A1. Questionnaire about chlorhexidine contact allergy

-
- 1a. Do you know what product caused your allergy to chlorhexidine?
Yes; No
- 1b. If yes in 1a: What product caused the allergy?
Cream; hair product; make-up or make-up remover; wet wipe or other skin disinfectant; mouth wash; wound bandage; product used at the hospital or by a doctor; product used by a dentist; other: _____
- 2a. Have you been exposed to chlorhexidine since the allergy was diagnosed?
Yes; No; Don't know
- 2b. If yes in 2a: What product was it?
Cream; hair product; make-up or make-up remover; wet wipe or other skin disinfectant; mouth wash; wound bandage; product used at the hospital or by a doctor; product used by a dentist; other: _____
- 2c. If yes in 2a: What symptoms did you experience?
Rash; itching skin; urticaria; breathing difficulties; feeling unwell; fainting; other: _____
- 2d. If yes in 2a: When did you experience the symptoms?
0–60 min; 1–24 hr; more than 24 hr
- 3a. Have you been exposed to chlorhexidine in your current job or in a previous job?
Yes; No; Don't know
- 3b. If yes in question 3a: What was your job?

- 3c. If yes in question 3a: What product contained chlorhexidine?

- 3d. If yes in question 3a: Did you have to quit your job?
Yes; No
- 4a. Do you know that chlorhexidine can be used in some cosmetic products?
Yes; No
- 4b. Do you check whether there is chlorhexidine in a cosmetic product before you use it?
Yes; No
- 5a. Do you know that chlorhexidine is used at hospitals and by dentists?
Yes; No
- 5b. Do you mention your allergy when you are at a hospital or at your dentist?
Yes; No
- 6a. Does the allergy cause limitations in your everyday life?
Yes; No
- 6b. If yes in 6a: In what way does the allergy limit you?

-

Translated from Danish.



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Allergy to chlorhexidine

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Chlorhexidine in cosmetic products - a market survey

The PhD student's contribution to the article: (please use the scale (A,B,C) below as benchmark*)	(A,B,C)
1. Formulation/identification of the scientific problem that from theoretical questions need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable by experiments	C
2. Planning of the experiments and methodology design, including selection of methods and method development	B
3. Involvement in the experimental work	B
4. Presentation, interpretation and discussion in a journal article format of obtained data	C

*Benchmark scale of the PhD student's contribution to the article		
A. refers to:	Has contributed to the co-operation	0-33 %
B. refers to:	Has contributed considerably to the co-operation	34-66 %
C. refers to:	Has predominantly executed the work independently	67-100 %

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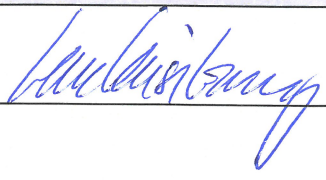
Title of PhD thesis:
Allergy to chlorhexidine

This declaration concerns the following article:
Contact allergy to chlorhexidine in a tertiary dermatology clinic in Denmark

The PhD student's contribution to the article: (please use the scale (A,B,C) below as benchmark*)	(A,B,C)
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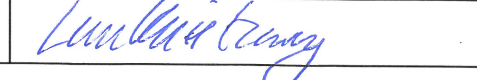
Title of PhD thesis:
Allergy to chlorhexidine


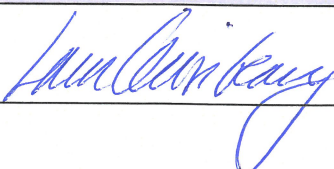
This declaration concerns the following article:
Standardized testing with chlorhexidine in perioperative allergy - a large single-centre evaluation

The PhD student's contribution to the article: (please use the scale (A,B,C) below as benchmark*)	(A,B,C)
1. Formulation/identification of the scientific problem that from theoretical questions need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable by experiments	C
2. Planning of the experiments and methodology design, including selection of methods and method development	C
3. Involvement in the experimental work	A
4. Presentation, interpretation and discussion in a journal article format of obtained data	C

*Benchmark scale of the PhD student's contribution to the article		
A. refers to:	Has contributed to the co-operation	0-33 %
B. refers to:	Has contributed considerably to the co-operation	34-66 %
C. refers to:	Has predominantly executed the work independently	67-100 %

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DECLARATION OF CO-AUTHORSHIP

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Title of PhD thesis:
Allergy to chlorhexidine

This declaration concerns the following article:
Dynamics of specific IgE in chlorhexidine allergic patients with and without accidental re-exposure

The PhD student's contribution to the article: (please use the scale (A,B,C) below as benchmark*)	(A,B,C)
1. Formulation/identification of the scientific problem that from theoretical questions need to be clarified. This includes a condensation of the problem to specific scientific questions that is judged to be answerable by experiments	C
2. Planning of the experiments and methodology design, including selection of methods and method development	C
3. Involvement in the experimental work	B
4. Presentation, interpretation and discussion in a journal article format of obtained data	C

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