FACULTY OF HEALTH SCIENCES
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Ph.D. Thesis

Allergic Reactions during Anaesthesia and Surgery

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Dedicated to my father

Ole Heise

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IV. Garvey LH, Belhage B, Krøigaard M, Husum B, Malling HJ, Mosbech H. Treatment with epinephrine (adrenaline) in suspected anaphylaxis during anesthesia in Denmark. *Submitted*

Contents

Preface

The work presented in this thesis was carried out at the Danish Anaesthesia Allergy Centre, Allergy Clinic 4222, Rigshospitalet/Department of Dermato-Allergology KAA-816, Copenhagen University Hospital, Gentofte Hospital and Department of Anaesthesia 4231, Centre of Head and Orthopaedics, Copenhagen University Hospital, Rigshospitalet, Denmark in the period January 2007- March 2010.

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Abbreviations

DAAC	Danish Anaesthesia Allergy Centre
EAACI	European Academy of Allergy and Clinical Immunology
ENDA	European Network on Drug Allergy
HCW	Health care workers
HR-test	Histamine release test
IDT	Intradermal test
IM	Intramuscular
IV	Intravenous
NHS	National Health Service (United Kingdom)
NMBA	Neuromuscular Blocking Agent
PO	Peroral
SC	Subcutaneous
SPT	Skin prick test
STN	Skin test negative
STP	Skin test positive
T_{basal}	Basal level serum tryptase
T_{react}	Serum tryptase taken at the time of suspected allergic reaction

Summary

Allergic reactions during anaesthesia are rare, but dramatic occurrences which are remembered by both patients and attending personnel. Subsequent investigation to determine the mechanism and cause of the reaction is important to reduce the risk of potentially lifethreatening recurrences. The Danish Anaesthesia Allergy Centre (DAAC) was established in 1998 to investigate such patients and this thesis is based on results from the first 10 years of the Centre's existence. The thesis focuses on major aspects of allergic reactions during anaesthesia, with special emphasis on in-vitro diagnosis and treatment.

More specifically the aim was to answer the following research questions:

Is chlorhexidine allergy based on an IgE-mediated mechanism? (Study I); does surgery and anaesthesia affect serum values of mast cell tryptase? (Study II); can using the intra-individual difference in serum tryptase improve sensitivity of serum tryptase as a diagnostic marker for anaphylaxis during anaesthesia? (Study II and III); do basal levels of serum tryptase vary with age? (Study II and III); and how often is adrenaline used in the treatment of patients with suspected anaphylaxis during anaesthesia – and is the timing of treatment important? (Study IV).

The mechanism behind chlorhexidine allergy was explored by retrospective analysis of sera from 22 patients with clinical reactions suggestive of anaphylaxis to chlorhexidine. Patients were divided into a chlorhexidine skin test positive (STP) group (n=12) and a skin test negative (STN) group (n=10). Stored serum was analysed using a recently developed specific IgE analysis and Histamine Release test. In the STP group 11 of 12 had elevated specific IgE and 7 of 11 had positive Histamine Release test. None of the patients in the STN group had elevated specific IgE or positive Histamine Release test (study I).

The effect of surgery and anesthesia on serum tryptase was studied in 120 patients undergoing elective orthopaedic surgery in general anaesthesia. Blood samples for tryptase analysis were drawn shortly before anaesthesia and after anaesthesia/surgery. Mean serum tryptase before surgery was 5.0µg/L with a mean decrease of 0.5µg/L ($p<0.0001$, 95% CI 0.3-0.8) postoperatively. All patients received intravenous fluid perioperatively; median value 750 ml (range 200-2000ml).

There was no significant effect of gender or ASA group on mean serum tryptase. The intra-individual difference in serum tryptase was observed to be small, with 95% of patients expected to have a change in serum tryptase in the interval between a decrease of 3.33 µg/L and an increase of 2.23 µg/L (Study II). It was hypothesised, that this intra-individual difference could be utilised diagnostically to improve the sensitivity of serum tryptase as a diagnostic marker for anaphylaxis during anaesthesia. In study III we thus looked at sensitivity and specificity of serum tryptase as a marker for anaphylaxis in patients investigated at the DAAC. A total of 180 patients were included. As different criteria for positivity are applied in the literature we analysed upper limits of 11.4 µg/L and 15 µg/L and 25 µg/L and compared with using the intra-individual difference found in study II.

When looking at all reaction grades, including grade 1 mild reactions, sensitivity was relatively low using all criteria for positivity. Sensitivity improved markedly by only including reaction grade 3 (symptoms of anaphylactic shock) and grade 4 (cardiac arrest). Sensitivity when using the intra-individual difference was only marginally higher than the sensitivity using the currently recommended upper limit of 11.4 μg/L.

The possible variation in basal levels of serum tryptase with age was studied both in the 120 elective orthopaedic surgery patients included in study II, and in 318 patients with suspected allergic reactions during anesthesia. Linear regression showed no significant correlation between age and basal levels serum tryptase in elective orthopaedic surgery patients in the absence of signs and symptoms of anaphylaxis ($r=0.05$, $p=0.6$) (study II), but a significant increase with increasing age was observed in patients with suspected allergic reactions during anaesthesia ($r=0.23$; $p<0.0001$) (study III).

The use of adrenaline in the treatment of anaphylaxis during anaesthesia was investigated in a descriptive study based on information from referral papers and anaesthetic charts from 270 patients referred to the DAAC in the period 1999-2008.

Reactions had been graded by one of two anaesthesiologists. Treatment with adrenaline would be expected in grade 3 (anaphylactic shock with circulatory compromise) and grade 4 (cardiac arrest) reactions. A total of 122 (45.2%) of referred patients had grade 3 or 4 reactions and of those 101 (82.8%) received adrenaline. Route of administration was iv in 95 (94%) patients. Median time from onset of reported hypotension to treatment with adrenaline was 10 minutes (range 1-70 minutes). Defining adrenaline treatment ≤ 10 minutes as early, and > 10 minutes as late, and using the need for subsequent infusion of adrenaline as a parameter for a protracted reaction, we found that infusion was needed in 12 of 60 patients (20%) treated early vs. 12 of 35 patients (34.3%) treated late (OR 2.087; 95% CI 0.814-5.353).

From the above studies we concluded that: (I) Allergy to chlorhexidine is IgE-mediated; (II) a small decrease in serum tryptase is seen in connection with surgery/anaesthesia most likely due to a dilutional effect of intravenous fluid; (III) serum tryptase values increase with age in patients with suspected allergic reactions during anaesthesia, but not in patients scheduled for elective orthopaedic surgery; (II,III) sensitivity of using serum tryptase as a marker for anaphylaxis, in patients investigated at the DAAC, is only marginally improved by using the intra-invididual variation as opposed to the currently recommended population-based upper limit of normal; (IV) delays in the use of adrenaline in treatment of anaphylaxis during anaesthesia seems to increase the risk for prolonged treatment in the form of intravenous adrenaline infusion.

Resumé

Allergiske reaktioner i forbindelse med anæstesi er sjældne, men dramatiske hændelser, som bliver husket af både patienter og personale. Efterfølgende udredning af patienten med henblik på at påvise mekanisme og årsag til reaktionen er vigtig, for at forebygge potentielt livstruende reaktioner ved fremtidig anæstesi. Dansk Anæstesi Allergi Center (DAAC) blev etableret i 1998 og har som hovedformål at undersøge denne type patienter. Denne phd afhandling er baseret på 10 års erfaringer fra DAAC og omhandler en række aspekter af emnet allergiske reaktioner i forbindelse med anæstesi med specielt fokus på in-vitro diagnostik og behandling. Mere specifikt vil følgende spørgsmål blive søgt besvaret: Er klorhexidin allergi IgE-medieret? (studie I); påvirkes mast celle tryptase af kirurgi og anæstesi? (studie II); kan sensitiviteten af serum tryptase som markør for anafylaksi i forbindelse med anæstesi øges ved at benytte den intra-individuelle variation i testen? (studie II og III); er der sammenhæng mellem basal niveau af serum tryptase og alder? (studie II og III); bliver adrenalin brugt i behandlingen af anafylaksi i forbindelse med anæstesi og er tidsrelationen mellem reaktion og påbegyndt behandling vigtig? (studie IV)

Mekanismen bag klorhexidin allergi blev undersøgt ved retrospektiv analyse af serum på 22 patienter, med kliniske reaktioner som var forenelige med allergi overfor klorhexidin. Patienterne blev opdelt i en klorhexidin hudtest positiv gruppe STP (n=12) og en hudtest negativ gruppe STN (n=10). Serum blev analyseret med en nyligt udviklet specifik IgE analyse, samt Histamine Release test. I STP gruppen fik 11 af 12 påvist specifik IgE og 7 af 11 havde positiv Histamine Release test. Ingen af patienterne i STN gruppen fik påvist specifik IgE og alle havde negativ Histamine Release test (studie I).

Mulig effekt af kirurgi og anæstesi på serum tryptase blev undersøgt hos 120 patienter, som fik foretaget elektivt ortopædkirurgisk indgreb i generel anæstesi. Blodprøver taget før og efter operation og anæstesi blev analyseret for serum tryptase. Mean serum tryptase før kirurgi var 5.0μg/L med et fald på 0.5μg/L ($p<0.0001$, 95% CI 0.3-0.8) efter indgrebet. Alle patienter fik intravenøs væske peroperativt, median 750 ml (range 200-2000ml).

Der var ingen effekt af køn eller ASA gruppe på serum tryptase. Den intra-individuelle forskel i serum tryptase var lille; 95% af patienterne havde en ændring i serum tryptase mellem prøver taget før og efter operation i intervallet fra et fald på 3.33 μg/L til en stigning på 2.23 μg/L (studie II). I studie III blev det herefter undersøgt om denne intra-individuelle forskel kunne bruges diagnostisk og om det ville øge sensitiviteten af serum tryptase, som markør for allergiske reaktioner i forbindelse med anæstesi, hos patienter udredt i DAAC. I alt 180 patienter blev inkluderet. Da der i litteraturen bruges forskellige værdier som øvre grænse for normalområdet, samt forskellige kriterier for hvornår testen er positiv, inkluderede vi øvre værdier på 11.4 μg/L, 15 μg/L og 25 μg/L og sammenlignede med den intra-individuelle forskel fundet i studie II.

Når alle reaktionsgrader, både milde og alvorlige reaktioner, blev inkluderet i analysen var sensitiviteten relativt lav for alle kriterier for positivitet. Sensitiviteten blev øget markant hvis analysen kun indeholdt reaktionsklasse 3 (anafylaktisk shock) og 4 (hjertestop). Sensitiviteten ved brug af den intra-individuelle variation var kun marginalt bedre end sensitiviteten ved den aktuelt rekommenderede øvre værdi på 11.4 µg/L.

En mulig variation i basal niveau af serum tryptase med alderen blev undersøgt hos både 120 elektive ortopædkirurgiske patienter, der ikke have haft allergiske reaktioner i forbindelse med anæstesi (fra studie II), samt hos 318 patienter med mistænkte allergiske reaktioner i forbindelse med anæstesi (studie III). Der var ingen statistisk signifikant sammenhæng mellem alder og basal niveau serum tryptase hos elektive ortopædkirurgiske patienter ($r=0.05$, $p=0.6$) (studie II), men hos patienter med mistænkte allergiske reaktioner i forbindelse med anæstesi sås en statistisk signifikant stigning med stigende alder ($r= 0.23$, $p<0.0001$) (studie III).

Brug af adrenalin i behandlingen af allergiske reaktioner i forbindelse med anæstesi blev undersøgt i et deskriptivt studie baseret på information fra udfyldte henvisninger, anæstesiskemaer og andet relevant journalmateriale på 270 patienter henvist til DAAC i perioden 1999-2008.

Reaktioner blev scoret i reaktionsklasser af én af to anæstesiologer i DAAC. Behandling med adrenalin ville være forventelig ved reaktioner i reaktionsklasse 3 (anafylaktisk shock med kredsløbspåvirkning) og 4 (hjertestop). I alt 122 (45.2%) af de henviste patienter havde klasse 3 eller 4 reaktioner og 101 af disse (82.8%) blev behandlet med adrenalin. Administrationsvejen var intravenøs i 95 (94%) tilfælde. Median tid fra start af rapporteret hypotension til behandling med adrenalin var 10 minutter (range 1-70 minutter). Adrenalin behandling $\leq$ 10 minutter efter hypotension blev defineret som "tidlig" og behandling > 10 minutter efter blev defineret som "sen". Behov for efterfølgende infusion af adrenalin blev defineret som tegn på protraheret reaktion og der var behov for infusion hos 12 af 60 patienter (20%) ved tidlig behandling og hos 12 af 35 patienter (34.3%) ved sen behandling (OR 2.087; 95% CI 0.814-5.353).

Fra ovennævnte undersøgelser har vi konkluderet at (I) klorhexidin allergi er IgE medieret; (II) der ses et lille fald i serum tryptase i forbindelse med anæstesi og operation pga. en fortyndingseffekt af intravenøs væskeindgift; (II og III) basal niveau af serum tryptase stiger med stigende alder hos patienter med allergiske reaktioner i forbindelse med anæstesi, men ikke hos elektive ortopædkirurgiske patienter uden allergisk reaktion i forbindelse med anæstesi; (II and III) brug af den intra-individuelle variation i serum tryptase i diagnostikken af anafylaksi i forbindelse med anæstesi, hos patienter henvist til DAAC, øger kun sensitiviteten marginalt i forhold til den populationsbaserede øvre værdi; (IV) forsinkelse af brug af adrenalin i behandlingen af anafylaktisk shock i forbindelse med anæstesi ser ud til at øge risikoen for behov for efterfølgende intravenøs infusion af adrenalin.

Introduction

Allergic reactions occur rarely and unexpectedly during anaesthesia and surgery, but remain one of the serious and potentially lifethreatening adverse events in this setting. A good outcome depends on prompt diagnosis and correct treatment.[1] However, as symptoms and signs are masked by the effect of anaesthetic drugs and patient comorbidity, the diagnosis is often difficult to make, causing potential delays in treatment. In addition, the patient has been exposed to a large number of substances and it is not possible to identify the cause in the clinical situation.[2]

Patients who have experienced an allergic reaction during anaesthesia are at increased risk of reactions during subsequent anaesthesia[3] and investigation to determine the cause and mechanism of the reaction is necessary to reduce the risk of a potentially lifethreatening re-exposure to the causative agent.

In countries such as France, Australia and UK systematic investigations of allergic reactions during anaesthesia were initiated in the late 1970'ies[4-7] and in 1998 the Danish Anesthesia Allergy Centre (DAAC) was established at Gentofte Hospital, Copenhagen, to centralise and standardise investigations of suspected allergic reactions during anaesthesia in Denmark. During the period 1998-2003 the centre was based on a close cooperation between the Department of Anaesthesiology and the Department of Dermatology. In this way the ability of the anaesthesiologist to decipher the anaesthetic chart, understand the clinical situation and give advice for future anaesthetics was combined with the dermatologist's ability to advice on, perform and interpret allergological tests. Patients were investigated following a standardised investigation protocol based on the available literature and comprising mainly in vitro tests (serum tryptase and specific IgE antibodies) and skin tests (skin prick tests SPT, intradermal tests IDT and patch tests).[8]

In 2004 DAAC moved to Rigshospitalet, Copenhagen and the organisational structure changed to be based on close cooperation between anaesthesiologists, now based in the Department of Anaesthesia, Centre for Head and Orthopaedics and Allergologists based in the Allergy Clinic. Several improvements were made to the investigation protocol, the most important being the addition of systematic drug provocation and HR-test.

The subject of allergic reactions during anaesthesia is vast, not least due to the combination of two specialities with differing areas of interest.

This thesis therefore concerns three areas that have proved to deserve special attention:

1) Chlorhexidine as an allergen 2) Serum tryptase and 3) Treatment with adrenaline in suspected anaphylaxis during anaesthesia in Denmark

1) Chlorhexidine as an allergen

Chlorhexidine is a disinfectant, used routinely during anaesthesia and surgery in Denmark. The first patient investigated in DAAC in 1999 was found to have an allergy towards chlorhexidine and

subsequently skin testing with chlorhexidine was included in the standardised investigation protocol. Although still a rare allergy it has proven to be the most commen cause of allergic reactions during anaesthesia in Denmark.[1] This has led to an increased interest in chlorhexidine as an allergen, with regard to clinical picture, diagnostic tests and identification of high-risk groups of both patients and health care workers.[9-11] A study to confirm an IgE-mediated mechanism behind allergy to chlorhexidine is included in this thesis.

2) Serum tryptase

Serum tryptase is released from mast cells during severe allergic reactions and was incorporated in the standardised investigation protocol in DAAC in 1999. An increase in serum tryptase during a suspected allergic reaction during anaesthesia is helpful in confirming an allergic mechanism.[1] A control sample to establish basal levels of serum tryptase has also been part of standard investigations in DAAC since 1999. It has become evident, that potential effect of anaesthesia and surgery on serum tryptase has only been studied sporadically in small studies.[12-13]

In addition no studies could be found on the characteristics of basal levels of serum tryptase in a population of patients with suspected allergic reactions during anaesthesia. More specifically, in an early study we had hypothesised that serum tryptase increased with increasing age.[8]

Lastly, using the intraindividual variation in serum tryptase in contrast to using an absolute cut-off value has been suggested to improve the sensitivity of serum tryptase as a diagnostic marker for anaphylaxis.[14]

3) Treatment with adrenaline in suspected anaphylaxis during anaesthesia in Denmark

Due to the rare and unexpected occurrence of anaphylaxis during anaesthesia the literature on anaesthesiologist's treatment patterns is scarce. Questionnaire studies and studies of the treatment of anaphylaxis in other settings have suggested that treatment may be suboptimal and that education on the diagnosis and treatment of anaphylaxis is necessary to improve awareness of anaphylaxis.[15-17] Only one relevant study could be found, suggesting that diagnosis and treatment strategies for anaphylaxis by anaesthesiologists were suboptimal.[18]

In recent years increasing interest in the treatment of anaphylaxis has led to the publication of guidelines emphasising the role of adrenaline both during anaesthesia[1,19-21] and outside the operating room.[22-24]

In DAAC information has been gathered on the management of suspected allergic reactions during anaesthesia in patients referred for investigation in the period 1999-2009. Detailed information on treatment regarding drugs used, timing, dosing and route of administration is therefore available for study.

Aims

This thesis has the following aims:

Study I

- To determine the mechanism behind allergy to chlorhexidine
- To describe the characteristics of patients with allergy to chlorhexidine

Study II

- To investigate the effect of surgery and anaesthesia on serum tryptase in patients undergoing orthopaedic surgery in general anaesthesia, in the absence of allergic reactions
- To examine the effect of age on serum tryptase in the same population

Study III

- To examine the effect of age on basal levels serum tryptase in patients investigated for suspected allergic reactions during anaesthesia
- To examine the effect of using the intra-individual difference in serum tryptase on the sensitivity and specificity of serum tryptase as a marker for anaphylaxis during anaesthesia

Study IV

- To describe how often adrenaline is used in the treatment of anaphylaxis during anaesthesia in Denmark
- To investigate if timing of treatment with adrenaline is important

Background

Allergy and anaphylaxis

The earliest documented observation of the phenomenon of anaphylaxis probably goes back to 1839 when the French physiologist Francois Magendie described that injection of ovalbumin into animals produced no reaction on first injection, but caused sudden death of the animal on a second injection after an interval of a few days.[25,26] The term ”allergy” was first suggested by the Austrian paediatrician Clemens Von Pirquet in 1906 to describe any form of altered biological reactivity. In 1912 the French physiologist Charles Richet coined the term ”anaphylaxis” to describe the absence of protection against foreign substances leading to a heightened sensitivity to such substances.[27] He received the Nobel prize in 1913 in recognition for his work on anaphylaxis. Since then many significant discoveries have been made in the field of Allergology; one of the most significant was the simultaneous discovery of the IgE class antibody by Japanese and Scandinavian research groups in 1966-67 laying the foundation for todays knowledge of the mechanism of IgE mediated allergy.[28,29]

Terminology

Many different terms such as anaphylactic, anaphylactoid, pseudoallergic, histaminoid etc have been used in the literature, reflecting differences in definition and underlying mechanisms.
In the anaesthesia-allergy literature from France, initially the term anaphylactoid was used about all reactions referred for investigation, and reactions with a positive test result were re-named anaphylactic reactions.[30] In DAAC Gentofte this terminology was initially adopted and was used in the first publications.[8] However, this terminology was later abandoned when attempts at standardising the definitions of hypersensitivity reactions were made.[31,32] According to the new definitions the overall term ”hypersensitivity reaction” should be used. Hypersensitivity reactions can be divided into allergic and non-allergic hypersensitivity reactions and the allergic hypersensitivity reactions can be further divided into IgE-mediated and non-IgE mediated reactions. Anaphylaxis is used as an overall term for severe, generalised and lifethreatening reactions, and divides into the same categories as mentioned above. According to this definition the overall term covering all reactions in patients referred to DAAC should be ”hypersensitivity reactions” as they cover a multitude of different mechanisms, both allergic and non-allergic. However, the main emphasis in this thesis is on reactions where a cause is found, and in the majority of these cases the mechanism is allergic (either IgE mediated or non-IgE mediated). The following terms will therefore be used throughout his thesis: ”Allergic reactions” as an overall term and ”anaphylaxis” in case of severe lifethreatening reactions.

Allergic reactions during anaesthesia

Incidence

Allergic reactions during anaesthesia are rare, but the true incidence is difficult to estimate due to factors such as underdiagnosis, underreporting and differences in definitions and investigation protocols. The estimated incidence of allergic reactions during anaesthesia based on referrals to anaesthesia-allergy centres range between 1:1250 anaesthetics and 1:13.000 anaesthetics.[33] A recent French prospective study, with systematic allergological follow-up of adverse events/reactions during 70.000 anaesthetics in a single hospital, over a two-year period, resulted in an incidence of hypersensitivity reactions of 1:3180 anaesthetics.[34]

In a study from Norway 19 reactions were referred from one hospital over a six-year period where approx 113000 anaesthetics were performed. This gives an incidence of a referred reaction per approx 1:6000 anaesthetics.[35] There are no published estimates of incidence in Denmark, but during 1999-2003, when DAAC was located in Gentofte Hospital, a total of 40 referrals were made from our own hospital and 17 of these (43%) tested positive. A total of 73707 anaesthetics were performed during that period, giving an incidence of referred reactions of approx 1:2000 anaesthetics and an incidence of reactions with a suspected IgE mediated allergy of 1:5000 anaesthetics.

Mechanisms

The clinical presentation and management of suspected allergic reactions during anaesthesia is the same regardless of the underlying mechanism. IgE mediated allergic reactions occur when the allergen causes crossbridging of the high affinity specific IgE receptors on the surface of mast cells leading to degranulation and release of vasoactive mediators. Non-IgE mediated allergic reactions and mast cell degranulation can also be triggered via IgG, C5a and C3a, neuropeptides and certain drugs.[36] Non-allergic reactions can be caused by direct pharmacological or “toxic” stimulation of mast cells or basophils.[31] Determining the mechanism is helpful for giving advice for future anaesthesia. In cases of proven IgE mediated allergy the identified allergen should always be avoided, but in cases of, usually less severe, non allergic histamine release, reactions may be prevented by slow injection and/or pretreatment with antihistamines.[37]

Signs and symptoms

Signs and symptoms of allergic reactions during anaesthesia comprise a combination of cardiovascular symptoms, respiratory symptoms, skin symptoms and gastrointestinal symptoms, but the perioperative setting hampers the diagnosis by introducing concurrent potential causes of both cardiovascular and respiratory symptoms. Gastrointestinal and skin symptoms are at risk of being overlooked altogether. Severity of reactions can depend on the mechanism behind the reaction, the allergen and the route of

administration of the allergen.[1,38] IgE mediated reactions to drugs administered/exposed intravenously are more commonly associated with severe hypotension and cardiovascular collapse/arrest than non-IgE mediated reactions.[1,38-41]

Investigation

Systematic investigation of suspected allergic reactions during anaesthesia were initiated in the 1970ies and early 1980ies in anaesthesia/allergy centres in France, Australia and the UK, and a standardised investigation procedure was agreed in Nancy in 1983[42] and followed up 10 years later by the same group.[43-45] This investigation procedure was based on detailed clinical history, blood tests (serum tryptase, specific IgE and Histamine release) and skin testing (skin prick test and intradermal test) and still provides the basis for the investigation protocols recommended today.[1,21,46]

Causes

The literature on allergic reactions during anaesthesia goes back to the 1960'es and initial reports were case reports on allergic reactions to substances used during anaesthesia at that time, such as thiopentone and suxamethonium.[47,48] More recently reviews on the subject have emphasised the fact, that not only all drugs administered, but also other substances used during anaesthesia such as latex, colloids, disinfectants, contrast materials, blue dyes etc. can cause allergic reactions.[1,38,49-51]

The causes of allergic reactions during anaesthesia in Denmark differ from results from countries like France, Norway and Australia, where neuromuscular blocking agents (NMBA) predominate.[33,35,52] In Denmark the leading cause has proved to be chlorhexidine and NMBA's cause only few reactions.[1,8] This difference could be partly explained by the "pholcodine hypothesis". This hypothesis states that exposure to pholcodine, a substance with structural similarities to NMBA's, have caused cross sensitisation of patients to NMBA's in countries such as France and Norway, where pholcodine containing antitussive drugs have been available. Pholcodine is not marketed in Denmark and this is likely to be part of the explanation for the low incidence of allergic reactions to NMBA's in Denmark.[53,54]

Danish Anaesthesia Allergy Centre

The Danish Anesthesia Allergy Centre was established in 1998 at Gentofte Hospital. Prior to this time investigation of patients with suspected allergic reactions had been sporadic, but as Denmark is a geographically small country with easy infrastructure and a small population (5.5 million) it has been possible to make DAAC the main Danish Centre investigating suspected allergic reactions during anaesthesia. The organisational structure of DAAC is based on close collaboration between anaesthesiologists and allergologists combining the expertise across the specialties. Also the centralisation of investigations of these rare reactions has maximised the potential to improve knowledge and carry out research in the field.

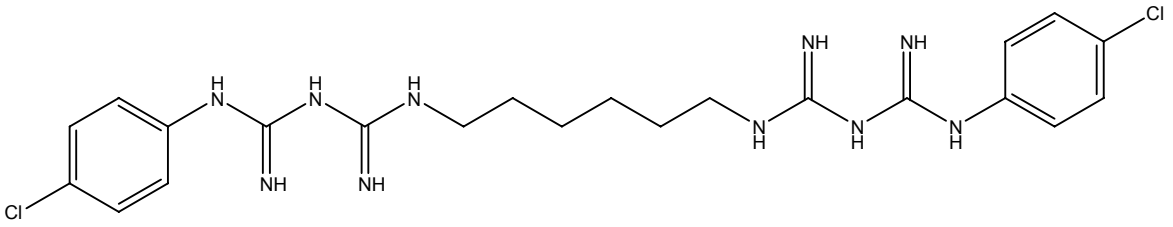
The main purpose of DAAC is to investigate patients with suspected allergic reactions during anaesthesia from all of Denmark, but other important purposes are

- To advice patients and anaesthestic personnel on all aspects of allergic reactions during anaesthesia
- To inform and teach anaesthetic personnel about diagnosis, treatment and referral procedures for patients with suspected allergic reactions during anaesthesia
- To initiate and participate in relevant research and guideline work both locally, nationally and internationally

Chlorhexidine as an allergen

Chlorhexidine digluconate is a cationic bis-biguanide disinfectant with broad-spectrum anti-microbial activity and low toxicity when used externally in mammals. Its clinical usefulness was first described in 1954.[55] The symmetrical molecular structure is shown in figure 1.

Figure 1. Chlorhexidine molecule.



Chlorhexidine digluconate is used all over the world and also has a wide variety of uses in the Danish health care system, including skin disinfection prior to surgery and invasive procedures ranging from simple blood tests to major operations. It is used by health care workers for disinfection of hands between patient contacts and prior to sterile procedures, and is commonly used in connection with and after dental

procedures. In addition, it is an ingredient in exploration gel used for vaginal and rectal examinations and in urethral gel used before inserting catheters. Outside the healthcare system, it is found in simple, over-the-counter products for disinfecting minor wounds and scratches in the home. It is also an ingredient in a large number of home care and personal hygiene products such as cleaning fluids, toothpastes, mouth rinses, plasters and dressings, ointments and suppositories. Chlorhexidine diacetate has been used in some countries as a commercial disinfectant in connection with food handling (USA Environmental Protection Agency, 1996), disinfection of udders before milking livestock, and veterinary disinfection. It has also been used as a preservative in products as diverse as lozenges, antacid preparations and contact lens cleaning fluids.
A large proportion of the Danish population is therefore exposed to chlorhexidine with varying degrees of exposure.

The first report of skin hypersensitivity to chlorhexidine was published in The Lancet in 1965[56] but the problem was concluded to be rare when taking into account the substance's widespread use.[57] In the early 1980ies, reports from Denmark described contact dermatitis caused by chlorhexidine in patients with skin disorders, and an increased incidence was seen in patients who had used chlorhexidine for disinfecting chronic leg ulcers.[58,59] Subsequently the first report of a severe anaphylactic reaction to chlorhexidine in an anaesthetised patient appeared from Japan.[60] In the period 1967-1984, nine reports of anaphylactic shock related to the use of chlorhexidine on mucous membranes were collected in Japan, which led to an official recommendation to avoid using the substance on mucous membranes. It was also recommended to use the lowest bactericidal concentration (0.05%) for contact with broken skin or mucous membranes.[61]

Sporadic case reports from all over the world have since followed. These concern severe anaphylaxis to chlorhexidine from different modes of exposure during surgery and anaesthesia, e.g. urological procedures and urinary catheter insertion[62], other surgical procedures[63] and central venous line insertion.[64]

All patients undergoing anaesthesia and surgery in Denmark are exposed to chlorhexidine and thus skin testing with chlorhexidine has been included as a standard investigation in the DAAC investigation protocol since 1999.

Tryptase

Tryptases are serine peptidases with a tetrameric structure, which share enzymatic, structural and phylogenetic features with the trypsin family. Tryptases are found in most mast cells in the human body – regardless of mast cell type and tissue location. Basal level of serum tryptase is made up primarily of pro-α tryptase and pro-β tryptase[65], which are continously spontaneously secreted from resting mast cells, and serum tryptase in a subject without signs or symptoms of anaphylaxis is likely to reflect mast cells numbers in the body.[66]

Mature β-tryptase is retained in secretory granules in the mast cell in its active form, complexed with proteoglycans, such as heparin or chondroitin sulphate[67], and is only released during mast cell activation and degranulation. Mast cell degranulation can be triggered by a variety of stimuli such as C5a and C3a, neuropeptides and certain drugs, but most commonly occurs when a specific antigen causes cross-linking of specific IgE molecules bound to the high affinity IgE receptor on the surface of mast cells.[36] An increase from basal level serum tryptase is therefore highly suggestive of IgE-mediated mast cell activation, when seen in connection with signs and symptoms of anaphylaxis.[68]

The only other leukocyte that contains tryptase is the basophil, which contains tryptase at levels of only 0.4% of that expressed in lung mast cells[69] or < 1 % of that expressed in tissue mast cells.[70]

Tryptase is therefore thought to be quite a specific marker for mast cell activation and has been used in clinical practice as a marker for anaphylaxis since the first immunoassay became available in 1987.[67]

Serum tryptase levels peak at 60-90 minutes after onset of anaphylaxis[71] and some studies indicate that elevated levels can be measured as early as 10 minutes after onset.[72]

The use of serum tryptase in anaphylaxis during anaesthesia was first described 1991.[73] It has since been recognised as a useful marker in suspected allergic reactions during anaesthesia, where the clinical diagnosis can be difficult to make; it has been recommended by several groups[35,74,75] and in international guidelines.[1,21]

When interpreting serum tryptase values in samples taken at the time of a suspected allergic reaction, ideally a basal level serum tryptase should be available for comparison.

Treatment with adrenaline in suspected anaphylaxis during anaesthesia

Due to anaphylaxis being a rare, potentially life threatening condition, which occurs unexpectedly, carrying out randomised controlled studies on the diagnosis and treatment of anaphylaxis is considered both impractical and unethical. New knowledge is therefore most likely to be gained from retrospective studies, case series or prospective cohort studies over a long time period. A recent systematic review of gaps in the management of anaphylaxis covering a large number of databases over a time period spanning 1966-2008 could only identify 59 relevant studies.[76] Useful information can be gained from post mortem studies where mortality is the outcome. One post mortem study from the UK identified main reasons for death from anaphylaxis to be 1) no use of adenaline or 2) late administration of adrenaline.[39]

There are no studies of mortality and morbidity from anaphylaxis during anaesthesia in Denmark, but a closed claims study analysing deaths related to anaesthesia in the period 1996-2004 in Denmark did not identify any cases of suspected perioperative anaphylaxis leading to a claim in the patient insurance association.[77] A study from the UK analysing claims against the NHS related to drug errors and anaphylaxis during in anaesthesia in England in the period 1995-2007 found five deaths and two cases of long term neurological damage attributed to anaphylaxis over a 12 year period. They estimated that six million anaesthetics were carried out every year giving a mortality of 1 death per 14.4 million anaesthetics.[78] However these figures only relate to reactions leading to litigation, and are likely underestimated, as mortality estimates are prone to error.

When anaphylaxis occurs outside the operating room the allergen is often singular and known eg. an insect sting or injection of a drug, and the patient is in a pre-hospital setting or in a general hospital ward. The diagnosis can be made swiftly and reliably in most cases and the recommended first line treatment of anaphylaxis in this setting is intramuscular adrenaline 0.3 mg. [22,23,79] The rationale behind this is, that it is a reasonably safe treatment in an unmonitored patient without iv access, and it is also the treatment given to patients with verified anaphylaxis to eg. insect venom or peanut, for self administration.

The setting of anaphylaxis during anaesthesia is quite different. Several potential allergens are administered simultanously, at a time when anaesthetic drugs have a potent effect on the circulation and manipulation of the airways can produce airway irritation. The diagnosis is thus difficult to make and the potential allergen cannot be identified immediately.[2] However, the patient is usually fully monitored, has an iv access and is being managed by anaesthetic personnel specially trained in emergency situations. This should give optimal conditions for prompt treatment once anaphylaxis is diagnosed. Treatment recommendations for anaphylaxis during anaesthesia are therefore aimed at anaesthetic personnel and first line treatment is intravenous adrenaline 0.01-0.05 mg.[1,21]

Research on anaphylaxis during anaesthesia is made difficult by its rare and unexpected occurrence and only few relevant studies can be found in the literature. One of these was a study of 21 anaesthetist teams undergoing simulation training at the Danish Institute for Medical Simulation. They were presented with

a clinical scenario of anaphylaxis, and none of the teams made the diagnosis within 10 minutes. After hints from the instructor 6/21 teams made the diagnosis, but no teams had a structured plan for treating anaphylaxis.[18] This could indicate a lack of awareness of anaphylaxis as a possible diagnosis in the anaesthetic community at that time. In the literature we found only one study examining the management of anaphylaxis during anaesthesia. The main conclusion from this study was, that there was a striking reluctance to administer adrenaline even when anaphylaxis is suspected.[80]

Methodological considerations

Classification of suspected allergic reactions during anaesthesia

All suspected allergic reactions in referrals made to DAAC are classified according to severity of the reaction. All classifications have been carried out by one of two anaesthesiologists in DAAC (either by Dr. Mogens Krøigaard or the author).

The classification used in DAAC is a modification of previously published classifications [81,82] and was first published in 2001.[8] It is based on a combination of symptom severity and need for treatment. Class 4 has since been added to distinguish the few reactions resulting in cardiac arrest. However, due to the small number of class 4 reactions, for the purpose of statistical analysis in the studies included in this thesis, class 3 and 4 have been added together.

Class 1	Mild reactions resolving spontaneously, usually only involving one organsystem, e.g. urticaria, angioedema, rash
Class 2	More severe reactions resolving within 10-20 min, with or without treatment, usually involving one or more organsystems, e.g. hypotension, bronchospasm and urticaria
Class 3	Very severe reactions requiring prolonged treatment, e.g. anaphylactic shock, usually, but not always, involving two or more organsystems
Class 4	Cardiac arrest

Investigation of suspected allergic reactions during anaesthesia in DAAC

Investigations of patients with suspected allergic reactions during anaesthesia in DAAC have followed standardised protocols based on the best available evidence from the literature. Three groups from France, Australia and the UK respectively, dominated the literature in the 1980'ies and 1990'ies. The investigation protocol used in DAAC when testing was started in 1999, was based on a combination of recommendations from all three centres[8]. The protocol was based on a step-by step approach using a combination of in-vitro and in-vivo tests (see appendix 1) Both mild and severe reactions were investigated and *all* drugs and substances (incl. premedication, substances used by the surgeon, low molecular weight heparins, colloids etc) the patient was exposed to prior to the reaction were investigated in one or more test modalities. If a test was positive in one testmodality it was deemed positive and no further testing with that drug was carried out. One anaesthesiologist received the referrals and ensured that full copy of anaesthetic charts; recovery charts and relevant medical notes were retrieved. The individual investigation protocols were planned on the basis of this information in cooperation with the dermatologist. One lab technician performed fresh dilutions for skin testing and one nurse carried out skin testing. If a skin test was thought to be positive it was repeated to ensure reproducability. Due to the

widespread use of natural rubber latex and chlorhexidine in the health care system, all patients were investigated for allergy to latex and chlorhexidine.

In 2004 DAAC moved to Rigshospitalet and cooperation with allergologists replaced the cooperation with dermatologists. A number of changes were made to the organisational structure and to the investigation protocol (see appendix 2). The investigation protocol was extended to include Histamine Release Testing and challenge testing with all drugs and substances possible and the latter has extended the investigation time considerably. Consequently restrictions on the drugs considered for testing were applied such that drugs administered intravenously > 1 hr before the reaction and drugs administered by other routes (oral, subcutaneous, spinal/epidural etc) > 2 hrs before the reaction are not considered for testing. The rationale behind this is that the aim is to diagnose immediate hypersensitivity reactions and these should feasibly occur within the above time limits. All patients are still investigated for allergy to latex and chorhexidine and in 2008 Specific IgE testing for ethylene oxide was introduced in all patients due to widespread exposure to this sterilising agent during anaesthesia and surgery. The step-by-step approach is still applied and drugs are tested in all available test modalities, but further testing may be carried out despite a positive result, as false positive tests are not uncommon on skin testing. All skin test are now carried out in duplicate. Eight nurses take turns to perform skin testing and dilutions are prepared every morning by two nurses. One allergologist and two anaesthesiologists plan the individual investigation protocols and five allergologists are involved in overseeing testing.

Diagnostic methods

Specific IgE

Identifying specific IgE antibodies in serum can prove sensitisation to a small number of drugs. Bloodsamples for specific IgE analysis are quick and easy to handle and without risk to the patient. Specific IgE can be especially useful in cases where skin testing and provocation cannot be carried out (dermographism, patients on immunosuppressants, children) or can be used as an adjunct to skin testing.

However, analyses are relatively expensive and sensitivity and specificity varies with the allergen tested. For the purpose of investigation of allergic reactions during anaesthesia specific IgE can be detected only for some of the substances used. In DAAC the ImmunoCAP® specific IgE immunoassay (Phadia, Uppsala, Sweden) is used, with access not only to the presently commercially available allergens (suxamethonium, morphine, penicillin G+V, amoxicilloyl, ampicilloyl, chlorhexidine, latex, ethylene oxide, pholcodine) but also to allergens only used for research purposes where the IgE mediated mechanism is not proven in all cases (thiopental, rocuronium, penicillin minor determinants, cefuroxime, ceftriaxone, mepivacain, methylprednisolone, paracetamol). Some groups advocate the use of IgE to the quarternary ammonium ion[83,84] or morphine[85] as a marker for IgE to NMBA's. However, these are rather unspecific tests with a high rate of false positives[52] and are primarly used in countries with a relatively high prevalence of NMBA reactions (France and Australia). As prevalence of NMBA reactions is low in Denmark these tests are not used at present.

A cut-off of < 0.35 kUA/l is used, as recommended by the manufacturer. As shown in **study I** levels of IgE to chlorhexidine can be elevated already at the time of the reaction and this has also been shown for NMBA's[86,87] and ethylene oxide.[88,89] Also shown in **study I** a decline is seen in IgE levels over time in the absence of exposure to the allergen. Levels may fall below 0.35 kUA/l and this has also been shown for penicillins[90], insect venom[91] and chlorhexidine in health care workers.[92]

All relevant specific IgE analyses are routinely requested depending on which drugs the individual patient has been exposed to. Blood tests are requested 2-3 months after the reaction and are taken locally and sent for analysis at the laboratory for Medical Allergology, Rigshospitalet. Sera from all patients have been routinely analysed for specific IgE to latex since 1999, chlorhexidine (since 2006, when assay was made available to us) and ethylene oxide (since November 2008 when we became aware of ethylene oxide as a possible allergen).

Serum tryptase

Serum tryptase has been included in the investigation protocol since 1999 using the ImmunoCAP immunoassay, measuring total tryptase ie. the sum of pro-β tryptase, pro-α tryptase and mature β-tryptase (Phadia ImmunoCAP® Tryptase, Phadia, Uppsala, Sweden).
The recommendations have been, that a sample is taken 1-4 hours after the reaction and that a basal level serum tryptase is requested 4-6 weeks after the reaction, at a time when the patient has no allergic signs or symptoms. The upper limit of normal < 11.4 µg/l, currently recommended by Phadia, is used.

All analyses in DAAC patients have been carried out on the UniCAP100 system using commercially available reagents. During statistical analysis of data for study III in November 2009 it became apparent that median values of serum tryptase in patients investigated in DAAC Gentofte in the period 1999-2003 were significantly higher that median values for patients investigated in DAAC Rigshospitalet 2003-2009 (6.32 µg/l vs 4.65 µg/l) p=0.0033. As procedures for patient selection were unchanged and no differences could be found in age or gender of the patient population, contact was made to the manufacturer of the tryptase test. It became evident that in 2001 changes had been made to the antibody content in the reagent, causing values in the lower range to be markedly lower in the new assay used after 2001 (personal communication Bjarne Kristensen, Phadia, Allerød, November 2009). This had led to the manufacturers reference values to be changed from median 5.6 µg/l and upper limit 13.5 µg/l, to median 3.8 µg/l and upper limit 11.4 µg/l (ref product info). This discovery led to re-analysis of DAAC samples analysed before October 2001, using the new reagent to ensure that all samples used for analyses in study III were performed using the same reagent. Re-analysis led to a reduction in median serum tryptase values for patients analysed in DAAC Gentofte from 6.32 µg/l to 4.25 µg/l, and the significant difference between hospitals, which could have led to potentially erroneous conclusions in study III, disappeared (4.25 µg/l vs 4.65 µg/l) p=0.20.

Skin testing

In skin testing (skin prick testing (SPT) and intradermal testing (IDT)) mast cells in the skin are exposed to a suspected allergen and positive wheal and flare responses are interpreted as an indication of an IgE mediated mechanism leading to mast cell degranulation. It is generally recommended that skin tests are performed minimum six weeks after the suspected reaction.[21]
SPT is based on a small volume of high concentration being pricked into the epidermis, which is devoid of bloodvessels. IDT is based on a larger volume, but lower concentration, being injected into the dermis, which is vascularised. The small risk of systemic reactions from skin testing is therefore higher from IDT than from SPT. In addition, the risk of false negative test results is greatest for SPT and IDT carries a higher risk of false positive test results.[93,94]

There are many sources of error related to performance and interpretation of skin testing related to:

1. **The subject being tested** (Skin characteristics; skin reactivity (excess/reduced); test location (arm/back))[95]
2. **The allergen** (some drugs eg opioids and NMBA's cause unspecific histamine release; decreased drug reactivity when "out of the bottle"; wrong dilution used)
3. **Materials/methods used**[96,97,98] (different materials lancets/syringes used; different techniques for both SPT and IDT; different positive controls eg. histamine, codeine, morphine)
4. **Tester** (Larger inter-tester variation even among experienced testers; IDT more technically difficult than SPT)
5. **Reading and interpretation of tests** (timing of reading; differing diagnostic criteria; record keeping (tracing and documentation))[99]

Due to this vast number of potential sources of error it is very important that a standardised protocol is followed when performing skin testing. Ideally testing should be kept on as few hands as possible, but unfortunately this is rarely practical in a clinical setting. In DAAC the methods, materials and diagnostic criteria are standardised, and since 2004 all skin tests have been performed in duplicate. (At DAAC Gentofte only positives were reproduced). Dilutions of relevant drugs are freshly prepared each day.

Diagnostic criteria used in DAAC are based on criteria recommended by the EAACI interest group on drug hypersensitivity[21]:

- SPT - wheal ≥ 3 mm greater than negative control and ≥ ½ the diameter of the positive control (histamine 10 mg/ml)
- IDT - wheal ≥ 8mm and ≥ double the diameter of negative control/induced wheal (should be 3-4 mm)

When testing NMBA's the dilutions recommended by GERAP in France are used.[100] Due to the reported risk of cross sensitivity in this group of drugs, all available NMBA's are tested if a positive test is found to the NMBA used during the suspected reaction.[19] Testing for cross reactivity is not carried out if the NMBA used during the suspected allergic reaction tests negative.

The occurrence of systemic reactions to skin testing in DAAC is very limited. In the ten years prick- and intradermal testing has been a part of the standard investigation protocol only 2 mild systemic reactions have occurred in > 320 patients tested with an average of 3-4 substances in three concentrations. Both reactions were managed with antihistamines only. Systemic reactions to skin testing are reported in the literature[101,102], but the risk is decreased if an investigation protocol with titrated doses is followed and testing is carried out by specially trained personnel.

Drug provocation

Intravenous/Subcutaneous/Oral

While drug provocation is considered the ultimate ”gold standard” by many allergologists, in the past by it has been considered hazardous by leading experts in the field of anaesthesia-allergy.[52] However as the other available test modalities have a number of limitations intravenous drug provocation was included in the standard investigation protocol in 2004. The protocol was made based on the recommendations from an EAACI position paper on drug provocation testing.[103]

The overall principles of the drug provocation protocol are:

- Written informed consent from patient
- Full resuscitation back-up and intravenous access
- Close monitoring of blood pressure, pulse and peak flow
- Placebo controlled
- Route of administration same as in suspected reaction iv/sc/po (not spinal/epidural)
- Incremental dosage regimen – time interval for observation between doses
- Drugs with powerful pharmacological effect (anaesthetics, opioids, vasoactive drugs) tested to a maximum dose 1/10 of therapeutic dose
- NMBA’s, chlorhexidine, latex, ethylenoxide not tested as no suitable provocation method
- Other drugs tested to maximum of normal therapeutic dose

Introducing drug provocation into the standard investigation protocol has shown to have obvious ***benefits*** as it can diagnose reactions of all underlying mechanisms including 1) non allergic mechanisms as is seen for reactions to eg. oxytocin 2) IgG mediated allergic reactions, which cannot be diagnosed on skin testing, as is seen for reactions to dextrans 3) other non IgE mediated allergic mechanisms such as reactions to opioids 4) IgE mediated allergic mechanisms which could not be diagnosed on conventional testing due to either negative results or inconclusive results and 5) reactions to metabolites.

Also, in several cases drugs that have tested positive on skin test on the highest concentrations of intradermal testing only, a negative iv provocation has indicated that skin test results may have been false positive.

Drug provocation testing has already proved to be useful in practice, especially for drug groups such as opioids where skin test results can be inconclusive and IgG mediated reactions to eg. dextrans where skin testing is false negative.

Limitations of provocation testing are 1) it is very timeconsuming 2) it cannot diagnose reactions where a combination of eliciting factors is needed eg. other drugs, proteins, allergens, physical stress, dehydration, fever etc. 3) criteria for positive provocation are difficult to standardise 4) it cannot, presently, be

performed for inhalational agents and NMBA's 5) Maximum doses may be too low to elicit a response in those drugs only tested with 1/10 of therapeutic dose 6) Theoretical risk of inducing rapid desensitisation and thereby not eliciting reaction despite prior sensitisation to the allergen.

Basophil Histamine Release test

The basophil histamine release test measures histamine released in response to exposure to a suspected allergen. The analysis is carried out on fresh heparinised blood and all substances can in theory be tested, even catheters or other solid substances, which can be incubated with blood. It detects both IgE and non-IgE mediated reactions. Histamine release can be performed with passive sensitisation (incubation of donor basophils with patient serum prior to exposure to suspected allergen) and this method detects IgE mediated reactions.[104] The HR-test (Reference Laboratory, Copenhagen, Denmark) has been included as a supplement to our standard investigations since 2003. The fact that solid substances can be tested, has proved useful in proving an allergy to a Swan-Ganz catheter.

Patients

Study I, III and IV are based on the patients referred to DAAC for investigation of suspected allergic reactions during anaestesia. Due to the relatively small number of children investigated, only adult patients ≥ 18 yrs have been included.

One female aged 27 with a basal level serum tryptase of 81.3 µg/l was excluded from the study pending further investigations for mastocytosis.

Study II was based on a population of elective surgical patients with no signs of an allergic reaction during anaesthesia. Exclusion criteria for study II were pregnancy, previous allergic reactions during anesthesia, corticosteroid treatment of > 2.5 mg daily and known hematological disease including mastocytosis. If a patient had developed a suspected allergic reaction during anesthesia in the study, that would also have led to exclusion.

Statistical analysis

Serum tryptase values are not normally distributed and thus all statistical analyses of serum tryptase were carried out after logarithmic transformation to approximate a normal distribution. Continuous data are reported as median and interquartile range. In study II pre- and postoperative values were compared using paired Student t-test. Comparisons were made using unpaired Student t-test or Analysis of Variance after logarithmic transformation. The relation between age and serum tryptase was assessed using linear regression after logarithmic transformation in study II and III.

Multiple logistic regression was used to investigate the risk factors for severe reactions in study III. In all studies p values < 0.05 were considered statistically significant. Data analysis and statistical evaluation were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC).

Ethical considerations

The regional ethics committee approved study II and patients were included after giving written informed consent. The Data Protection Agency also approved storage of data for study II.

As studies I, III and IV were based on retrospective analysis of data gathered during routine clinical practice in DAAC ethical committee approval was not necessary. All patients had given written informed consent before skin testing and drug provocation procedures.

Results and discussion of study I-IV

Results and discussion - Chlorhexidine as an allergen (Study I)

The diagnosis of allergy to chlorhexidine was initially based on results of skin testing (skin prick test and intradermal test) combined with clinical features of reactions occuring 20-40 minutes into surgery and, in some cases, repeated reactions on several occasions. In January 2007 a specific IgE test was made available and an IgE mediated mechanism behind chlorhexidine allergy was confirmed in a study of retrospective analysis of sera from 22 patients with clinical reactions suggestive of anaphylaxis to chlorhexidine (**Study I)**. Patients were divided into a chlorhexidine skin test positive (STP) group (n=12) and a skin test negative (STN) group (n=10). Stored serum was analysed using the recently developed specific IgE analysis and Histamine Release test. In the STP group 11 of 12 had elevated specific IgE and 7 of 11 had positive Histamine Release test. None of the patients in the STN group had elevated specific IgE or positive Histamine Release test (figures 2a and 2b).

Figure 2a. Specific IgE to chlorhexidine in skin test positive (STP) and skin test negative patients (STN).

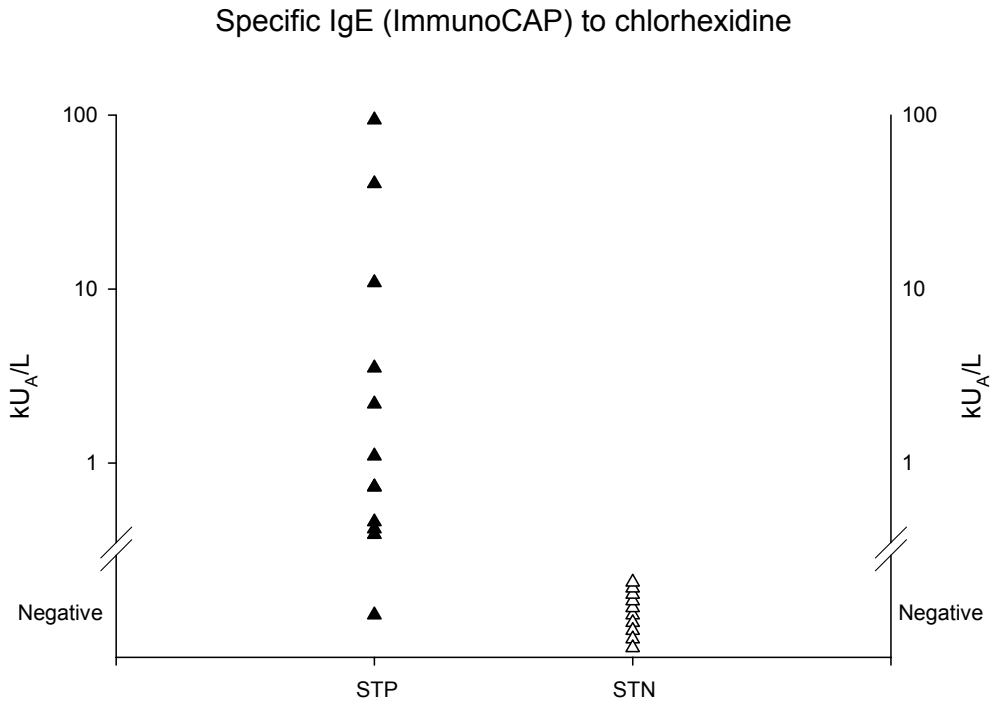
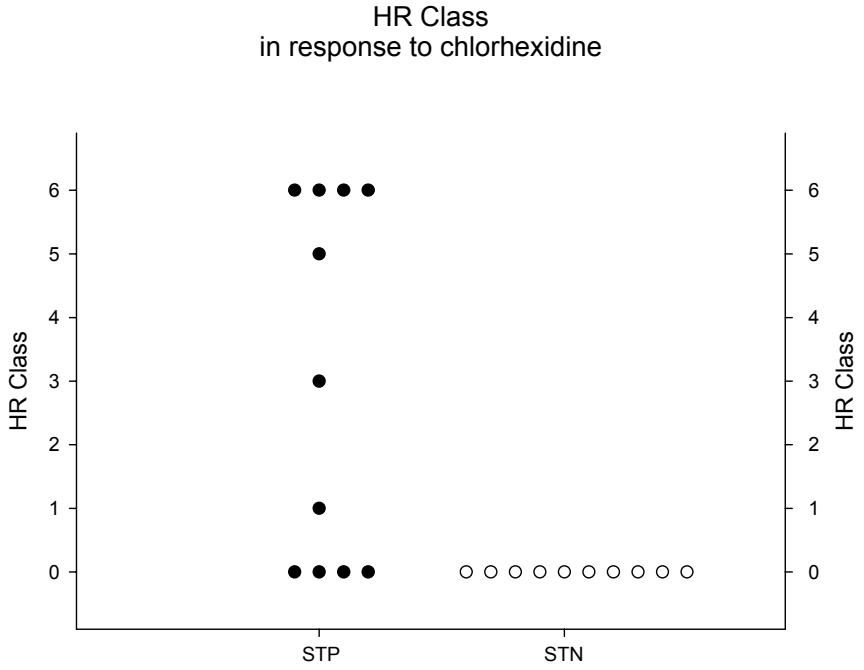


Figure 2b. HR class in skin test positive (STP) and skin test negative patients (STN).



Since the specific IgE test for chlorhexidine became available it has been included in the standard investigations in DAAC. In addition sera were analysed retrospectively on patients investigated in 2005-2006. A total of 205 patients investigated in DAAC, Rigshospitalet has had specific IgE to chlorhexidine measured and as all have been skin tested with chlorhexidine it is thus possible to examine the sensitivity and specificity of the specific IgE analysis vs skin test results combined with detailed clinical history (table 1).

Table 1. Four-by-four table showing results of specific IgE to chlorhexidine vs. skin test result in 205 patients investigated in DAAC, Rigshospitalet 2005-2009. IgE positive if > 0.35 kUA/l. (NPV Negative Predictive Value; PPV Positive Predictive Value)

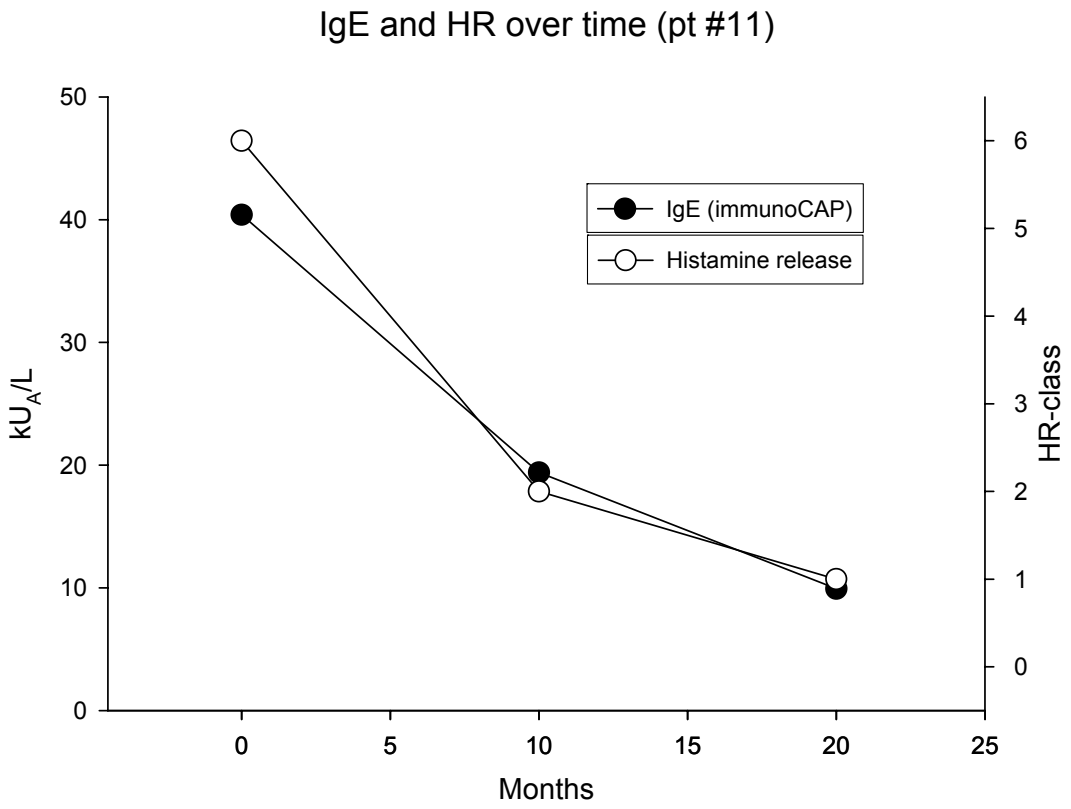
	IgE negative	**IgE positive**		
Skin test positive	2	19	21	**Sensitivity 90.5%**
Skin test negative	181	3	184	**Specificity 98.4%**
	183	22	205	
	NPV 98.9%	**PPV 86.4%**		

Specific IgE to clorhexidine has a very high sensitivity and specificity in this population of patients. The three patients with false positive IgE tests had IgE values of 0.37, 0.43 and 0.46 kUA/l, respectively ie just above the cut-off limit. Two patients had false negative IgE. One had a doubtful skin test result on initial testing but subsequently developed another allergic reaction during anaesthesia and was re-referred and re-investigated. On the second investigation she had postive IgE and skin testing to chlorhexidine. This emphasises the question of when sensitisation and IgE formation actually occurs in relation to exposure, something that could be further explored by serial measurements of IgE.
Chlorhexidine is the single most common agent causing allergic reactions during anaesthesia in Denmark. A total of 28 of 149 patients (18.8%) testing positive to one or more substances tested positive to chlorhexidine. Expressed in another way chlorhexidine accounts for 15.5% of all positive tests in patients referred to DAAC in the period 1999-June 2009. However, considering that all 450.000 patients who have an anaesthetic each year in Denmark, has been exposed to chlorhexidine, this still makes it a very rare allergy occuring in approximately 1:170.000 anaesthetics.

As recommended by the manufacturer a cut-off of < 0.35 kUA/l is used in DAAC. It was shown in **study I** that levels of IgE to chlorhexidine can be elevated already at the time of the reaction and this has also been shown for NMBA's[86,87] and ethylene oxide.[88,89] Also shown in **study I** a decline is seen in IgE levels over time in the absence of exposure to the allergen, which is matched by a decline in HR class (figure 3). Levels may fall below 0.35 kUA/l and this has also been shown for penicillins[90], insect venom[91] and chlorhexidine in health care workers.[92]

The first four cases of chlorhexidine allergy diagnosed in DAAC were published in 2001.[9] Some interesting common features were observed: 1) All patients were males aged >50 years 2) all had severe anaphylaxis in connection with anaesthesia and surgery/invasive procedures. One patient even had a cardiac arrest on two separate occasions. 3) Reactions occurred 20 to 40 minutes into the operation and 4) all patients had a history of previous milder reactions in connection with exposure to chlorhexidine.
In **study I** the clinical features of the first 12 chlorhexidine positive patients were further explored. It was found that chlorhexidine positive patients were predominantly male, had a higher median age than controls and that the majority of reactions were severe (reaction class 3), nearly half occurring during urological surgery.

Figure 3. Decline in specific IgE and HR class over time in a patient sensitised to chlorhexidine.



Examining the total of 28 chlorhexidine positive patients found in the period 1999-2009 they are predominantly male (22M/6F), with a median age of 59 yrs (17-85 yrs) and most cases 10/28 (35.7%) still occurred during urological surgery, but intestinal surgery now account for 8/28 (28.6%).
The vast majority of reactions 21/28 (75%) are class 3, but four cases are mild class 1 reactions; typically with a rash or urticaria appearing at the end of surgery or in recovery. As described in an earlier publication[9] and in **Study I** several of the patients with severe reactions had one or more identifiable milder cases, such as localised urticaria or generalised rash after previous exposure to chlorhexidine. The fact that we diagnose IgE-mediated allergy to chlorhexidine in some patients with very mild reactions, strengthens the argument that even patients with mild reactions after exposure to chlorhexidine should be investigated to prevent severe reactions on subsequent exposure.

Allergy to chlorhexidine display several features that are comparable to those of latex allergy: 1) The allergen probably needs to pass through a broken skin or mucosa barrier for a reaction to occur and this accounts for the reactions occurring 20 to 40 minutes after initial exposure 2) It causes mild non-specific reactions on some occasions, but has the potential to cause serious life-threatening anaphylaxis 3) As

chlorhexidine and latex are not drugs, they can be overlooked as the cause of anaphylaxis during anaesthesia increasing the risk of potentially dangerous re-exposure 4) In some patients, a combined IgE and lymphocyte-mediated allergy has been noted.

The possible parallels to latex allergy have been explored in a study of chlorhexidine allergy in health care workers (HCW)[11], who, like patients, are widely exposed to chlorhexidine as well as to latex. A total of 248 members of staff in the Department of Anaesthesiology at Gentofte Hospital were invited to participate in the study, which comprised a questionnaire plus testing to determine IgE-mediated allergy (SPT with 0.5% and IDT with 0.0002% chlorhexidine digluconate), and lymphocyte-mediated allergy (patch test with chlorhexidine digluconate and chlorhexidine acetate, both 1% in water). A total of 104 individuals were tested and none were positive in any of the test modalities. It was concluded that since numbers were small, the results could not rule out a statistically significant incidence of allergy to chlorhexidine in HCW, even at the percent level, and that larger studies were needed.[11]

In 2008 a British allergologist identified HCW with allergic symptoms and IgE-mediated allergy to chlorhexidine. In 2009 these British cases were published as the first proven cases of IgE mediated allergy to chlorhexidine in HCW[92] concluding that 1) increasing use of chlorhexidine in health care workers over the past years may be reflected in an increase in cases of allergy to chlorhexidine 2) investigation of HCW with allergy symptoms at work should include both latex and chlorhexidine and 3) on lack of exposure levels of specific IgE to chlorhexidine may fall under the recommended lower limit of normal of 0.35 kUA/l and thus levels > 0.2 kUA/l may be significant for sensitisation in individuals who have experienced symptoms.

Presently there is no knowledge of the prevalence of chlorhexidine allergy in the Danish population. Data of chlorhexidine IgE on sera from 196 atopics from Denmark included in the ISPHO study on sensitisation to Pholcodine[54] showed that 8 out of 196 persons (4.1%) had specific IgE to chlorhexidine > 0.35 kUA/l (personal communication Erik Florvaag/SGO Johansson). No clinical information on allergic symptoms on contact with chlorhexidine is available and thus the clinical relevance is uncertain. A total of 130/196 (66.3%) of persons had IgE levels > 0.1 kUA/l (Table 2), perhaps indicating widespread exposure to chlorhexidine in the Danish population.

Table 2. Prevalence of IgE to chlorhexidine in 196 atopic patients (defined as positive specific IgE to at least one inhalational allergen) from Denmark.

IgE to chlorhexidine (kUA/l)	n (%)
≥ 0.10	130 (66.3)
≥ 0.20	11 (5.6)
≥ 0.35	8 (4.1)

However, there is some indication from studies performed by the manufacturer that high levels of total IgE (< 800) could cause spurious elevation of the chlorhexidine specific IgE to levels above 0.2 kUA/l but not above 0.35 kUA/l (personal communication Lennart Venemalm, Phadia, Uppsala). Thus background from elevated total IgE cannot be ruled out as a cause of specific IgE of > 0.2 kUA/l but < 0.35 kUA/l in some of the atopics mentioned above.

Results and discussion - Serum tryptase (Study II and III)

Serum tryptase has been used routinely in our investigation protocol since 1999 and recommendations have been for anaesthetists to take a sample at the time of the reaction (T_{react}), which was then compared to a basal level (T_{basal}) taken at the start of investigations in DAAC.

As the serum tryptase test was not marketed for use specifically in allergic reactions during anesthesia not much literature could be found on a potential effect of surgery and general anaesthesia on serum tryptase. This was therefore investigated in **study II,** which included 120 patients (median age 54 yrs (range 19-94)) undergoing elective orthopaedic surgery in general anaesthesia. Blood samples for tryptase analysis were drawn shortly before anaesthesia and after anaesthesia/surgery. Median duration of anaesthesia was 105 min (range 44-263 min); median interval between blood samples was 139 min (range 39-370 min). It was found that 1) serum tryptase did not increase during surgery and anaesthesia 2) a mean decrease of 0.55 µg/l was observed, most likely due to dilutional effect of intravenous fluid 3) serum tryptase showed only a small intraindividual variation, even during surgery and anaesthesia; 95% of patients had a change between pre- and postoperative values of serum tryptase in the interval between a decrease of 3.33 µg/l and an increase of 2.23 µg/l. It was also hypothesised, that clinically significant increases in serum tryptase could occur within the upper limit of normal of 11.4 µg/l.

In **study III** it was examined whether using the intraindividual difference could increase the sensitivity of serum tryptase as a diagnostic marker for allergic reactions during anaesthesia. As **study II** had shown that only 2.5% of patients would be expected to have an increase of > 2.2 µg/l, in the absence of anaphylaxis, this value was chosen. Sensitivity and specificity using this diagnostic criterion was compared to cut-off values of 11.4 µg/l, 15 µg/l and 25 µg/l (table 3a and 3b).

These values were chosen as they are the upper limits of normal recommended 1) By the manufacturer in the Phadia product information (ImmunoCAP® Tryptase in anaphylaxis, 2007) 2) in the American literature on serum tryptase[68] and 3) by some groups in anaesthesia allergy.[21,35]

When analysing all reaction classes together the highest sensitivity of 66.7 % was found using an intra-individual difference of > 2.2 µg/l between serum tryptase taken at the time of reaction (T_{react}) and basal level serum tryptase (T_{basal}). The second highest sensitity of 62.4% was achieved using the currently recommended upper limit of normal of 11.4 µg/l (table 3a). It has previously been reported that serum tryptase elevation does not always occur in mild reactions and findings in **study III** supported this as the sensitivity of serum tryptase increased markedly on only including severe reactions in the analysis (reaction class 3 and 4) (table 3b). The highest sensitivity of 82.8% was again achieved using (T_{react} - T_{basal}) > 2.2 µg/l, but a cut-off of 11.4 µg/l gave a very similar sensitivity of 81.0 %. However, using the approach of comparing T_{react} with T_{basal} potentially gives some additional benefits compared to using a single value: 1) an elevated basal level will be detected and 2) clinically relevant increases in serum tryptase within the upper limit of normal will be detected.

Table 3a.
Sensitivity, specificity and positive and negative predictive value for serum tryptase using different criteria for positivity in patients with suspected allergic reactions during anaesthesia (all four reaction classes, n=180).

Criteria for positive serum tryptase	Sensitivity %	PPV %	Specificity %	NPV %
(T_{react} - T_{basal}) > 2.2 µg/l	66.7	76.5	78.2	68.7
Treact > 11.4 µg/L	62.4	82.9	86.2	68.2
Treact > 15.0 µg/L	52.7	83.1	88.5	63.6
Treact > 25.0 µg/L	34.4	88.9	95.4	57.6

Table 3b.
Sensitivity, specificity and positive and negative predictive value for serum tryptase using different criteria for positivity in patients with suspected allergic reactions during anaesthesia (reaction class 3 and 4 only, n=102).

Criteria for positive serum tryptase	Sensitivity %	PPV %	Specificity %	NPV %
(T_{react} - T_{basal}) > 2.2 µg/l	82.8	76.2	65.9	74.4
Treact > 11.4 µg/L	81.0	82.5	77.3	75.6
Treact > 15.0 µg/L	70.7	82.0	79.6	67.3
Treact > 25.0 µg/L	50.0	87.9	90.9	58.0

PPV positive predictive value
NPV negative predictive value
Treact serum tryptase taken at time of reaction
Tbasal basal level serum tryptase

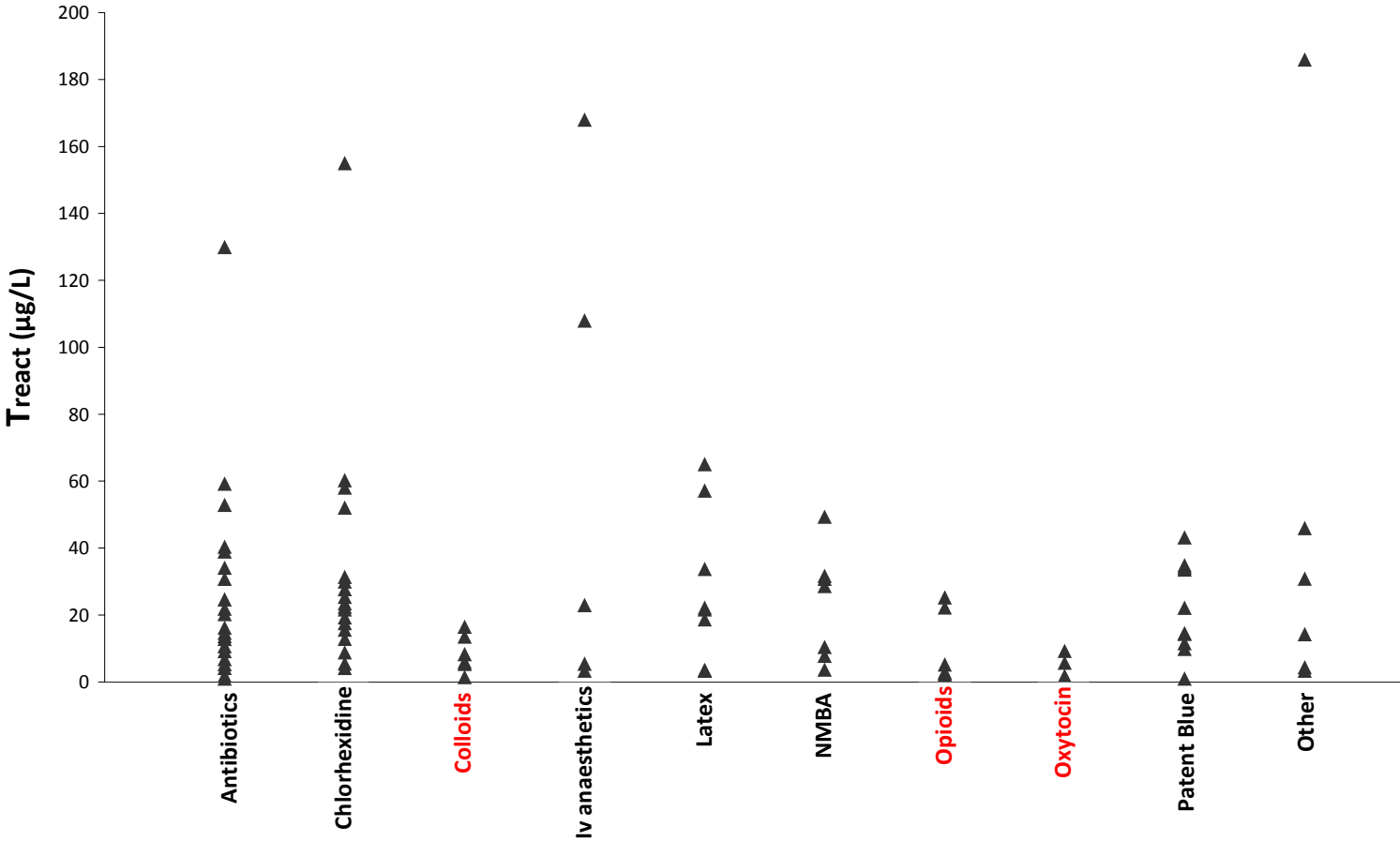
In the setting of anaesthesia allergy it is important to avoid false negative tests as this may lead to a patient being re-exposed to potential allergens during subsequent anaesthesia. Therefore patients with a clinical suspicion of an allergic reaction during anaesthesia, but negative serum tryptase, should still be considered for subsequent allergological investigation. In our view, for the safety of the patient, the aim is therefore to achieve the highest possible sensitivity for serum tryptase, if necessary at the expense of the specificity of the test. Thus we would recommend using the intra-individual difference (T_{react} - T_{basal}) > 2.2 µg/l for suspected allergic reactions during anaesthesia.
Further studies in patients with suspected anaphylaxis outside the operating room would be required to determine whether our findings could be extrapolated to that setting.

The increase in serum tryptase in connection with anaphylaxis depends on several factors such as time of sampling of serum tryptase in relation to reaction, severity of reaction, the allergen and the mechanism of the reaction.[68] In **study III** we found that elevated levels of serum tryptase can be found as early as 25

min after the reaction, but also as late as nearly 6 hours after the reaction. We also found a significant increase in serum tryptase with increasing reaction class in patients testing positive on subsequent investigation in DAAC. This suggests that the sensitivity of serum tryptase is lower for mild reactions as is also concluded above.

In study III we also found that serum tryptase values appeared higher in patients testing positive to allergens with a known IgE mediated mechanism (figure 4), than in patients testing positive to oxytocin, colloids and opioids where the mechanism is different. Reactions to oxytocin are caused by an exaggerated physiological response resulting in flushing and hypotension in most cases and these are non-allergic reactions with no accompanying increase in serum tryptase.[105,106] Reactions to colloids were predominantly severe reactions (C3) to dextrans. The reaction mechanism is IgG mediated[107] and presumably does not stimulate mast cell degranulation, as we saw little or no increase in serum tryptase despite reactions being severe. Reactions to opioids are mainly caused by direct histamine release, presumably mainly from basophils as we saw only small elevations in serum tryptase in these patients.

Figure 4. T_{react} according to allergen found on subsequent investigation.



A statistically significant increase in serum tryptase with increasing age was found on linear regression in DAAC patients (r=0.23; p<0.0001) but not in elective patients (r=0.05; p=0.6). Also a higher proportion of DAAC patients had an elevated basal level serum tryptase (T_{basal} > 11.4 µg/l) than elective patients (8.2% vs 5.8%). This corresponds well to findings in venom allergic patients from a recent multicenter study which showed that 8.4% of patients had a T_{basal} > 11.4 µg/l.[108] This large multicenter study also concluded, that the risk of severe reactions in patients with venom allergy was increased with T_{basal} > 11.4 µg/l, increasing age and male sex among other parameters. In **study III** this was investigated in our population of patients with suspected allergic reactions during anaesthesia using multiple logistic regression. We found that the risk of severe reactions was increased with T_{basal} > 11.4 µg/l, increasing age, male sex and positive result on subsequent investigation (table 4). Thus some parallels can be drawn between patients with venom allergy and patients with anaesthesia (drug) allergy.

Table 4. Risk factors for severe reactions in patients with suspected allergic reactions during anaesthesia using multiple logistic regression (n = 299).

Variable	p value	Odds ratio	95% CI
Tbasal > 11.4 µg/L	0.0079	4.815	1.509 - 15.367
Male sex	0.0328	1.709	1.045 - 2.793
Age (per year)	0.0131	1.022	1.005 - 1.039
Positive investigation result in DAAC	0.0055	1.917	1.211 - 3.036

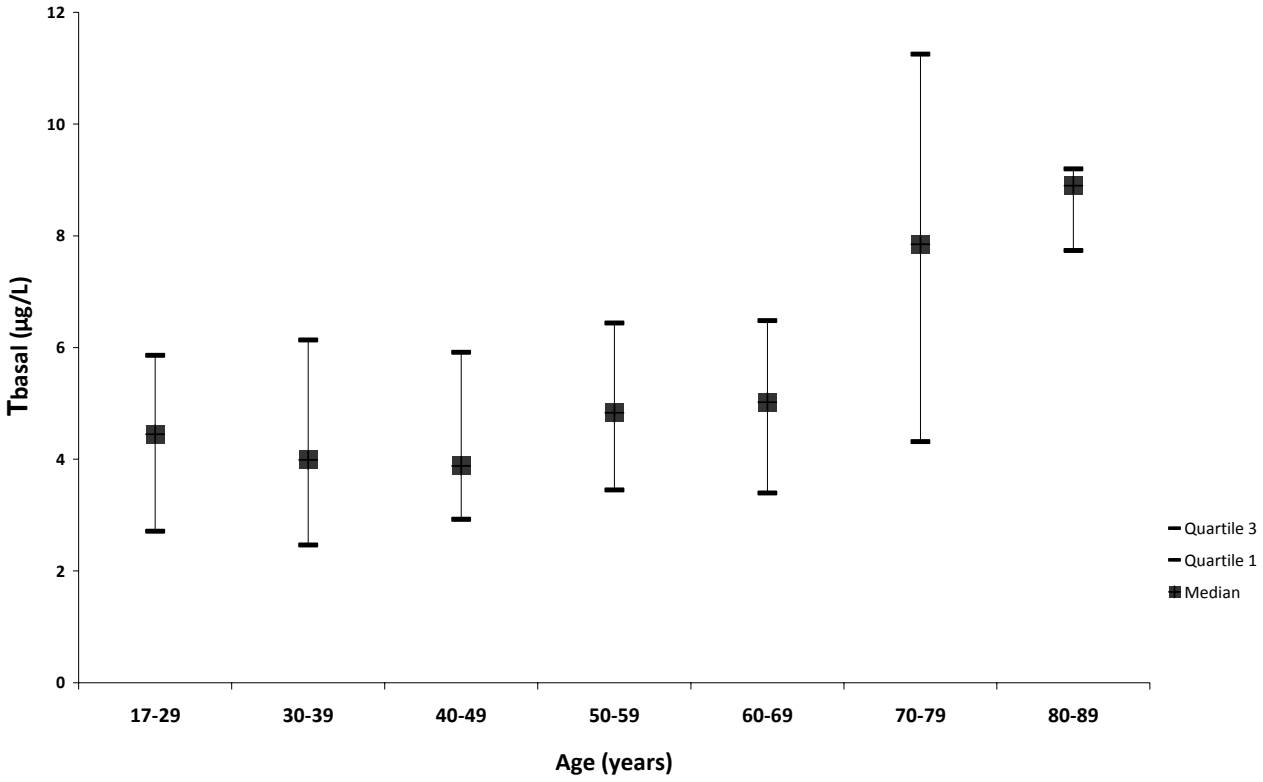
All variables were included in the model simultaneously.

The clinical implications of these findings are, that patients at higher risk of severe reactions can be identified and relevant advice and precautions can be taken during future anaesthesia.

The cause of elevated T_{basal} in this group of patients is not known, but in a study in venom allergic patients, where bone marrow biopsy was performed in all patients with T_{basal} > 11.4 µg/l, a very high proportion of patients showed signs of mast cell disorder or mastocytosis.[109]

In **study III** we also found an increase in T_{basal} with increasing age, especially patients aged > 60 and an alternative explanation for this may be, that the increase is related to the increasing co-morbidity in older patients (figure 5).

Figure 5. T_{basal} according to age.



Animal and human studies have established that mast cells and serum tryptase are involved in numerous physiological and pathological processes as diverse as angiogenesis, tissue remodelling, bloodclotting, inflammation and pathogen defense.[110-112] However, no studies have correlated these findings with measuring serum tryptase. On the other hand elevated basal level serum tryptase has been found in conditions such as mastocytosis and myeloid haematological malignancy[113,114] and some patients in DAAC are likely to fall into these categories. Unfortunately clinical detail about comorbidity were not analysed in the present study, so we cannot identify patients with known haematological malignancy or other relevant disease.

Conditions involving 1) angiogenesis, such as malignant tumours, and 2) atherosclerosis, such as coronary artery disease and aortic aneurysms are all more common in the older population. It could be speculated, that the higher basal level serum tryptase in some of these patients reflected an increased mast cell activity. In DAAC the patients undergoing cardiovascular surgery for ischaemic disorders, and urological and intestinal surgery, mainly for malignant disease, have higher median basal level serum tryptase than other surgical categories (table 5). Differences in age and gender also play a role, and further larger studies of these patient populations would be needed to confirm such speculation.

Table 5. Median basal level serum tryptase according to surgery type.

	Patients n (%)	Serum tryptase µg/L Median (IQ range)	p¤	Age Median (IQ range)	p§
Overall group	318 (100)	4.56 (3.14 - 6.60)		50 (36-62)	
Surgery type (n=315)					
cardiovascular	36 (11.4)	5.43 (3.37 - 8.93)	0.0301	61.5 (55-66)	<0.0001
endocrine	23 (7.3)	4.06 (3.14 - 9.18)		56 (50-66)	
gut	44 (14)	5.25 (3.26 - 8.79)		51.5 (37-62.5)	
gynecological	51 (16.2)	3.99 (2.15 - 5.28)		35 (29-429	
head and neck	29 (9.2)	4.64 (3.45 - 5.86)		46 (33-60)	
organ	15 (4.8)	3.58 (2.39 - 5.99)		43 (30-55)	
orthopedic	50 (15.9)	4.25 (2.92 - 6.60)		50 (40-60)	
urological	35 (11.1)	5.98 (3.79 - 7.76)		66 (43-72)	
other	32 (10.1)	4.99 (3.37 - 6.44)		48 (36.5-57)	

¤ unpaired t-test or ANOVA after logarithmic transformation

§ unpaired t-test or ANOVA

Results and discussion – treatment with adrenaline in suspected anaphylaxis during anaesthesia (Study IV)

It is well known that adrenaline is the first line treatment of anaphylaxis. Numerous guidelines have been published regarding the dose and route of administration of adrenaline for the treatment of anaphylaxis both outside and inside the operating room.[1,21-24,79] However, no studies could be found describing the use of adrenaline, in practice, in suspected anaphylaxis during anaesthesia. In DAAC detailed information on the use of adrenaline regarding timing, dose and route of administration has been gathered since 1999. Study IV is a descriptive study based on information from referral papers and anaesthetic charts from 270 patients (158F/112M) referred to DAAC in the period 1999-2008. Reactions had been graded into four reaction classes (C1-C4) by one of two anaesthesiologists as described previously under methodological considerations. Patient characteristics can be seen in table 6.

Table 6. Patient characteristics for 270 patients with suspected anaphylaxis during anaesthesia.

	Patients n (%)	Age Median (range)	p¤
All patients	270 (100)	49.5 (17-86)	
Gender			
Female	158 (58.5)	43.0 (17-85)	< 0.0001
Male	112 (41.5)	57.5 (17-86)	
ASA classification			
I	105 (38.9)	38.0 (17-76)	< 0.0001
II	131 (48.5)	56.0 (18-86)	
III	34 (12.6)	62.5 (45-78)	
IV	0	0	
Reaction class			
1	83 (30.7)	40 (17-78)	< 0.0001*
2	65 (24.1)	46 (18-86)	
3	113 (41.9)	56 (18-86)	
4	9 (3.3)	57 (33-62)	

¤ unpaired t-test or ANOVA

* reaction class 3 and 4 added together for statistical analysis

Severe reactions (C3 and C4) with circulatory symptoms were seen in 122/270 patients (45.2%) and of these 101 (82.8%) received adrenaline. There was a trend towards increasing total dose with increasing reaction class (table 7).

Table 7. Total adrenaline dose in the 123 of 270 patients where adrenaline was administered. Information on dose available for n=102.

Reaction class	Adrenaline given	Total adrenaline dose in mg	
	n (%)	n	Median (range)
1	4/83 (4.8)	1	0.01
2	18/65 (27.7)	10	0.125 (0.03-1.0)
3	93/113 (82.3)	83	0.2 (0.002-2.0)
4	8/9 (88.9)	8	1.95 (0.6-2.0)
Total	123/270 (45.6)	102	

Total doses (total bolus doses only) used in C2 and C3 reactions were high at 0.125 mg (range 0.03-1.0 mg) and 0.2 mg (range 0.002-2.0 mg), respectively, compared with current recommendations to start with doses of 0.01-0.05 mg and titrate to response.[1,21] In some cases the relatively high total doses were due to repeated need for bolus injection, but in many cases only a single injection of 0.1-0.5 mg was administered.

Route of administration was primarily intravenous for C3 and C4 reactions where 94% of patients received intravenous adrenaline.

In 33 out of 123 cases (26.8%) across all four reaction classes antihistamine and steroid treatment were given **before** treatment with adrenaline. For C3 and C4 reactions only, a total of 17 out of 101 patients (16.8%) with hypotension were treated with antihistamine and steroid **before** treatment with adrenaline. In most C3 reactions other vasoactive drugs such as ephedrine and phenylephrine were first line treatment, except in 22 cases (23.7%) where adrenaline was the first vasoactive drug administered.

Median time from onset of reported hypotension to treatment with adrenaline was 10 minutes (range 1-70 minutes). Defining adrenaline treatment ≤ 10 minutes as early, and > 10 minutes as late, and using the need for subsequent infusion of adrenaline as a parameter for a protracted reaction, we found that infusion was needed in 12 of 60 patients (20%) treated early vs. 12 of 35 patients (34.2%) treated late (OR 2.087; 95% CI 0.814-5.353). Thus this finding did not reach statistical significance, probably due to the small number of patients studied or to the chosen cut-off of 10 minutes.

There was no significant difference in distribution of age and gender in groups treated early and late. A serum tryptase was taken in 70 of 95 cases (73.7%) and median serum tryptase was 16.45 µg/l (IQ range 5.84-32.75) in patients treated early vs 16.1µg/l (IQ range 4.83-25.6) in patients treated late (p=0.72). This may suggest that serum tryptase is related to initial severity of the reaction, but not to the prolonged inflammatory response associated with prolonged reactions.

As far as we are aware no other studies have looked at the timing of the use of adrenaline in anaphylaxis during anaesthesia. Some relevant information may be extracted from a recent closed claims study from

the UK.[78] In this study 31 allergic reactions to drugs were reported. In 20/31 cases a drug was given despite known allergy – and no deaths or sequelae occurred in this group. The remaining 11 cases were caused by unexpected anaphylaxis from an unknown allergen and in this group 5 deaths and 4 cardiac arrests occurred, of which two resulted in severe neurological damage. One could speculate that when a drug is given despite a known allergy it is either an oversight or a conscious decision on part of the anaesthesiologist. In both cases a rapid diagnosis of anaphylaxis may be made due to a high index of suspicion and treatment will be initiated promptly. The fact that there were no deaths or long-term sequelae in that group, supports this notion. On the other hand unexpected anaphylaxis, especially during anaesthesia, may be difficult to diagnose and the delay in diagnosis and treatment might be speculated to be the cause of the poorer outcome in this group. The findings of **study IV** could indicate that delayed treatment with adrenaline may lead to protracted reactions requiring prolonged treatment with adrenaline. We chose to allow 10 minutes for the diagnosis to be made and for treatment to be initiated. With a 10 minute cut-off the risk of needing intravenous infusion of adrenaline was doubled in the group treated later than 10 minutes after circulatory symptoms appeared. However, this did not reach statistical significance and larger studies would be needed to confirm this finding.

We are aware of only one case of long term neurological damage in patients referred to DAAC. Deaths in connection with anaphylaxis would not be referred to DAAC for obvious reasons, and we have not received information on any cases of death from anaphylaxis during anaesthesia in the period 1999-2009. In addition a closed claims study analysing deaths related to anaesthesia in the period 1996-2004 in Denmark did not identify any cases of suspected perioperative anaphylaxis leading to a claim in the patient insurance association.[77]

Findings of **study IV** are generally encouraging as anaphylaxis is diagnosed and treated within 10 minutes in 2/3 of cases and because intravenous adrenaline is used in the vast majority of cases. However, there is still some room for improvement and continued education, perhaps ideally in a simulated setting, on the diagnosis and correct treatment of anaphylaxis is an important factor in ensuring continued improvement.

As the diagnosis of anaphylaxis is difficult to make during anaesthesia, we suggest that anaphylaxis should be considered in cases of circulatory instability, not responding to the usual maneouvres such as ephedrine, phenylephrine, fluid administration and elevation of the patients legs within a maximum of 10 minutes. If no other obvious cause is present and anaphylaxis cannot be ruled out, the treatment should be intravenous adrenaline starting at 0.01 mg and titrating to effect. As adrenaline is a life saving, but also extremely potent drug with potentially lethal side effects[39,115] all anaesthetists should be trained in the correct dilution of and administration of adrenaline. It should be the aim to administer the minimum effective dose to decrease the risk of side effects from overdose. In Denmark the standard strength of

adrenaline for adults is 1mg/ml and the only time this should be used undiluted is during cardiac arrest or severe circulatory collapse. In all other cases dilution to a 0.05 mg/ml or 0.01 mg/ml solution should be performed. However during a stressful emergency situation, and especially in inexperienced hands, this may be difficult to carry out correctly and the risk of error is present.[116] Therefore intravenous administration of adrenaline should be reserved for treatment of monitored patients by specially trained personnel such as anaesthesiologist/intensivists and doctors in emergency departments. In some countries a weaker solution of 0.1 mg/ml adrenaline is available for emergencies other than cardiac arrest and it could be argued that this would decrease the risk of serious side effects from too high doses of adrenaline during treatment of anaphylaxis.

Conclusions and future perspectives

Chlorhexidine as an allergen

Results from this thesis has helped prove that chlorhexidine allergy is IgE mediated and has shown that chlorhexidine is the single most common cause of allergic reactions during anaesthesia in Denmark. Many issues remain to be clarified in further studies - among these the potential risk of sensitisation to chlorhexidine in health care workers. As prevalence is likely to be low the study could be undertaken in a subpopulation with allergic symptoms.

Also further studies of IgE to chlorhexidine, even at levels below 0.35 kUA/l (keeping in mind the need for a total IgE measurement), correlated to exposure and clinical symptoms in patients, health care workers and atopics from other countries would help elucidate potential differences in exposure and sensitisation in different populations.

Further studies of serial samples of specific IgE to chlorhexidine in patients with verified allergy to chlorhexidine could further explore the dynamics of variation in IgE with exposure and perhaps correlation with IgG/IgG4 levels as an indicator for exposure in non-allergic patients.

Lastly, a questionnaire study in patients with verified allergy to chlorhexidine may shed a light on potential modes of sensitisation.

Serum tryptase

The studies on serum tryptase in this thesis have shown, that the intraindividual variation in serum tryptase is very small in the absence of allergic signs and symptoms, but that a small dilutional decrease is seen in connection with surgery and anaesthesia. In the population of patients with suspected allergic reactions during anaesthesia studied here the sensitivity and specificity for serum tryptase as a marker for anaphylaxis was highest when using the intraindividual difference between tryptase taken at time of reaction and patients own basal level. When using an absolute value the recommended 11.4 µg/l yielded the highest sensitivity and specificity.

We also found a statistically significant increase in basal level serum tryptase with increasing age in patients with suspected allergic reactions during anaesthesia, but not in patients undergoing elective orthopaedic surgery.

Lastly, an elevated basal level serum tryptase increased the risk of severe reactions in patients with suspected allergic reactions during anaesthesia.

These conclusions lead to following ideas for further studies:

The relevance of elevated serum tryptase in DAAC patients could be further explored by a follow-up study of patients with elevated basal level serum tryptase to monitor development in serum tryptase,

question about symptoms and signs of mastocytosis/haematological disease and other relevant co-morbidity.
Large population studies of serum tryptase could give more precise indications of differences related to age, gender and different disease categories.
The use of basal level serum tryptase as a potential marker for changed activity in ischaemic heart disease and aortic aneurysms could be further explored in populations of patients with stable/unstable coronary artery disease and aortic aneurysms.

Treatment with adrenaline

Data from our records of the use of adrenaline in the treatment of anaphylaxis during anaesthesia in Denmark has shown, that there is some room for improvement regarding the timing and dosing of adrenaline. Also, our results indicate that treatment with adrenaline initiated later than 10 minutes after onset of hypotension seems to increase the risk of needing intravenous infusion of adrenaline to stabilise the patient.
Larger studies of the timing of treatment with adrenaline would be needed to explore this further and confirm the findings of our study.
Further and continued education on the diagnosis and treatment of anaphylaxis during anaesthesia is needed in order to ensure the safe, correct and prompt treatment. Teaching on anaphylaxis is integrated in the late stages of specialist training in anaesthesiology in Denmark, and it may be interesting to assess the effect of training in anaphylaxis management in a full-scale simulator before, and at different time intervals after training. The hypothesis is, that like with other rarely occuring emergencies, only a limited amount of information is retained over time and therefore regular training may be needed.

References

1. Krøigaard M, Garvey LH, Gilberg L, Johansson SGO, Mosbech H, Florvaag E et al. Scandinavian Clinical Practice Guidelines on the diagnosis, management and follow-up of anaphylaxis during anaesthesia. Acta Anaesthesiol Scand 2007; 51: 655-70

2. Krøigaard M, Garvey LH, Menné T, Husum B. Allergic reactions in anaesthesia: Are suspected causes confirmed on subsequent testing? Br J Anaesth 2005; 95: 468-71

3. Fisher MM, Doig GS. Prevention of anaphylactic reactions to anaesthetic drugs. Drug Safety 2004; 27: 393-410

4. Sigiel M, Laxenaire MC, Moneret-Vautrin D, Condi M, Jacob F, Picard JM. 3 cases of anaphylactic shock to celocurine. Anesth Analg (Paris) 1975; 32: 497-62

5. Fisher MMcD. Intradermal testing in the diagnosis of acute anaphylaxis during anaesthesia – results of five years experience. Anaesth Intens Care 1979: 5: 58-61

6. Watkins J. Adverse anaesthetic reactions an update from a proposed national reporting and advisory service. Anaesthesia 1985; 40: 797-800

7. Watkins J. Investigation of allergic and hypersensitivity reactions to anaesthetic agents. Br J Anaesth 1987; 59: 104-11

8. Garvey LH, Roed-Petersen J, Menné T, Husum B. Danish Anaesthesia Allergy Centre – premiminary results. Acta Anaesthesiol Scand 2001; 45: 1204-9

9. Garvey LH, Roed-Petersen J, Husum B. Anaphylactic reactions in anaesthetised patients – four cases of chlorhexidine allergy. Acta Anaesthesiol Scand 2001; 45: 1290-4

10. Garvey LH, Roed-Petersen J, Husum B. Klorhexidinallergi – en vigtig differentialdiagnose. Ugeskr Læger 2004; 166: 382-3

11. Garvey LH, Roed-Petersen J, Husum B. Is there a risk of sensitisation and allergy to chlorhexidine in health care workers? Acta Anaesthesiol Scand 2003; **47**: 720-724.

12. Fayaz KM, Pugh S, Balachandran S, Sudheer PS, Hall JE. Histamine release during adult cardiopulmonary bypass. Anaesthesia 2005; 60: 1179-84

13. Storjord E, Nielsen EW. Tryptase levels after suxamethonium administration and defibrillation. Acta Anaesthesiol Scand 2008; 52: 838-840

14. Brown SGA, Blackman KE, Heddle RJ. Can serum mast cell tryptase help diagnose anaphylaxis? Emergency medicine Australasia 2004; 16: 120-4

15. Thain S, Rubython J. Treatment of anaphylaxis in adults: results of a survey of doctors at Dunedin Hospital, New Zealand. N Z Med J 2007; 120: U2492

16. Gompels LL, Bethune C, Johnston SL, Gompels MM. Proposed use of adrenaline in anaphylaxis and related conditions: a study of senior house officers starting accident and emergency posts. Postgrad Med J 2002; 78: 416-48

17. Haymore BR, Carr WW, Frank WT. Anaphylaxis and epinephrine prescribing patterns in a military hospital: underutilization of the intramuscular route. Allergy and asthma proc 2005; 26:361-5

18. Jacobsen J, Lindekær AL, Østergaard T, Nielsen K, Østergaard D, Laub M, Jensen PF, Johannesen N. Management of anaphylactic shock evaluated using a full-scale anaesthesia simulator. Acta Anaesthesiol Scand 2001; 45: 315-9

19. Reducing the risk of anaphylaxis during anaesthesia. Abbreviated text. Ann Fr Anesth Reanim 2002; 21: 7s-23s

20. Harper NJ, Dixon T, Dugué P, Edgar DM, Fay A, Gooi HC et al. Suspected anaphylactic reactions associated with anaesthesia - guidelines of the Association of Anaesthetists of Great Britain and Ireland. Anaesthesia 2009; 64: 199-211

21. Mertes PM, Laxenaire MC, Lienhart A, Aberer W, Ring J, Pichler WJ et al. Reducing the risk of anaphylaxis during anaesthesia: guidelines for clinical practice. J Invest Allergol Clin Immunol 2005; 15: 91-101

22. Kemp SF, Lockey RF, Simons FER et al. Position paper. Epinephrine: the drug of choice for anaphylaxis. A statement of the World Allergy Organization. Allergy 2008; 63: 1061-70

23. Mirakian R, Ewan PW, Durham SR, Youlten LJ, Dugué P, Friedmann PS, English JS, Huber PA, Nasser SM; BSACI. BSACI guidelines for the management of drug allergy. Clin Exp Allergy 2009; 39: 43-61.

24. Simons FER. Anaphylaxis. J Allergy Clin Immunol 2010; 125: S161-81

25. Editorial. The history of anaphylaxis. J Am Med Assoc. 1921;76: 380

26. Saavedra-Delgado AM. Francois Magendie on anaphylaxis. Allergy Proc. 1991;12:355-356

27. Jackson M. Allergy – the history of a modern malady. Reaction books ltd., London, United Kingdom 2006

28. Ishizaka K, Ishizaka T, Terry WD. Antigenic structure of gamma-E-globulin and reaginic antibody. J Immunol 1967; 99: 849-858

29. Stanworth DR, Humphrey JH, Bennich H, Johansson SGO. Specific inhibition of the Prausnitz-Küstner reaction by an atypical human myeloma protein. Lancet 1967;2:330-332

30. Gueant JL, Aimone-Gastin I, Namour F, Laroche D, Bellou A, Laxenaire MC. Diagnosis and pathogenesis of the anaphylactic and anaphylactoid reactions to anaesthetics. Clin Exp Allergy 1998 ;28: 65-70

31. Johansson SGO, Hourihane J O'B, Bousquet J et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. Allergy 2001; 56: 813-24

32. Johansson SGO, Bieber T, Dahl R et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Comittee of the World Allergy Organization, October 2003. Journal of Allergy and Clinical Immunology 2004; 113: 832-6

33. Mertes PM, Aimone-Gastin I, Guéant-Rodriguez RM et al. Hypersensitivity reactions to Neuromuscular Blocking Agents. Current Pharmaceutical Design 2008; 14: 2809-25

34. Malinovsky J-M, Decagny S, Wessel F, Guilloux L, Mertes PM. Systematic follow-up increases incidence of anaphylaxis during adverse reactions in anesthetized patients. Acta Anaesthesiologica Scandinavica 2008; 52: 175-81

35. Harboe T, Guttormsen AB, Irgens A, Dybendal T, Florvaag E. Anaphylaxis during anaesthesia in Norway. A 6-year single-center follow-up study. Anesthesiology 2005; 102: 897-903

36. Hallgren J, Pejler G. Biology of mast cell tryptase. An inflammatory mediator. FEBS Journal 2006; 273: 1871-95

37. Moss J. Muscle relaxants and histamine release. Acta Anaesthesiol Scand 1995; 39: 7-12

38. Mertes PM, Lambert M, Guéant-Rodriquez RM, Aimone-Gastin I, Mouton-Faivre C, Moneret-Vautrin DA, Guéant JL, Malinovsky JM, Demoly P. Perioperative anaphylaxis. Immunol Allergy Clin N Am 2009; 29: 429-451

39. Pumphrey RSH. Lessons for management of anaphylaxis from a study of fatal reactions. Clin Exp Allergy 2000; 30: 1144-50

40. Whittington T, Fisher MM. Anaphylactic and anaphylactoid reactions Balliere's Clinical Anesthesiology 1998; 12: 301-21

41. Mertes PM, Laxenaire MC, GERAP. Anaphylactic and anaphylactoid reactions occurring during anaesthesia in France. Seventh epidemiologic survey (January 2001-December 2002. Ann Fr Anesth Reanim 2004; 23: 1133-43

42. Laxenaire MC, Moneret-Vautrin DA, Watkins J. Diagnosis of the causes of anaphylactoid anaesthetic reactions. A report of the recommendations of the joint Anaesthetic and Immuno-allergological Workshop, Nancy, France: 19 March 1982. Anaesthesia 1983; 38: 147-148

43. Laxenaire MC et al. Drugs and other agents involved in anaphylactic shock occuring during anaesthesia. A French multicenter epidemiological inquiry. Ann Fr Anaesth Reanim 1993; 12: 91-96

44. Fisher MM, Baldo BA. The incidence and clinical features of anaphylactic reactions during anaesthesia in Australia. Ann Fr Anesth Reanim 1993; 12: 97-104

45. Watkins J, Wild G. Improved diagnosis of anaphylactoid reactions by measurement of serum tryptase and urinary methylhistamine. Ann Fr Anaesth Reanim 1993; 12: 169-172

46. Dewachter P, Mouton-Faivre C. What investigation after an anaphylactic reaction during anaesthesia? Curr Opin Anaesthesiol 2008; 21: 363-8

47. Currie TT, Whittingham S, Ebringer A, Peters JS. Severe anaphylactic reaction to thiopentone: case report. BMJ 1966; 1: 1462-1463

48. Kepes ER, Haimovici H. Allergic reaction to succinylcholine. J Am Med Assoc. 1959; 171: 548-9.

49. Hepner DL, Castells MC. Anaphylaxis during the perioperative period. Anesth Analg 2003; 97: 1381-95

50. Ebo DG, Fisher MM, Hagendorens MM, Bridts CH, Stevens WJ. Anaphylaxis during anaesthesia: diagnostic approach. Allergy 2007; 62: 471-87

51. Dewachter P, Mouton-Faivre C, Emala CW. Anaphylaxis and anaesthesia – controversies and new insights. Anesthesiology 2009; 111: 1141-50

52. Fisher MM, Baldo BA. Anaphylaxis during anaesthesia: Current aspects of diagnosis and prevention. European Journal of Anaesthesiology 1994; 11: 263-284.

53. Florvaag E, Johansson SG. The Pholcodine Story. Immunol Allergy Clin North Am 2009; 29: 419-27

54. Johansson SGO, Florvaag E, Öman, Poulsen LK, Mertes PM, Harper N, Garvey LH, van Wijk RG, Metso T, Irgens A, Dybendal T, Halsey J, Guttormsen AB. Pholcodine consumption and prevalence of IgE-sensitization, an international multicentre study. Allergy 2010; 65: 498-502

55. Davies GE, Francis J, Martin AR, Rose FL, Swain G. 1:6-Di-4'-chlorophenyldiguanidohexane (hibitane); laboratory investigation of a new antibacterial agent of high potency. Br J Pharmacol Chemother 1954; 9: 192-6

56. Birdwood B. Reaction to Chlorhexidine and Cetrimide. Lancet 1965; 43: 651-2

57. Nickel KM, Marsden CW. Reaction to chlorhexidine and cetrimide. Lancet 1965; 1: 821-2

58. Osmundsen PE. Contact dermatitis to chlorhexidine. Contact Dermatitis 1982; 2: 81-3

59. Lasthein AB, Brandrup F. Contact dermatitis from chlorhexidine. Contact Dermatitis 1985 ; 13: 307-9

60. Cheung J, O'Leary JJ. Allergic reaction to chlorhexidine in an anaesthetised patient. Anaesth Intensive Care 1985; 13: 429-30

61. Okano M, Nomura M, Hata S, Okada N, Sato K, Kitano Y, et al. Anaphylactic symptoms due to chlorhexidine gluconate. Arch Dermatol 1989; 125: 50-2

62. Visser LE, Veeger JH, Roovers MH, Chan E, Stricker BH. Anaphylaxis caused by chlorhexidine following cystoscopy or urethral catheterization. Ned Tijdschr Geneeskd 1994; 138: 778-80

63. Knight BA, Puy R, Douglass J, O'Hehir RE, Thien F. Chlorhexidine anaphylaxis: a case report and review of the literature. Intern Med J 2001; 31: 436-7

64. Stephens R, Mythen M, Kallis P, Davies DW, Egner W, Rickards A. Two episodes of life-threatening anaphylaxis in the same patient to a chlorhexidine-sulphadiazine-coated central venous catheter. Br J Anaesth 2001; 87: 306-8

65. Caughey GH. Tryptase genetics and anaphylaxis. J Allergy Clin Immunol 2006; 117: 1411-4

66. Schwartz LB, Min HK, Ren S, Xia HZ, Hu J, Zhao W, Moxley G, Fukuoka Y. Tryptase precursors are preferentially and spontaneously released, whereas mature tryptase is retained by

HMC-1 cells, Mono-Mac-6 cells, and human skin-derived mast cells. Journal of Immunology 2003;170: 5667-73

67. Schwartz LB, Metcalfe DD, Miller JS, Earl H, Sullivan T. Tryptase levels as an indicator of mast-cell activation in systemic anaphylaxis and mastocytosis. NEJM 1987; 316: 1622-6

68. Schwartz LB. Diagnostic value of tryptase in anaphylaxis and mastocytosis. Immunol Allergy Clin N Am 2006; 26: 451-63

69. Castells MC, Irani AM, Schwartz LB. Evaluation of human peripheral blood leukocytes for mast cell tryptase. Journal of Immunology 1987; 138: 2184-89

70. Jogie-Brahim S, Min HK, Fukuoka Y, Xia HZ, Schwartz LB. Expression of α-tryptase and β-tryptase by human basophils. J Allergy Clin Immunol 2004; 113: 1086-92

71. Schwartz LB, Yunginger JW, Miller J, Bokhari R, Dull D. Time course and disappearance of human mast cell tryptase in the circualtion after anaphylaxis. J Clin Invest 1989; 83: 1551-5

72. Dybendal T, Guttormsen AB, Elsayed S, Askeland B, Harboe T, Florvaag E. Screening for mast cell tryptase and serum IgE antibodies in 18 patients with anaphylactic shock during general anaesthesia. Acta Anaesthesiol Scand 2003; 47: 1211-18

73. Laroche D, Vergnaud MC, Sillard B, Soufarapis H, Bricard H. Biochemical markers of anaphylactoid reactions to drugs. Comparison of plasma histamine and tryptase. Anesthesiology 1991; 75: 945-9

74. Fisher MM, Baldo BA. Mast cell tryptase in anaesthetic anaphylactoid reactions. Br J Anaesth 1998; 80: 26-9

75. Payne V, Kam PCA. Mast cell tryptase: a review of its physiology and clinical significance. Anaesthesia 2004; 59: 695-703

76. Kastner M, Harada L, Waserman S. Gaps in anaphylaxis management at the level of physicians, patients and the community: a systematic review of the literature. Allergy 2010; 65: 435-44

77. Hove LD, Steinmetz J, Krogh Christoffersen J, Møller A, Nielsen J, Schmidt H. Analysis of deaths related to anesthesia in the period 1996-2004 from closed claims registered by the Danish Patient Insurance Association. Anesthesiology 2007; 106: 675-80

78. Cranshaw J, Gupta KJ, Cook TM. Litigation related to drug errors in anaesthesia: An analysis of claims against the NHS in England 1995-2007. Anaesthesia 2009; 64:1317-23

79. Soar J, Pumphrey R, Cant A, Clarke S, Corbett A, Dawson P, Ewan P, Foëx B, Gabbott D et al. Statement paper. Emergency treatment of anaphylactic reactions – Guidelines for healthcare providers. Resuscitation 2008; 77: 157-9

80. Currie M, Kerridge RK, Bacon AK, Williamson JA. Crisis management during anaesthesia: anaphylaxis and allergy. Qual saf Health Care 2005; 14:e19

81. Ring J, Messmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. Lancet 1977:1:466-9

82. Galletly DC, Treuren BC. Anaphylactoid reactions during anaesthesia. Seven years' experience of intradermal testing. Anaesthesia 1985; 40: 329-33

83. Laxenaire MC, Mertes PM. Anaphylaxis during anaesthesia. Results of a two-year survey in France. Brit J Anaesth 2001; 87: 549-58

84. Mertes PM, Laxenaire MC, Alla F. Anaphylactic and anaphylactoid reactions occuring during anaesthesia in France in 1999-2000. Anesthesiology 2003; 99:536-45

85. Fisher MM, Baldo BA. Immunoassays in the diagnosis of anaphylaxis to neuromuscular blocking drugs. The value of morphine for the detection of IgE antibodies in allergic subjects. Anaesth Intens Care 2000; 28:167-70

86. Laroche D, Lefrancois C et al. Early diagnosis of anaphylactic reactions to neuromuscular blocking drugs. British journal of Anaesthesia 1992; 69: 611-614

87. Guttormsen AB, Johansson SG, Oman H, Wilhelmsen V, Nopp A. No consumption of IgE antibody in serum during allergic drug anaphylaxis. Allergy 2007; 62: 1326-30

88. Poothullil J, Shimizu A, Day RP, Dolovich J. Anaphylaxis from the product(s) of ethylene oxide gas. Ann Intern Med 1975; 82: 58-60

89. Opstrup-Petersen M, Mosbech H, Garvey LH. Allergic sensitization to ethylene oxide in patients with suspected allergic reactions during anaesthesia and surgery. Accepted Journal of investigational allergology and clinical immunology May-June 2010;20:

90. Blanca M, Garcia J, Vega JM, Miranda A, Carmona MJ, Mayorga C, et al. Anaphylaxis to penicillins after non-therapeutic exposure: an immunological investigation. Clin Exp Allergy 1996; 26: 335-40

91. Mosbech H, Christensen J, Dirksen A, Søborg M. Insect allergy. Predictive value of diagnostic tests: a three-year follow-up study. Clin Allergy 1986; 16: 433-40

92. Nagendran V, Wicking J, Ekbote A, Onyekwe T, Garvey LH. IgE-mediated chlorhexidine allergy: a new occupational hazard? Occup Med 2009;59:270-2

93. Leynadier F, Sansarricq M, Didier JM, Dry J. Prick tests in the diagnosis of anaphylaxis to general anaesthetics. Br J Anaesth 1987; 59: 683-9

94. Wood M, Watkins J, Wild G, Levy CJ, Harrington C. Skin testing in the investigation of reactions to intravenous anaesthetic drugs. A prospective trial of atracurium and tubocurarine. Ann FrAnaesth Reanim 1985; 4: 176-9.

95. Brockow K, Romano A. Skin tests in the diagnosis of drug hypersensitivity reactions. Current pharmaceutical design 2008; 14: 2778-91

96. Malling HJ, Andersen CE, Boas MB, Holgersen F, Munch EP, Weeke B. The allergy pricker. Qualitative aspects of skin prick testing using a precision needle. Allergy 1982; 37: 563-567

97. Nelson HS, Lahr J, Buchmeier A, McCormick D. Clinical aspects of allergic disease. Evaluation of devices for skin prick testing. J Allergy Clin Immunol 1998; 101: 153-6

98. Malling HJ. Skin prick testing and the use of histamine references. Allergy 1984;39:596-601

99. Moneret-Vautrin DA, Laxenarie MC. Skin tests in the diagnosis of allergy to muscle relaxants and other anesthetic drugs. Assem ESK (ed): Allergic reactions to anaesthetics. Clinical and basic aspects. Monogr Allergy. Basel, Karger 1992; 30: 145-55

100. Mertes PM, Moneret-Vautrin DA, Leynadier F, Laxenaire MC. Skin reactions to intradermal neuromuscular blocking agent injections. A randomized multicenter trial in healthy volunteers. Anesthesiology 2007; 107: 245-52

101. Bagg A, Chacko T, Lockey R. Reactions to prick and intradermal skin tests. Ann Allergy Asthma Immunol 2009; 102: 400-2

102. Liccardi G, D'Amato G, Canonica GW, Salzillo A, Piccolo A, Passalacqua G. Systemic reactions from skin testing: literature review. J Investig Allergol Clin Immunol 2006; 16:75-8

103. Aberer W, Bircher A, Romano A, Blanca M, Campi P, Fernandez J, Brockow K, Pichler WJ, Demoly for ENDA and the EAACI interest group on drug hypersensitivity. Position paper. Drug provocation testing in the diagnosis of drug hypersensitivity reactions: general considerations. Allergy 2003; 58: 854-63

104. Poulsen LK, Quan S, Krag C, Platzer MH, Skov PS. The basophil granulocyte in allergic reactions: experimental models and their use for the identification of drugs with effects or side effects on basophils. Curr med Chem – Anti-inflammatory & Anti-allergy Agents 2004; 3: 167-80

105. Svanström MC, Biber B, Hanes M, Johansson G, Näslund U, Bålfors EM. Signs of myocardial ischaemia after injection of oxytocin: a randomized double-blind comparison of oxytocin and methylergometrine during Caesarean section. BJA 2008; 100: 683-9

106. Langesæter E, Rosseland LA, Stubhaug A. Haemodynamic effects of repeated doses of oxytocin during Caesarean delivery in healthy parturients. BJA 2009; 103: 260-2

107. Ljungström KG, Revenäs B, Smedegård G, Hedin H, Richter W, Saldeen T. Histopathological lung changes in immune complex mediated anaphylactic shock in humans elicited by dextran. Forensic Science International 1988; 38: 251-58

108. Ruëff F, Przybilla B, Biló MB, Müller U, Scheipl F, Aberer W, Birnbaum J, Bodzenta-lukaszyk A et al. Predictors of severe systemic anaphylactic reactions in patients with hymenoptera venom allergy: Importance of baseline serum tryptase – a study of the European Academy of allergology and Clinical immunology Interest Group on insect venom hypersensitivity. J Allergy Clin Immunol 2009; 124: 1047-54

109. Bonadonna P, Perbellini O, Passalacqua G, Caruso B, Colarossi S, Dal Fior D, Castellani L, Bonetto C, Frattini F, Dama A, Martinelli G, Chilosi M, Senna G, Pizzolo G, Zanotti R. Clonal mast cell disorders in patients with systemic reactions to Hymenoptera stings and increased serum tryptase levels. J Allergy Clin Immunol 2009; 123: 680-6

110. Nienartowicz A, Sobaniec-Lotowska ME, Jarocka-Cyrta E, Lemancewicz D. Mast cells in neoangiogenesis. Med Sci Monit 2006; 12: RA53-6

111. Fiorucci L, Ascoli F. Mast cell tryptase, a still enigmatic enzyme. Cell Mol Life Sci 2004; 61: 1278-95

112. McNeil HP, Adachi R, Stevens RL. Mast cell-restricted tryptases: Structure and function in inflammation and pathogen defense. J Bio Chem 2007; 282: 20785-9

113. Schwartz LB, Sakai K, Bradford TR, Ren S, Zweiman B, Worobec AS, Metcalffe DD. The α form of human tryptase is the predominant type present in blood at baseline in normal subjects and is elevated in those with systemic mastocytosis. J Clin Invest 1995; 96: 2702-10

114. Sperr WR, El-Samahi A, Kundi M, Girschikofsky M, Winkler S, Lutz D, Endler G, Rumpold H, Agis H, Sillaber C, Jäger U, Valent P. Elevated tryptase levels selectively cluster in myeloid neoplasms: a novel diagnostic approach and screen marker in clinical haematology. Eur J Clin Invest 2009; 39: 914-23

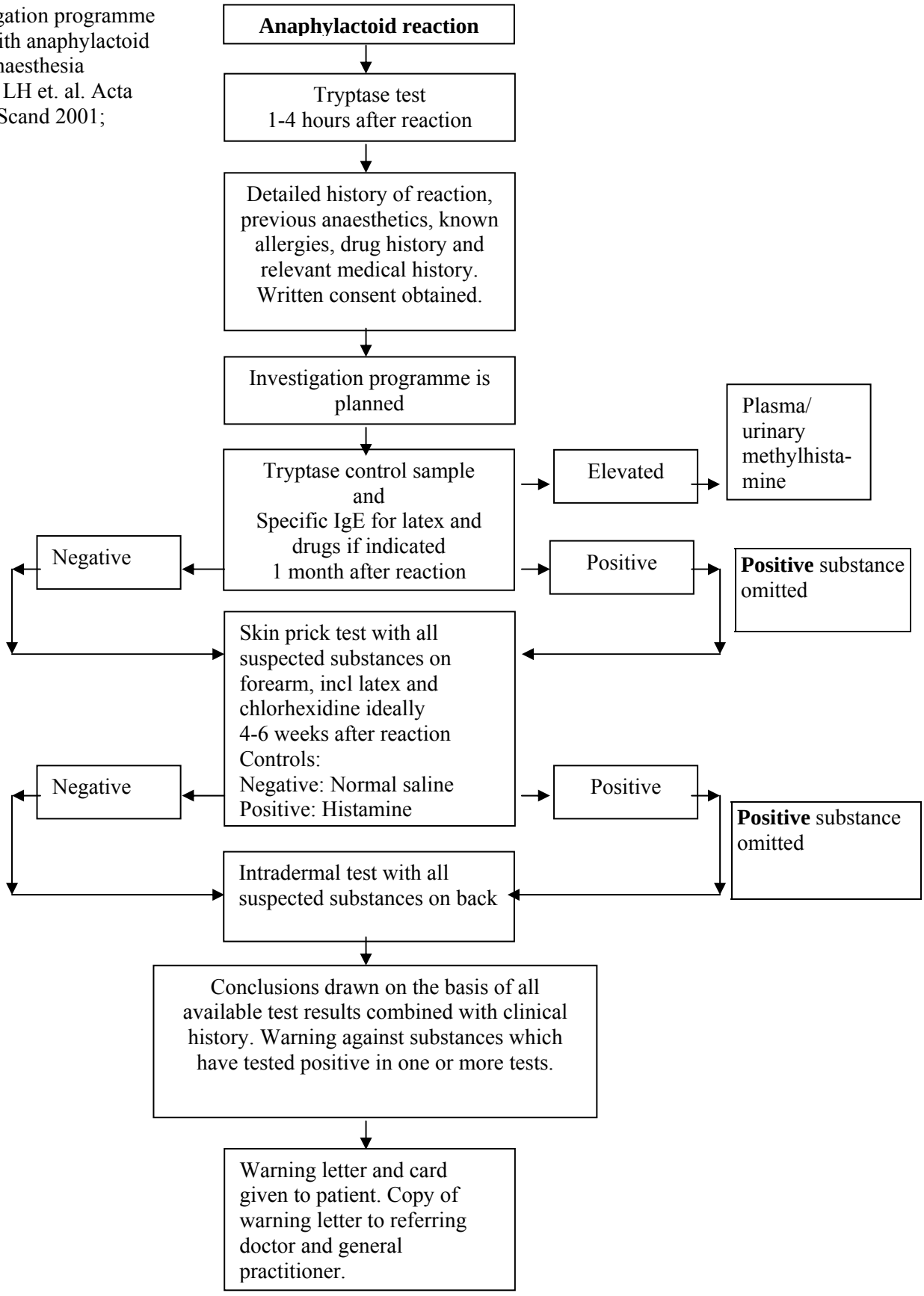
115. Johnston SL, Unsworth J, Gompels MM. Adrenaline given outside the context of life threatening allergic reactions. BMJ 2003; 326: 589-90

116. Wheeler DW, Carter JJ, Murray LJ, Degnan BA, Dunling CP, Salvador R, Menon DK, Gupta AK. The effect of drug concentration expression on epinephrine dosing errors. Ann Intern Med 2008; 148: 11-14

Appendices

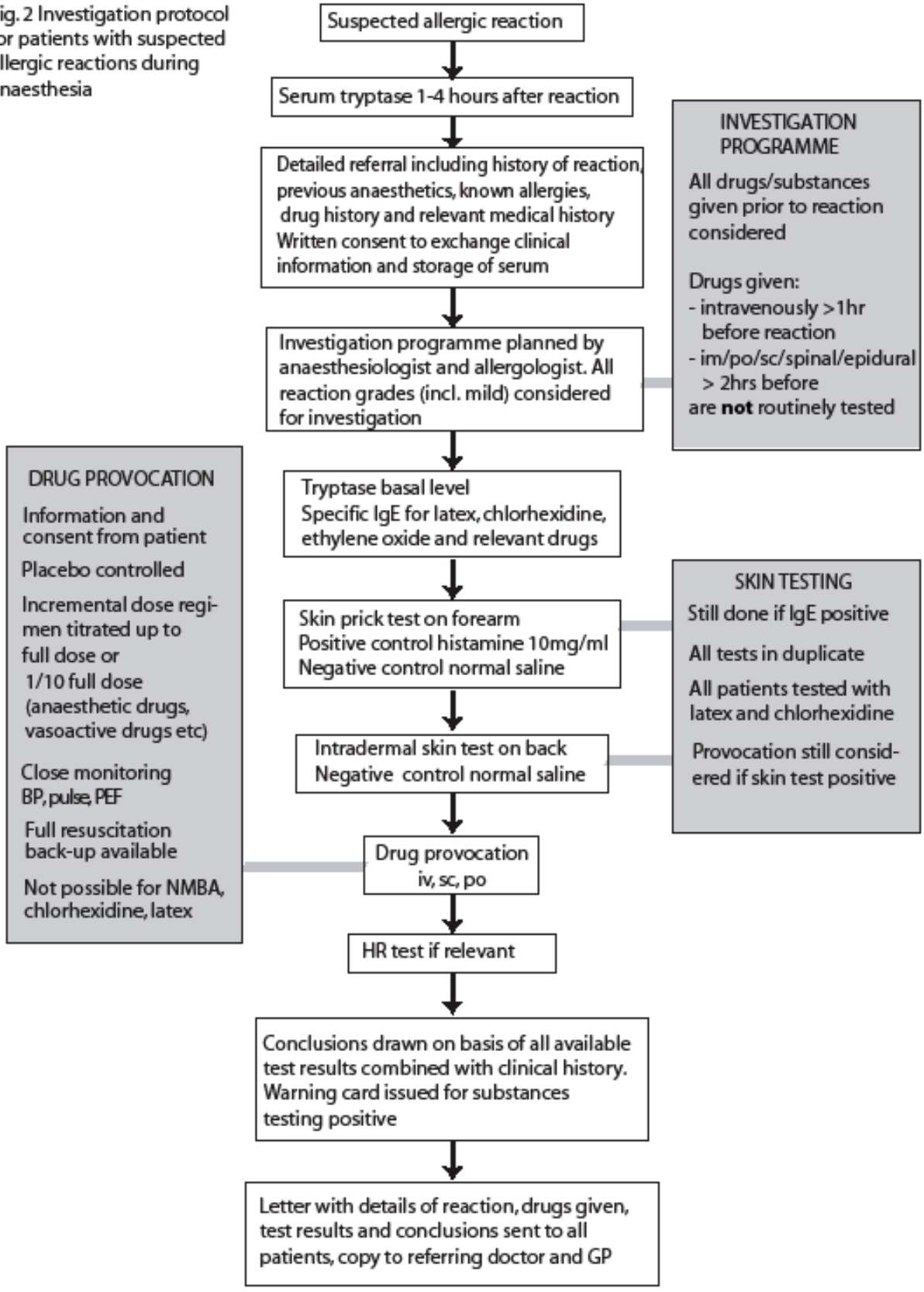
Appendix 1 DAAC investigation protocol 1999-2003

Fig. 1 Investigation programme for patients with anaphylactoid reactions in anaesthesia (from Garvey LH et. al. Acta Anaesthesiol Scand 2001; 45:1290-94)



Appendix 2 DAAC Investigation protocol 2004 -

Fig. 2 Investigation protocol for patients with suspected allergic reactions during anaesthesia



Papers

I. Garvey LH, Krøigaard M, Poulsen LK, Skov PS, Mosbech H, Venemalm L, Degerbeck F, Husum B. IgE-mediated allergy to chlorhexidine. *J Allergy Clin Immunol* 2007; 120: 409-415

II. Garvey LH, Bech B, Mosbech H, Krøigaard M, Belhage B, Husum B, Poulsen LK. Effect of general anesthesia and orthopedic surgery on serum tryptase. *Anesthesiology* 2010; 112: 1184-9

III. Garvey LH, Belhage B, Krøigaard M, Husum B, Poulsen LK, Mosbech H. Serum tryptase in patients with suspected allergic reactions during anaesthesia – ten years' experience from the Danish Anaesthesia Allergy Centre. *Submitted*

IV. Garvey LH, Belhage B, Krøigaard M, Husum B, Malling HJ, Mosbech H. Treatment with epinephrine (adrenaline) in suspected anaphylaxis during anesthesia in Denmark. *Submitted*

Paper I

IgE-mediated allergy to chlorhexidine

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Background: Investigations at the Danish Anesthesia Allergy Centre have included testing for allergy to chlorhexidine since 1999.

Objective: To investigate whether measurement of IgE and histamine release confirm an IgE-mediated mechanism for chlorhexidine allergy.

Methods: Twenty-two patients with clinical history suggestive of chlorhexidine allergy were included. Skin tests with chlorhexidine and tryptase measurements were performed during initial investigations. Sera were analyzed retrospectively for IgE and histamine release (passive sensitization) to chlorhexidine.

Results: Twelve patients were skin test positive and 10 were skin test negative. Of the skin test–positive patients, 11 of 12 had IgE to chlorhexidine and 7 of 11 had a positive histamine release test. None of the skin test–negative patients had specific IgE or positive histamine release to chlorhexidine. Skin test–positive patients had higher median age (64 vs 49 y) and were mainly male (11/12 vs 6/10). In both groups, 8 patients had hypotension, but bronchospasm mainly appeared in skin test–negative patients (1/12 vs 6/10). Reactions occurred more often during urologic surgery in skin test–positive patients (5/12 vs 0/10). Baseline tryptase was higher in skin test–positive patients (median, 11.5 vs 3.7 μg/L), and 6 of 7 patients had elevated IgE to chlorhexidine in serum at the time of reaction.

Conclusion: This study confirms that chlorhexidine allergy is IgE-mediated and that measurement of specific IgE and histamine release are good adjuncts to skin testing in patients with clinical history suggesting chlorhexidine allergy.

Clinical implications: IgE and histamine release can be used to support the diagnosis of allergy to chlorhexidine. (J Allergy Clin Immunol 2007;120:409-15.)

Key words: *Chlorhexidine, anaphylaxis, allergy, specific IgE, skin prick testing, intradermal testing, histamine release, anesthesia, drug allergy*

Abbreviations used
DAAC: Danish Anaesthesia Allergy Centre
HR: Histamine release
IDT: Intradermal test
STN: Skin test negative
STP: Skin test positive

The Danish Anaesthesia Allergy Centre (DAAC) has systematically investigated patients with suspected anaphylaxis during anesthesia since 1999. Patients are referred from all of Denmark and undergo standardized investigations with all drugs and substances they were exposed to during surgery and anaesthesia.[1] In Denmark, all patients are in contact with latex and chlorhexidine during surgery and anesthesia (unless allergy is suspected), and all patients referred to the DAAC are thus tested with these substances. Allergy to latex is thoroughly described in the literature, but allergy to chlorhexidine is less well known. Chlorhexidine digluconate is a disinfectant widely used in patients' homes and during contact with health services in Denmark and other countries. In the home, it is found in products such as mouthwash, toothpaste, plasters and dressings, ointments, and suppositories, and it is available as an over-the-counter solution for disinfection of minor cuts and wounds. In the health services, it is used in swabs for disinfection before venepuncture and intravenous cannulation. It is the standard skin disinfectant used before invasive procedures such as epidural/spinal anesthesia and before surgical incision. It is also found in a number of lubricant gels used in urinary catheterization and vaginal and rectal examination. Chlorhexidine diacetate is used as a preservative in products such as antacid preparations, contact lens fluid, and cosmetics and has also been used in commercial food handling. Exposure to chlorhexidine is thus extensive, but the incidence of allergy to chlorhexidine is largely unknown. Reactions to chlorhexidine have been reported in patients from all over the world since 1962[2] and range from contact dermatitis to anaphylactic shock with cardiac arrest.[3] Our group has previously published 4 cases of chlorhexidine allergy in association with anesthesia.[4] Skin testing has been the main diagnostic tool in chlorhexidine allergy,[1,5] and positive reactions suggest an IgE-mediated mechanism. This has been suggested previously, but only on the basis of single patients[6] or small groups of patients.[7-9] In this study, we investigate whether measurement of specific IgE and histamine release (HR) can confirm an IgE-mediated mechanism for allergy to

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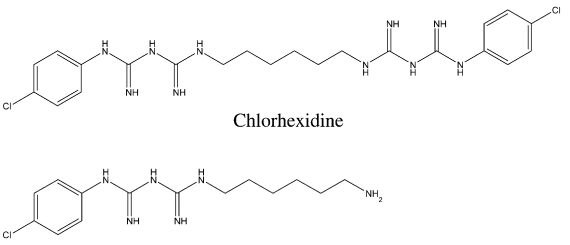

FIG 1. Molecular structure of chlorhexidine and the monovalent antigen used for binding IgE antibodies to the chlorhexidine ImmunoCAP.

chlorhexidine. In addition, we discuss the characteristics of patients with allergy to chlorhexidine.

METHODS

Patients

From 1999 to 2005, a total of 174 patients were investigated at the DAAC because of suspected allergic reactions in connection with anesthesia and surgery. All underwent standardized skin testing with all drugs and substances to which they had been exposed, including latex and chlorhexidine digluconate, before the suspected allergic reaction. Most patients had a serum tryptase sample drawn within the recommended interval of 1 to 4 hours after the allergic reaction, and all patients had a baseline tryptase sample drawn at a time when no allergic symptoms were present, typically 4 to 6 weeks after the reaction.

Twelve patients had a positive skin test result with chlorhexidine digluconate, and the remaining 162 patients tested negative. At the time of referral, allergy to chlorhexidine had been strongly suspected in the 12 skin test–positive patients and in 10 of the 162 skin test–negative patients because of the clinical picture at the time of reaction. These 22 patients were then retrospectively included in this study on the basis of one or both of the following criteria: (1) history of repeated allergic reactions in connection with anesthesia and/or surgical procedures and (2) allergic reaction occurring >10 minutes after induction of anesthesia, at the end of surgery, or postoperatively. At the time of skin testing, all patients had consented to storage of serum samples for future allergologic analysis.

Both measurement of IgE for chlorhexidine (ImmunoCAP; Phadia AB, Uppsala, Sweden) and HR-test (Reflab, Copenhagen, Denmark) have since 2005 been part of the standard investigations performed on all patients in the DAAC. Because IgE for chlorhexidine was made available only in early 2005, it was, for the purposes of this study, measured retrospectively, together with HR, on sera taken in connection with initial investigations at the DAAC.

For a number of patients, sera from several dates were available, all were analyzed, and the results from the serum sample drawn closest to the allergic reaction was used, except when looking at changes in chlorhexidine IgE over time.

Specific IgE against chlorhexidine

The chlorhexidine ImmunoCAP was prepared by covalent coupling of 1-[N^5-(*p*-chlorophenyl)biguanido]-6-aminohexane[10] to the cyanogen bromide-activated cellulose sponge. 1-[N^5-(*p*-chlorophenyl)biguanido]-6-aminohexane represents ½ of the symmetrical bivalent chlorhexidine molecule (Fig 1) and contains a primary amino group that facilitates immobilization to the solid phase.

IgE antibodies in sera from patients with suspected allergy to chlorhexidine were measured with ImmunoCAP 100 according to the directions for use (Phadia AB). A positive result was defined as a value greater than or equal to 0.35 kU_A/L.

The specificity of binding of IgE antibodies to the chlorhexidine ImmunoCAP was demonstrated by inhibition experiments. Varying concentrations of chlorhexidine digluconate were added to patient serum, and a 50% inhibition of the assay response was achieved at a chlorhexidine concentration of 0.3 μmol/L. An immunologically unrelated substance, chloramine T, gave no inhibition even at 44 mmol/L.

Chlorhexidine-induced HR

Histamine release to chlorhexidine was measured after passive sensitization of IgE stripped basophils with patients' sera. Buffy coat blood samples (from the blood bank) were used for the sensitization. Cells were screened and selected for the capability to elicit an anti-IgE response (HR > 30%) but with no HR reactivity toward 10 common inhalant allergens and 10 food allergens and no response to chlorhexidine. Sensitization of stripped basophils was performed as described previously.[11] Peripheral blood mononuclear cells (PBMCs) from the selected buffy coat were isolated by Lymphoprep gradient centrifugation (Nycomed, Oslo, Norway) and contained 1% to 2% basophils. Cell-bound IgE was removed by washing the PBMCs in a phosphate buffer (pH 3.55). Stripped basophils were then sensitized with patient sera and serum from a healthy nonallergic control. The passively sensitized cells were incubated with chlorhexidine digluconate in the following 6 final concentrations: 0.005%, 0.0014%, 0.0005%, 0.00014%, 0.00005%, and 0.000014%. HR was determined by the glass fiber method (HR-test) according to the manufacturer's standard procedure.[12] Results were expressed in percentage of total cellular histamine content, and a HR > 10% was considered a positive response. HR responses were classified according to the lowest sample dilution inducing > 10% HR. This classification implies that cells responding to the lowest concentration is a class 6 reaction and cells only responding to the highest concentration is a class 1 reaction. No reaction to any dilution is a class 0 reaction. Chlorhexidine in concentrations above 0.005% induced unspecific HR in healthy controls, and these concentrations were therefore not applied.

Skin testing

Skin testing was performed during initial investigations in the DAAC following a standardized investigation program. Skin testing was performed with chlorhexidine digluconate, because this is more widely used than chlorhexidine diacetate. A prick test with chlorhexidine digluconate was performed in all cases and intradermal test (IDT) was, in the first patients, only performed if the prick test result was negative. Prick tests were performed on the forearm with chlorhexidine digluconate 0.5%, a normal saline negative control, and a histamine positive control (Soluprick histamine 10 mg/mL; ALK-Abelló, Hørsholm, Denmark). Development of a wheal greater than ½ the diameter of the wheal of the positive control, in the presence of a negative reaction to the saline control, was considered positive.[13] In practice, this usually meant a wheal diameter of 3 mm or greater.

Intradermal test with chlorhexidine digluconate 0.0002% was performed on the back or forearm with a normal saline negative control. IDT was considered positive if a wheal greater than or equal to twice the diameter of the induced bleb developed, with surrounding flare.[13] In practice, the diameter of the bleb induced was usually 4 mm, and thus, positive tests usually had a wheal diameter of 8 mm or greater.

Concentrations used for skin testing are based on a large number of negative controls. We have > 800 negative controls to prick test with 0.5% chlorhexidine gluconate and > 300 negative controls to IDT with 0.0002% chlorhexidine gluconate (DAAC, unpublished data, January 2007).

Statistics

Data were analyzed for statistical significance using the Student unpaired *t* test. Probability values of < .05 were considered statistically significant.

RESULTS

Results of initial investigations at the DAAC

Twenty-two patients were included in this study on the strong clinical suspicion of allergy to chlorhexidine because of repeated or delayed reactions, and results of their initial investigations at the DAAC are shown in Table I. Skin test results for chlorhexidine (prick test or IDT or both) were positive in 12 patients (STP) and negative in 10 patients (STN). Ten of 12 in the STP group were positive in prick test, and 6 of 7 presented a positive IDT. Two patients who had a negative prick test had a positive IDT.

Five patients in the STN group tested positive to other substances such as latex, penicillin, mivacurium, and pancuronium, and 5 tested negative to all substances. Ten patients in the STP group tested negative to all substances other than chlorhexidine, and 2 had additional positive test results to morphine and penicillin (Table II). Both patients had repeated reactions on exposure to chlorhexidine without exposure to the other allergen.

Serum samples were analyzed for tryptase during initial investigations at the DAAC (Table I). Samples were taken at the time of reaction in 8 of 12 in the STP group, median 32.8 μg/L (range, 11.3-155 μg/L), and in 7 of 10 patients in the STN group, median 33.8 μg/L (range, 3.8-65.1 μg/L; $P = .41$). All patients had a baseline tryptase sample taken at a time with no allergic symptoms, and the median values were 11.5 μg/L (range, 3.79-42.3 μg/L) and 3.7 μg/L (range, 1.57-22.1 μg/L) for the STP group and the STN group, respectively ($P = .07$; Fig 2).

TABLE I. Initial investigations at the DAAC

	Skin testing		Tryptase μg/L	
Patient no.	**SPT**	**IDT**	**At baseline**	**At reaction**
STP patients				
P1	pos	ND	18.8	ND
P2	pos	ND	14.1	155
P3	neg	pos	18.9	ND
P4	pos	pos	29.4	34.1
P5	neg	pos	6.51	ND
P6	pos	ND	6.26	11.3
P7	pos	ND	3.79	ND
P8	pos	pos	7.57	58.1
P9	pos	pos	4.4	23.5
P10	pos	ND	11.6	21.7
P11	pos	neg	11.4	52.1
P12	pos	pos	42.3	31.4
STN patients				
C1	neg	neg	2.93	30.8
C2	neg	neg	5.55	46
C3	neg	neg	9.25	10.7
C4	neg	neg	1.57	33.8
C5	neg	neg	4.11	3.8
C6	neg	neg	3.34	36.8
C7	neg	neg	22.1	65.1
C8	neg	neg	1.99	ND
C9	neg	neg	3.18	ND
C10	neg	neg	13.6	ND

ND, Not done; *neg*, negative; *pos*, positive; *SPT*, skin prick test.

Results of specific IgE against chlorhexidine and HR-test

Specific IgE for chlorhexidine was positive in 11 of 12 in the STP group (Fig 3), and HR-test was positive in 7 of 11 in the STP group (Fig 4). One patient did not have a HR-test because of lack of serum. Ten of 10 patients in the STN group had negative specific IgE and HR-test for chlorhexidine, giving a specificity of 100% for both tests in this study. A further 80 patients investigated at the DAAC in 2005 to 2006 with negative skin test results for chlorhexidine digluconate had negative IgE for chlorhexidine, confirming the high specificity of this tests (DAAC, unpublished data, January 2007).

When serial samples were drawn, a decline in IgE levels and HR over time could be demonstrated, and in 1 patient, the rate of decline was seen to be almost identical in the 2 tests (Fig 5, *A*). Median time from reaction to IgE sampling (most distant sample) was 3 months (range, 0.5-7 months) in the STN group versus 4.75 months (range, 0.4-24.5 months) in the STP group.

Initial level and rate of decline of IgE showed great variability between patients, and IgE levels were raised in the sample taken at the time of the reaction in 6 of 7 cases with levels varying between 0.39 and 93.9 kU_A/L (Fig 5, *B*).

Patients and clinical features

Details about patients and clinical features of reactions can be seen in Table II. There appeared to be more males in the STP group than in the STN group, 11 of 12 versus 6 of 10, but this difference was not statistically significant. Median age was higher in the STP group (64 vs 49 y; $P = .03$), and there was no difference in IgE to inhalant allergens (Phadiatop; Phadia AB) with 3 of 12 patients in the STP group versus 5 of 9 in the STN group. One patient in the STN group was not tested because of lack of serum.

The severity of reactions was similar with 9 of 12 and 8 of 10 having severe anaphylaxis in the 2 groups, respectively, but symptoms varied. In both groups, 8 patients had hypotension during the reaction, whereas bronchospasm was present in only 1 of 12 in the STP group versus 6 of 10 in the STN group ($P = .008$). Allergic reactions occurred during urologic surgery or procedures in 5 of 12 patients in the STP group versus 0 of 10 in the STN group ($P = .02$).

DISCUSSION

This study confirms an IgE-mediated mechanism of allergy to chlorhexidine in 12 patients, the largest group of patients reported so far. We have retrospectively analyzed serum samples from patients with allergic reactions during

TABLE II. Patients and clinical features

		Reaction		Operation	Phadiatop	
Sex	Age (y)	Class*	Symptoms	Type of surgery	Class	Additional allergen
STP patients						
M	85	2	H, T, F/U	Prostate resection	0	Other tests negative
M	51	3	H, B	Cystectomy	0	Other tests negative
M	70	3+	CA, F/U	Cystoscopy	0	Penicillin
M	62	3	H, T, F/U	Hemicolectomy	4	Other tests negative
F	54	1	F/U, I	Hysterectomy	0	Other tests negative
M	39	3	H, D, F/U	Melanoma resection	2	Morphine
M	66	3	H, F/U	Prostate resection	0	Other tests negative
M	59	3+	B, CA, F/U, E	Laparotomy	0	Other tests negative
M	55	3	H, T, F/U	Skin cancer resection	0	Other tests negative
M	73	3	H, B	Bladder resection	0	Other tests negative
M	78	3	H, BR, I	Peripheral bypass	1	Other tests negative
M	72	2	F/U, I, E	Saphenous vein varices	0	Other tests negative
STN patients						
F	46	3+	H, B, BR, A	Appendectomy	4	Mivacurium, pancuronium
M	56	3	H, F/U	Heart surgery	0	Swan Ganz catheter
M	48	3	H, T, F/U, BR	Heart surgery	3	Latex, penicillin
M	66	3	H, T, BR	Aortic aneurysm repair	4	Latex
F	46	3	H, BR	Breast reconstruction	3	All tests negative
M	49	3	H, BR	Sigmoid resection	0	All tests negative
M	18	3	H, T, F/U, E	Laparotomy	2	Latex
M	59	2	A	Vascular surgery	0	All tests negative
F	18	1	F/U, I, E	Laparoscopy	0	All tests negative
F	76	3	H, B, BR	Rectum resection	ND	All tests negative

*Class 1, mild reactions resolving spontaneously; class 2, moderate reactions; class 3, severe reactions with prolonged treatment; class 3+, cardiac arrest.
A, Angioedema; *B*, bradycardia; *BR*, bronchospasm; *CA*, cardiac arrest; *D*, desaturation; *E*, edema; *F*, female; *F/U*, flushing/urticaria; *H*, hypotension; *I*, itching; *M*, male; *T*, tachycardia.

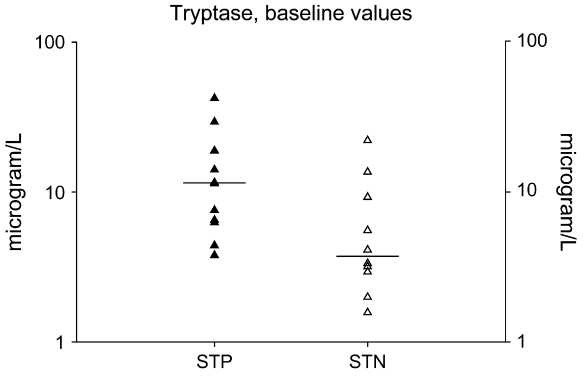

FIG 2. Plasma tryptase baseline values for patients with positive (*STP*) and negative (*STN*) skin test results for chlorhexidine.

anesthesia and surgery and examined the quality of the specific IgE analysis and the HR-test (passive sensitization) in 12 patients with chlorhexidine allergy verified by positive skin test results. We also analyzed serum samples from 10 patients with similar clinical presentation, but with negative skin test results to chlorhexidine, disproving allergy to chlorhexidine. There were no positive IgE or HR-test results in the STN group, and the specificity in this study was thus 100% for both tests. The apparently lower sensitivity of HR compared with IgE may be a result of 2 factors: (1) HR induced by chlorhexidine on passively sensitized basophils may have a reduced sensitivity compared with HR from the patient's own basophils, and (2) because of unspecific HR in concentrations above 0.005% of chlorhexidine, we were unable to test these higher concentrations, which might mean that patients with low sensitivity to chlorhexidine will have negative HR.

IgE values at the time of the reaction showed great individual variation, and levels showed decline over time with varying rate, not related to initial IgE level or severity of reaction. The recommended interval for sampling IgE is within 6 months of the reaction, and median times for IgE sampling in our study were within this time interval for both the STP and the STN group. A recent study of IgE to chlorhexidine measured by a prototype of the ImmunoCAP in patients with positive prick test results to chlorhexidine digluconate had a lower yield of positive IgE tests, but sera were taken an average of 29 months after the reaction.[14] A decline in the IgE antibody response to chlorhexidine has previously been measured in 1 patient,[8] and the same has been described for other allergens such as penicillin[15] and insect venom.[16] In this study, serial analyses for both IgE and HR in 1 patient showed an almost identical decline over time (Fig 5, *A*). Another patient, who was re-exposed to chlorhexidine several times before referral to the DAAC, maintained an IgE level above normal, not showing the gradual decline seen in

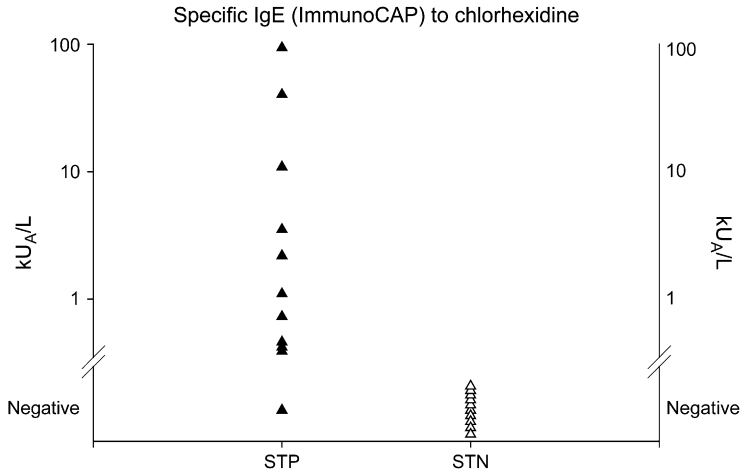

FIG 3. Results of specific IgE (ImmunoCAP) to chlorhexidine for patients with positive (*STP*) and negative (*STN*) skin test results for chlorhexidine. Values < 0.35 KU_A/L are negative.

other patients, presumably because of continued stimulation of IgE production (patient 9; Fig 5, *B*).

Previous studies have looked at the mechanism of chlorhexidine allergy, trying to elucidate what constitutes the allergen.[6,8,17,18] Chlorhexidine has a molecular weight of 505 d and is a symmetrical bis-biguanide with p-chlorophenyl end-groups, causing some authors to suggest that the entire chlorhexidine molecule constitutes the allergen.[6,18] The symmetrical structure of the chlorhexidine molecule (Fig 1) makes it possible, however, that the chlorguanide sites on both ends of the molecule could constitute allergenic sites by themselves, and inhibition studies suggest this.[8] Some authors speculate that the allergenic determinants could show structural heterogeneity, as is the case with antibiotics, for example.[6]

This could have implications for how sensitization to chlorhexidine occurs and what degree of exposure is needed for sensitization to occur. One group looked at induction of IgG antibodies to chlorhexidine in mice[19] and later found that IgG antibodies were present in both Japanese and British nurses.[7] They concluded that these antibodies had no clinical relevance and that the incidence of chlorhexidine allergy was decreasing. No work has been published in this area since.

In Denmark, chlorhexidine allergy constitutes a small but serious problem, especially in certain patient groups, and further studies are needed to try to elucidate the mechanisms of sensitization.

Since 1999 when we first suspected anaphylaxis to chlorhexidine in a patient, we have noticed, from own experiences and from the literature, that there are several features characterizing this patient group. These findings have become clearer in this study and may help identify high-risk patients and prevent future reactions to chlorhexidine.

It appears that most patients with allergy to chlorhexidine are males, and only few have atopy as defined by positive IgE to inhalational allergens (Phadiatop), but these findings

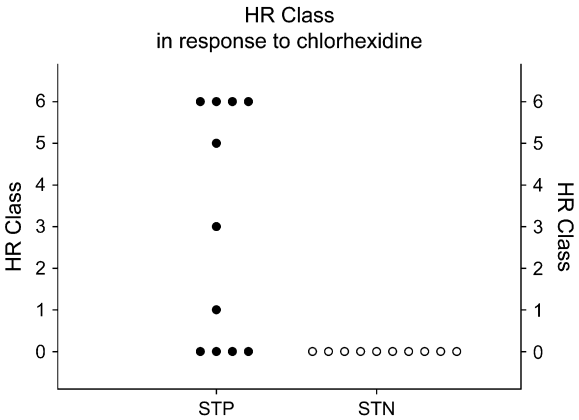

FIG 4. Results of HR-tests for patients with positive (*STP*) and negative (*STN*) skin test results for chlorhexidine.

were not statistically significant in this study. Median age, however, was higher in chlorhexidine-positive patients, and this finding was statistically significant.

On direct questioning, most chlorhexidine-positive patients have had previous mild reactions on exposure to chlorhexidine such as rash, swelling, itching, or generalized exanthema, and this has also been reported previously.[20-23] It has been reported that delayed-type hypersensitivity reactions and immediate-type reactions can be seen in the same patient.[4,24,25] This supports the need for investigation of patients with mild reactions on exposure to chlorhexidine to prevent future severe reactions.

In this study, severity of reactions was similar in the 2 groups, but symptoms varied, with hypotension occurring in most patients in both groups, whereas bronchospasm mainly occurred in the STN group. This was statistically significant, and the reasons for this finding are not clear.

Nearly half of reactions occurred during urologic surgery or procedures in the STP group, whereas this type of surgery did not feature in the STN group. This

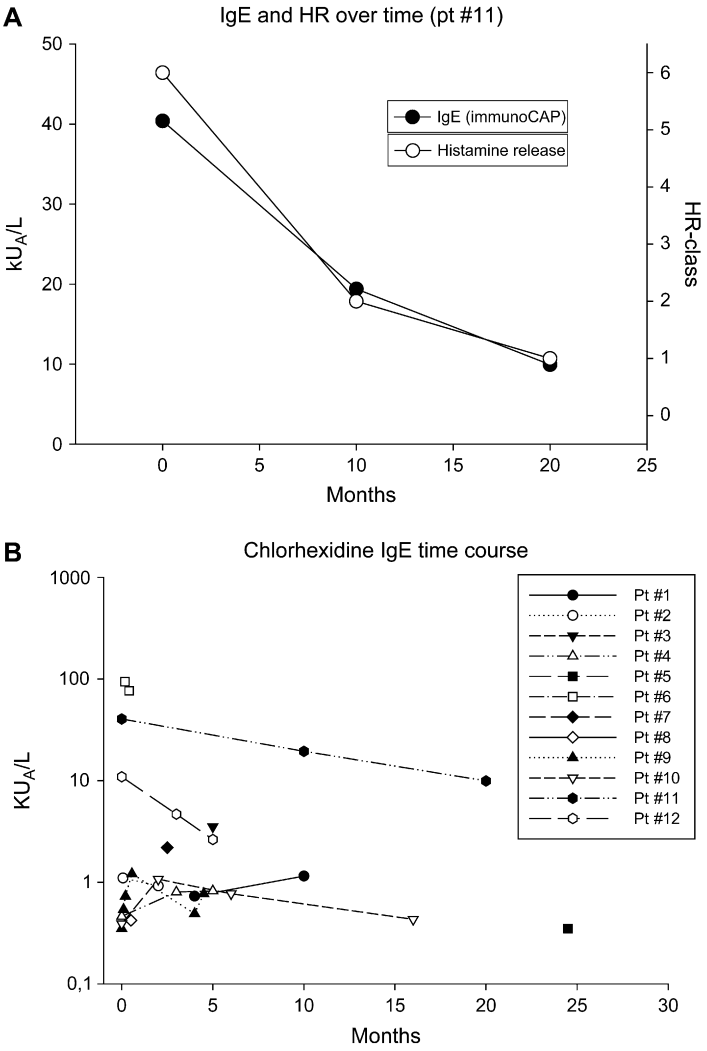

FIG 5. A, Specific IgE and HR values over time for patient with positive skin test results to chlorhexidine. **B,** Specific IgE values over time for patients *(Pt)* with positive skin test results to chlorhexidine.

finding was also statistically significant. During urologic procedures, exposure to chlorhexidine is on the urethral and bladder mucosa, which seems to be a very efficient way for the allergen to enter the bloodstream. Heinemann et al[5] reviewed the literature on anaphylactic reactions to chlorhexidine in 2002 and found that 21 out of 66 cases identified were caused by exposure via the urethral route. Reactions occurred through several different routes of exposure, but the urethral route was most common.

Concentration of tryptase at the time of reaction was similar in the 2 groups, but baseline tryptase values (taken when there was no allergic activity) appeared higher in the STP group than in the STN group. However, this difference was not statistically significant in this study ($P = .07$). If a difference did exist, there could be several explanations. In insect venom allergy, it has been suggested that baseline levels of tryptase may predict severity of the clinical response, especially severe hypotensive episodes.[26] Elevated baseline tryptase levels have also been suggested to be an indicator of low-grade inflammation as a marker for coronary artery disease,[27] and finally, our group has previously noted that baseline tryptase may increase with increasing age.[1] In this study, it may be a combination of all 3 factors, and further studies are needed in this area.

In conclusion, IgE testing and the HR-test are good adjuncts to skin testing and clinical history when diagnosing allergy to chlorhexidine. No single test has 100% sensitivity, and we therefore recommend a combination of several test modalities in combination with clinical history to achieve the highest possible sensitivity. The findings indicate that sampling time is critical, because IgE levels decline over time. Samples should probably be taken within 6 months of the reaction, or even earlier, and because IgE levels are already increased at the time of reaction, sampling could take place at this time. Analysis of IgE to chlorhexidine could thus be performed on the sample taken for tryptase analysis 1 to 4 hours after the reaction. Further studies with serial samples from patients with IgE against chlorhexidine should elucidate the timing aspect.

Allergy to chlorhexidine continues to be rare, but knowledge about incidence is lacking. Further studies should be performed to determine incidence and mode of sensitization of this often overlooked allergy. In addition, patients and health care workers should be made aware of chlorhexidine as a potential allergen, and patients who report reactions, however minor, in connection with exposure to chlorhexidine should be referred for specialized investigation. Last, we recommend that centers investigating patients with allergic reactions in connection with surgery and anesthesia routinely include testing with chlorhexidine because of its widespread use.

REFERENCES

1. Garvey LH, Roed-Petersen J, Menne T, Husum B. Danish Anaesthesia Allergy Centre: preliminary results. Acta Anaesthesiol Scand 2001;45: 1204-9.
2. Calnan CD. Contact dermatitis from drugs. Proc R Soc Med 1962;55: 39-42.
3. Krautheim AB, Jermann TH, Bircher AJ. Chlorhexidine anaphylaxis: case report and review of the literature. Contact Dermatitis 2004;50:113-6.
4. Garvey LH, Roed-Petersen J, Husum B. Anaphylactic reactions in anaesthetised patients: four cases of chlorhexidine allergy. Acta Anaesthesiol Scand 2001;45:1290-4.
5. Heinemann C, Sinaiko R, Maibach H. Immunological contact urticaria and anaphylaxis to chlorhexidine: overview. Exogenous Dermatol 2002;1:186-94.
6. Pham NH, Weiner JM, Reisner GS, Baldo BA. Anaphylaxis to chlorhexidine: case report: implication of immunoglobulin E antibodies and identification of an allergenic determinant. Clin Exp Allergy 2000;30:1001-7.
7. Layton GT, Stanworth DR, Amos HE. The incidence of IgE and IgG antibodies to chlorhexidine. Clin Exp Allergy 1989;19:307-14.
8. Ohtoshi T, Yamauchi N, Tadokoro K, Miyachi S, Suzuki S, Miyamoto T, et al. IgE antibody-mediated shock reaction caused by topical application of chlorhexidine. Clin Allergy 1986;16:155-61.
9. Ebo DG, Bridts CH, Stevens WJ. IgE-mediated anaphylaxis from chlorhexidine: diagnostic possibilities. Contact Dermatitis 2006;55:301-2.
10. Revelle LK, Rutter AM, Wilson JA. Synthesis of chlorhexidine digluconate impurities. J Agric Food Chem 1995;43:1299-301.
11. Dirks CG, Pedersen MH, Platzer MH, Bindslev-Jensen C, Skov PS, Poulsen LK. Does absorption across the buccal mucosa explain early onset of food-induced allergic systemic reactions? J Allergy Clin Immunol 2005;115:1321-3.
12. Untersmayr E, Poulsen LK, Platzer MH, Pedersen MH, Boltz-Nitulescu G, Skov PS, et al. The effects of astric digestion on codfish allergenicity. J Allergy Clin Immunol 2005;115:377-82.
13. Sfar Working Group. Clinical practice guidelines: reducing the risk of anaphylaxis during anaesthesia. Ann Fr Reanim 2002;21(suppl 1):7-23.
14. Aalto-Korte K, Mäkinen-Kiljunen S. Symptoms of immediate chlorhexidine hypersensitivity in patients with a positive prick test. Contact Dermatitis 2006;55:173-7.
15. Blanca M, Garcia J, Vega JM, Miranda A, Carmona MJ, Mayorga C, et al. Anaphylaxis to penicillins after non-therapeutic exposure: an immunological investigation. Clin Exp Allergy 1996;26:335-40.
16. Mosbech H, Christensen J, Dirksen A, Søborg M. Insect allergy: predictive value of diagnostic tests: a three-year follow-up study. Clin Allergy 1986;16:433-40.
17. Layton GT, Stanworth DR, Amos HE. The specificity of murine polyclonal and monoclonal antibodies to the haptenic drug chlorhexidine induced by chlorine-generated chlorhexidine-protein conjugates. Clin Exp Immunol 1987;69:157-65.
18. Baldo BA, Pham NH, Zhao Z. Chemistry of drug allergenicity. Curr Opin Allergy Clin Immunol 2001;1:327-35.
19. Layton GT, Stanworth DR, Amos HE. Factors influencing the immunogenicity of the haptenic drug chlorhexidine in mice, part I: molecular requirements for the induction of IgE and IgG anti-hapten antibodies. Mol Immunol 1987;24:133-41.
20. Garvey LH, Roed-Petersen J, Husum B. [Allergy to chlorhexidine: an important differential diagnosis]. Ugeskr Laeger 2004;166:382-3.
21. Ebo DG, Stevens WJ, Bridts CH, Matthieu L. Contact allergic dermatitis and life-threatening anaphylaxis to chlorhexidine. J Allergy Clin Immunol 1998;101:128-9.
22. Cheung J, O'Leary JJ. Allergic reaction to chlorhexidine in an anaesthetised patient. Anaesth Intensive Care 1985;13:429-30.
23. Thune P. [Two patients with chlorhexidine allergy: anaphylactic reactions and eczema]. Tidsskr Nor Laegeforen 1998;118:3295-6.
24. Lauerma AI. Simultaneous immediate and delayed hypersensitivity to chlorhexidine digluconate. Contact Dermatitis 2001;44:59.
25. Bergqvist-Karlsson A. Delayed and immediate-type hypersensitivity to chlorhexidine. Contact Dermatitis 1988;18:84-8.
26. Schwartz LB, Bradford TR, Rouse C, Irani AM, Rasp G, Van der Zwan JK, et al. Development of a new, more sensitive immunoassay for human tryptase: use in systemic anaphylaxis. J Clin Immunol 1994;14:190-204.
27. Deliargyris EN, Upadhya B, Sane DC, Dehmer GJ, Pye J, Smith SC Jr, et al. Mast cell tryptase: a new biomarker in patients with stable coronary artery disease. Atherosclerosis 2005;178:381-6.

Paper II

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Effect of General Anesthesia and Orthopedic Surgery on Serum Tryptase

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ABSTRACT

Background: Mast cell tryptase is used clinically in the evaluation of anaphylaxis during anesthesia, because symptoms and signs of anaphylaxis are often masked by the effect of anesthesia. No larger studies have examined whether surgery and anesthesia affect serum tryptase. The aim of this study was to investigate the effect of anesthesia and surgery on serum tryptase in the absence of anaphylaxis.

Methods: The study included 120 patients (median age, 54 yr; range, 19–94 yr) undergoing elective orthopedic surgery in general anesthesia. Exclusion criteria were allergic reactions during this or previous anesthesia, hematologic disease, or high-dose corticosteroid treatment. Blood samples for tryptase analysis (ImmunoCAP®; Phadia, Uppsala, Sweden) were drawn shortly before anesthesia and after anesthesia and surgery.

Results: Median duration of anesthesia was 105 min (range, 44–263 min). Median interval between blood samples was 139 min (range, 39–370 min). Mean tryptase before surgery was 5.01 μg/l, with a mean decrease of 0.55 μg/l ($P < 0.0001$; 95% CI, 0.3–0.8) postoperatively. All patients received intravenous fluid (median value 750 ml; range, 200–2000 ml) perioperatively. There was no significant effect of gender, age, American Society of Anesthesiologist's physical status classification, or self-reported allergy on serum tryptase.

Conclusions: Serum tryptase shows small intraindividual variation in the absence of anaphylaxis. A small decrease was observed postoperatively, likely due to dilution by intravenous fluid. On suspected anaphylaxis during anesthesia, tryptase values, even within the normal reference interval, should, when possible, be compared with the patient's own basal level taken more than 24 h after the reaction.

What We Already Know about This Topic

- Serum tryptase concentrations increase during anaphylaxis and have been used to confirm suspected anaphylaxis during anesthesia
- Whether anesthesia itself alters serum tryptase concentration is unknown

What This Article Tells Us That Is New

- In 120 subjects undergoing elective and uneventful surgery, there was a slight decrease in serum tryptase concentration, likely reflecting dilution from intravenous fluids
- Comparison of serum tryptase concentration within 1–4 hr of the event and at least 24 hr later can help confirm suspected anaphylaxis during anesthesia

TRYPTASE is a serine protease mainly stored in mast cell granules. Under normal conditions, serum tryptase consists of protryptase α and β, which are continuously secreted into the bloodstream, making up the basal level of serum tryptase, and this level is believed to be an indicator of the total number of mast cells in the body.[1] Increased number of mast cells leading to increased basal levels of serum tryptase can be seen in hematologic conditions such as mastocytosis.[2] Mature β tryptase is contained in granules in the mast cell and is released only during activation and degranulation of the mast cell. Mast cell degranulation can be triggered by a variety of stimuli, such as C5a and C3a, neuropeptides, and certain drugs, but most commonly occurs when a specific antigen causes cross-linking of specific IgE molecules bound to the high affinity IgE receptor on the surface of mast cells.[3] An increase in serum tryptase is, therefore, highly suggestive of IgE-mediated mast cell activation, when seen in connection with signs and symptoms of anaphylaxis.[4] Such a marker of anaphylaxis has proven to be

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particularly useful in cases where the clinical diagnosis is uncertain, for example, during anesthesia, where symptoms and signs of anaphylaxis are often masked or mimicked by the effect of anesthetics and patient comorbidity. The use of serum tryptase has, thus, been recommended when investigating patients with suspected anaphylactic reactions during anesthesia,[5,6] and the current recommendations are to take a sample 1–4 h after the reaction and compare with a basal level serum tryptase taken minimum 24 h after the reaction.[7] In the Danish Anaesthesia Allergy Centre, serum tryptase has been used routinely since 1999, when investigating patients with suspected allergic reactions during anesthesia. However, the possible effect of anesthesia and surgery *per se* on serum tryptase has only been partially elucidated in smaller studies. These indicate that there is no increase in serum tryptase during coronary bypass surgery using extracorporeal circulation[8] or during cardiac defibrillation with the administration of suxamethonium.[9] The aim of the current study was, therefore, to investigate the effect of anesthesia and surgery *per se* on serum tryptase concentration and to investigate the characteristics of basal levels of serum tryptase in a population of patients undergoing elective orthopedic surgery.

Materials and Methods

Patients

The study was approved by the Ethical Committee for the Capital Region, Copenhagen, Denmark, and included 120 patients undergoing elective orthopedic surgery in general anesthesia. Data storage was approved by the Data Protection Agency, Copenhagen, Denmark. Eligible patients were identified from operating lists the day before surgery. On the morning of scheduled surgery, before the administration of premedication, patients were included in the study, and the written informed consent was obtained. Inclusion took place on a total of 27 days at Bispebjerg Hospital, Copenhagen, Denmark, and a total of 120 patients were included. Inclusion criteria were as follows: patients aged 18 yr or older and scheduled elective orthopedic surgery in general anesthesia with a minimum expected duration of approximately 1 h. To cover the widest possible age range, it was intended to include 60 patients in the age group of 18–60 yr and 60 patients older than 60 yr, but this proved not to be possible because of the design with consecutive inclusion. Exclusion criteria were as follows: pregnancy, previous allergic reactions during anesthesia, corticosteroid treatment of more than 2.5 mg daily, and known hematologic disease including mastocytosis. Patients developing a suspected allergic reaction during anesthesia would also be excluded.

Twelve patients declined to participate in the study for the following reasons: fear of needles (seven patients), did not wish to participate (three patients), and did not speak Danish (two patients). After inclusion, two patients withdrew consent, and in three cases, surgery was cancelled. In these five cases, subsequent patients were included.

Before anesthesia, the following information was recorded: allergy status (self-reported allergy), type of orthopedic surgery, and American Society of Anesthesiologist's physical status classification. During anesthesia, a record was kept of the length of general anesthesia, all drugs administered, use of regional anesthesia, fluid administration, blood loss, administered blood products, and use of inotropes.

Postoperatively, patients and anesthetic charts were examined for signs of anaphylaxis. None of the 120 patients showed signs of an allergic reaction during or after anesthesia.

Because serum IgE was not measured for inhalant allergens, atopy was defined as self-reported allergy symptoms on contact with at least one inhalant allergen (tree/grass pollens, animal dander, house-dustmite, and ambrosia). Self-reported allergy to other allergens, such as drugs, plaster, nickel, were classified as other known allergen.

Blood Sampling

Preoperative blood samples for tryptase analysis were drawn shortly before anesthesia, in most cases, during insertion of the intravenous cannula. The postoperative blood sample was collected approximately at 1–4 h after anesthesia induction, as mast cell tryptase increase after anaphylaxis is seen in this time interval. Blood samples were collected, according to the standard procedure in our laboratory, as whole blood in plain collection tubes and were transported at room temperature. Samples were sent to the laboratory for analysis the following weekday.

Tryptase analysis was performed at the Laboratory for Allergy, Copenhagen University Hospital, Rigshospitalet, Denmark, using ImmunoCAP® (Phadia, Uppsala, Sweden) measuring total tryptase in serum, that is, all proforms of α tryptase and β tryptase, and mature β-tryptase. According to the manufacturer's product information (ImmunoCAP®, Tryptase in anaphylaxis, 2007), a study of 126 healthy individuals with an age range of 12–61 yr yielded an upper ninety-fifth percentile for basal levels of serum tryptase of 11.4 μg/l, and a median value of 3.8 μg/l with a lower detection limit of 1 μg/l. This forms the basis for the currently recommended normal reference interval.

Statistical Analysis

Analysis of sample size needed (n = 120) was carried out using the Altman nomogram.[10] From 118 measurements of baseline values of mast cell tryptase in the Danish Anaesthesia Allergy Centre patients, the SD was calculated to be 5 μg/l. Because there were no previous studies examining differences in preoperative and postoperative serum tryptase, we had no knowledge of the correlation between preoperative and postoperative values, and thus, the power calculation was based on unpaired analysis (a conservative procedure). The clinically relevant difference was determined to be more than or equal to 3 μg/l, that is, standardized difference 0.6 with power 0.9 and significance level 0.05.

Serum tryptase levels preoperatively and postoperatively were compared using paired Student *t* test. Because tryptase concentrations did not follow a normal distribution, differences in serum tryptase levels in groups of patients older than

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Table 1. Characteristics and Median Preoperative Serum Tryptase Values for Elective Orthopedic Surgery Patients (n = 120)

	Patients, n (%)	Serum Tryptase (μg/l), Median (Interquartile Range)	*P* Value
Age group, yr			
< 60	75 (62.5)	4.09 (3.08–5.04)	0.9*
≥ 60	45 (37.5)	4.04 (2.68–5.47)	
Sex			
Female	60 (50.0)	4.32 (2.47–6.03)	1.0*
Male	60 (50.0)	4.00 (3.19–5.11)	
ASA classification†			
I	60 (50.0)	4.02 (2.92–4.89)	0.7‡
II	51 (42.5)	4.09 (2.96–6.41)	
III	9 (7.5)	4.91 (3.88–5.34)	
IV	0	N/A	
Self-reported allergy			
Yes	43 (35.8)	3.85 (2.97–4.91)	0.6*
No	77 (64.2)	4.30 (2.96–5.64)	
Atopy			
Yes	21 (17.5)	3.98 (3.52–4.72)	0.5*
No	99 (82.5)	4.14 (2.73–5.64)	
Other known allergen			
Yes	28 (23.3)	3.87 (2.15–5.38)	0.8*
No	92 (76.7)	4.15 (3.10–5.32)	

* Unpaired *t* test after logarithmic transformation. † American Society of Anesthesiologists (ASA) physical status classification. ‡ Analysis of variance after logarithmic transformation.

and younger than 60 yr, between males and females, and in patients with or without self-reported allergy were compared using unpaired *t* test after logarithmic transformation. Association between tryptase values and American Society of Anesthesiologist's physical status classification was assessed using ANOVA after logarithmic transformation. Linear regression was performed looking for a relation between age and preoperative levels of serum tryptase after logarithmic transformation. *P* values of < 0.05 were considered statistically significant. All analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC).

Results

A total of 120 patients (60 men/60 women; median age 54 yr; range, 19–94 yr) were included. Median preoperative serum tryptase values can be seen in table 1. There was no significant effect of gender, age group, or American Society of Anesthesiologist's physical status classification on serum tryptase. Linear regression showed no correlation between age and preoperative levels of serum tryptase ($r = 0.05$, $P = 0.6$).

A total of 43% of patients reported to have one or more allergies. Atopy was present in 21% of patients, and 28% had self-reported allergy to drugs, mostly penicillin (12 patients) or other allergens, mostly plaster (7 patients). There was no significant effect of overall self-reported allergy status, atopy, or other known allergen on serum tryptase.

Variations in serum tryptase, both whole population and intraindividual variation, can be seen in table 2. Preoperative tryptase samples were used as a measure for the whole population variation with a median value of 4.07 μg/l (range,

Table 2. Variation in Serum Tryptase during Orthopedic Surgery

	Serum Tryptase (μg/l)	
n = 120	Median (Interquartile Range)	Mean (SD)
Whole population variation		
Preoperative sample* (range, 1.0 to 35.8)	4.07 (2.97 to 5.32)	5.01 (4.52)
Postoperative sample* (range, 1.0 to 31.9)	3.45 (2.67 to 4.74)	4.46 (3.80)
Intraindividual variation		
Postoperative–preoperative samples (range, 6.7 to −8.0)	−0.45 (−0.87 to 0.00)	−0.55 (1.39)

Whole population variation and intraindividual variation are shown.

* Paired Student *t* test, $P < 0.0001$ (95% CI 0.30–0.80).

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1.00–35.80 μg/l) and an upper ninety-fifth percentile of 13.05 μg/l. Mean value for preoperative samples was 5.01 μg/l, and mean value for postoperative samples was 4.46 μg/l, giving a statistically significant decrease in serum tryptase during surgery and anesthesia, mean decrease 0.55 μg/l ($P < 0.0001$, 95% CI 0.30–0.80).

The SD on the change between preoperative and postoperative value was 1.39 μg/L, giving a reference interval of (−3.33 to 2.23 μg/l), meaning that 95% of patients are expected to have a change in serum tryptase in the interval between a decrease of 3.33 μg/l and an increase of 2.23 μg/l.

Calculating the difference between postoperative and preoperative values after logarithmic transformation, a mean decrease of 9% (95% CI, 6–13%) was found. The reference interval for ratios was (0.59–1.39), meaning that 95% of patients are expected to have a change in serum tryptase in the interval between a 41% decrease and a 39% increase.

Only two patients had an increase in serum tryptase of more than 2.23 μg/l (mean + 2SD) with increases of 2.57 and 6.72 μg/l, respectively. Neither of them showed signs or symptoms of an allergic reaction nor reported any known allergies or atopy.

Preoperative serum tryptase values were more than the manufacturers recommended upper limit of 11.4 μg/l in 7 of 120 (5.8%) of patients (table 3). Four of these patients showed a decrease in tryptase of more than 3.33 μg/l (mean − 2SD). There was no statistically significant difference in the age between patients with serum tryptase more than and less than 11.4 μg/l.

Median duration of anesthesia was 105 min (range, 44–263 min), and median interval between blood samples before and after surgery was 139 min (range, 39–370 min). Because of unexpected surgical delay in four cases, the postoperative sample was taken before surgery was completed. In all cases, at least 2 h elapsed between presample and postsample, and mean difference in tryptase for these four patients was 0.57 μg/l, that is, very close to the overall result. In one case, the presample was taken more than 15 min after induction, and only 39 min elapsed between tests, but as pretest and posttest results were identical (less than 1 μg/l), this was not believed to affect the overall result. All patients were given intravenous fluids (median, 750 ml; range, 200–2000 ml) during surgery.

Table 3. Characteristics for Orthopedic Surgery Patients with Preoperative Serum Tryptase Values More than and Less than 11.4 μg/l* (n = 120)

	Tryptase ≤ 11.4 μg/l	Tryptase > 11.4 μg/l	*P* Value
Patients, n (%)	113 (94.2)	7 (5.8)	
Female, n (%)	55 (92)	5 (8)	
Male, n (%)	58 (97)	2 (3)	
Age (yr), mean ± SD	51.5 ± 17.6	61.6 ± 19.3	0.15†
Median serum tryptase, μg/l	3.98	14.8	

* Manufacturer's recommended upper limit for normal serum tryptase. † Unpaired *t* test for means.

Discussion

The aim of this study was to investigate the potential effect of anesthesia and surgery on serum tryptase. To minimize the possible confounding from type of surgery, we chose to investigate a population of patients undergoing elective orthopedic surgery in general anesthesia. A recent study of intestinal handling during abdominal surgery showed that mast cell activation and tryptase release were induced locally in peritoneal fluid, although this was not accompanied by an increase in serum tryptase.[11]

Comparing preoperative and postoperative samples of serum tryptase in a population of patients undergoing elective orthopedic surgery in general anesthesia, we found a mean decrease of 0.55 μg/l, and the most likely explanation is a dilutional effect of intravenous fluid therapy. Studies examining hemoglobin have found a dilutional effect of crystalloid fluids in healthy volunteers[12] and a small dilutional effect of general anesthesia, in the absence of fluid administration, during laparoscopic surgery.[13] No such studies have been performed for serum tryptase, and our study was not designed to examine the potential dilutional effect of intravenous fluids on serum tryptase. Thus, further studies would have to be undertaken to confirm the mechanism behind the decrease in serum tryptase.

The decrease observed in our study is statistically significant, but it is of minimal clinical importance in daily practice in the absence of suspected anaphylaxis. If intravenous fluids had not been administered, an even smaller change in serum tryptase may have been expected, as reported by Brown *et al.*[14] who found a mean difference in serum tryptase basal levels of 0.26 μg/l, when comparing samples taken from the same individual 14 weeks apart. The authors concluded that in the absence of anaphylaxis, tryptase values do not vary more than 2 μg/l in the same individual. Our study shows that during orthopedic surgery in general anesthesia, in the absence of anaphylaxis, 95% of patients showed a change between preoperative and postoperative values of serum tryptase in the interval between a decrease of 3.33 μg/l and an increase of 2.23 μg/l. Because changes were greater for higher serum tryptase values, calculations giving a percentage change might be more precise, and 95% of patients had a change in serum tryptase in the interval between a 41% decrease and a 39% increase. Thus, serum tryptase shows only small intraindividual variation even in the orthopedic surgical setting. In cases of clinical suspicion of anaphylaxis during anesthesia, current recommendations are that a serum tryptase sample should be taken within 1–4 h of the reaction and a note should be made of the timing of the sample in relation to the suspected allergic reaction. Also, this tryptase concentration should be compared with the patient's own basal level of serum tryptase taken at least 24 h after suspected

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anaphylaxis.[7] A preoperative basal level serum tryptase could also be used for comparison, but it is rarely available in clinical practice. Our data show that the variation in the whole population as expressed by SD in preoperative levels of serum tryptase is much greater than the intraindividual variation SD 4.52 *versus* 1.39 μg/l, respectively (table 2).

Because of this small intraindividual variation in serum tryptase level, clinically relevant increases within the normal reference range have been reported previously, and this should be kept in mind when interpreting tryptase values.[15,16] Also, in the setting of anaphylaxis, several liters of fluid may be administered, and the possible dilutional effect could potentially mask a discrete, but clinically significant increase in serum tryptase.

Our data show slightly higher tryptase concentrations compared with data in the manufacturers product information (ImmunoCAP® Tryptase in anaphylaxis, 2007), partly because a population of elective orthopedic surgery patients is not directly comparable with a population of healthy individuals. However, the age range of 19–94 yr (median 54 yr) in our population is broader than the age range of 12–61 yr (no median quoted) in the manufacturer's healthy individuals.

Preliminary results from the Danish Anaesthesia Allergy Centre previously suggested that in patients referred for investigation of allergic reactions during anesthesia, an increase in basal levels of serum tryptase might be seen with an increase in age.[17] In the literature, only two studies of patients with venom allergy has found an association between increasing age and basal levels of serum tryptase#.[18] No literature could be found on a possible age effect on basal levels of serum tryptase in patients investigated for drug allergy or anesthesia allergy.

In this study, samples taken preoperatively, before any intervention on the patient, are used as a measure for basal levels of serum tryptase in this patient population. Looking at these samples, there was no significant effect of gender, age group (less than or more than 60 yr), or American Society of Anesthesiologist's group on mean serum tryptase level. In addition, linear regression showed no correlation between age and preoperative levels of serum tryptase. A possible relation between age and serum tryptase could, thus, not be found in this population of elective orthopedic surgery patients, and studies of patients with suspected allergic reactions during anesthesia would be needed to further elucidate this.

We found no statistically significant association between self-reported allergy or atopy and preoperative levels of serum tryptase. Previous studies have shown no association between basal levels of serum tryptase and atopy.[15] In our study, only two patients showed an increase in serum tryptase level more than 2.23 μg/l (mean + 2SD), and neither reported any known allergy or atopy. The increase in serum tryptase level could, therefore, be either an expression of the extremes of the normal variation in the test or a sign of some other mechanism of mast cell degranulation. In clinical practice, this phenomenon would most likely go unnoticed, as mast cell tryptase would only be measured in the context of a suspected allergic reaction during anesthesia. It is important, however, to be aware that increases in tryptase level can be seen in the absence of allergic symptoms and signs.

A preoperative level of serum tryptase more than the recommended reference was found in 5.8% of patients in the absence of signs or symptoms of anaphylaxis or mastocytosis (table 3). The relevance of this finding is uncertain. In venom allergic patients with previous severe reactions, studies have indicated that an increased basal level of serum tryptase could be an early sign of mast cell disorder,[19,20] but no such studies have been carried out in patients without signs or symptoms of allergic disease or in patients investigated for drug allergy or anesthesia allergy. Further studies of these groups of patients will be needed to investigate whether findings in venom allergic patients can be extrapolated to other groups of patients.

In conclusion, no increase in serum tryptase concentration was observed in connection with orthopedic surgery in general anesthesia. On the contrary, there was a small, but statistically significant decrease of 0.55 μg/l, most likely due to the dilutional effect of intravenous fluid administration.

In the absence of anaphylaxis, serum tryptase shows only small intraindividual variation, and in the setting of anesthesia and orthopedic surgery, 95% of patients showed a change between preoperative and postoperative values of serum tryptase in the interval between a decrease of 3.33 μg/l and an increase of 2.23 μg/l. Because the recommended upper limit of normal for serum tryptase is 11.4 μg/l, it could be speculated that during suspected anaphylaxis, clinically relevant increases could occur within the normal limits, but this would have to be further studied in a population of patients with suspected allergic reactions during anesthesia and surgery. Until such data are available, it would seem prudent to continue to follow the current recommendations to compare a serum tryptase level taken 1–4 h after a suspected allergic reaction, with the patients' own basal level serum tryptase, in a sample taken minimum 24 h after the reaction.[7]

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Guenova E, Herberts T, Hoetzenecker W, Genova S, Burow G, Mitev V, Biedermann T: Basal serum tryptase and age as crucial risk factors for severe hymenoptera sting reactions. Abstract from the XXVII Congress of the European Academy of Allergology and Clinical Immunology. Barcelona, Spain, June 7–11, 2008.

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References

1. Caughey GH: Tryptase genetics and anaphylaxis. J Allergy Clin Immunol 2006; 117:1411–4
2. Müller UR: Elevated baseline serum tryptase, mastocytosis and anaphylaxis. Clin Exp Allergy 2009; 39:620–2
3. Hallgren J, Pejler G: Biology of mast cell tryptase. An inflammatory mediator. FEBS J 2006; 273:1871–95
4. Schwartz LB: Diagnostic value of tryptase in anaphylaxis and mastocytosis. Immunol Allergy Clin N Am 2006; 26: 451–63
5. Fisher MM, Baldo BA: Mast cell tryptase in anaesthetic anaphylactoid reactions. Br J Anaesth 1998; 80:26–9
6. Laroche D, Vergnaud MC, Sillard B, Soufarapis H, Bricard H: Biochemical markers of anaphylactoid reactions to drugs. Comparison of plasma histamine and tryptase. ANESTHESIOLOGY 1991; 75:945–9
7. Krøigaard M, Garvey LH, Gillberg L, Johansson SGO, Mosbech H, Florvaag E, Harboe T, Eriksson LI, Dahlgren G, Seeman-Lodding H, Takala R, Wattwil M, Hirlekar G, Dahlén B, Guttormsen AB: Scandinavian Clinical Practice Guidelines on the diagnosis, management and follow-up of anaphylaxis during anaesthesia. Acta Anaesthesiol Scand 2007; 51:655–70
8. Fayaz KM, Pugh S, Balachandran S, Sudheer PS, Hall JE: Histamine release during adult cardiopulmonary bypass. Anaesthesia 2005; 60:1179–84
9. Storjord E, Nielsen EW: Tryptase levels after suxamethonium administration and defibrillation. Acta Anaesthesiol Scand 2008; 52:838–40
10. Altman DG. Practical Statistics for Medical Research, 1st edition. London, Chapman and Hall, 1991, pp 456
11. The FO, Bennink RJ, Ankum WM, Buist MR, Busch ORC, Gouma DJ, van der Heide S, van den Wijngaard RM, de Jonge WJ, Boeckxstaens GE: Intestinal handling-induced mast cell activation and inflammation in human postoperative ileus. Gut 2008; 57:33–40
12. Drobin D, Hahn RG: Kinetics of isotonic and hypertonic plasma volume expanders. ANESTHESIOLOGY 2002; 96:1371–80
13. Olsson J, Svensén CH, Hahn RG: The volume kinetics of acetated Ringer's solution during laparoscopic cholecystectomy. Anesth Analg 2004; 99:1854–60
14. Brown SGA, Blackman KE, Heddle RJ: Can serum mast cell tryptase help diagnose anaphylaxis? Emerg Med Australas 2004; 16:120–4
15. Schwartz LB, Bradford TR, Rouse C, Irani AM, Rasp G, Van der Zwan JK, Van der Linden PWG: Development of a new, more sensitive immunoassay for human tryptase: Use in systemic anaphylaxis. J Allergy Clin Immunol 1994; 14: 190–204
16. Enrique E, García-Ortega P, Sotorra O, Gaig P, Richart C: Usefulness of UniCAP-Tryptase fluoroimmunoassay in the diagnosis of anaphylaxis. Allergy 1999; 54:602–6
17. Garvey LH, Roed-Petersen J, Menné T, Husum B: Danish Anaesthesia Allergy Centre—preliminary results. Acta Anaesthesiol Scand 2001; 45:1204–9
18. Kucharewicz I, Bodzenta-Lukaszyk A, Szymanski W, Mroczko B, Szmitkowski M: Basal serum tryptase level correlates with severity of hymenoptera sting and age. J Investig Allergol Clin Immunol 2007; 17:65–9
19. Bonadonna P, Perbellini O, Passalacqua G, Caruso B, Colarossi S, Dal Fior D, Castellani L, Bonetto C, Frattini F, Dama A, Martinelli G, Chilosi M, Senna G, Pizzolo G, Zanotti R: Clonal mast cell disorders in patients with systemic reactions to Hymenoptera stings and increased serum tryptase levels. J Allergy Clin Immunol 2009; 123: 680–6
20. Metcalfe DD, Schwartz LB: Assessing anaphylactic risk? Consider mast cell clonality. J Allergy Clin Immunol 2009; 123:687–8

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Paper III

Serum tryptase in patients with suspected allergic reactions during anaesthesia –

ten years' experience from the Danish Anaesthesia Allergy Centre

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Abstract:

Background

Serum tryptase is a marker for anaphylaxis during anaesthesia, but no studies have investigated the characteristics of serum tryptase in these reactions.

Objectives

1) To investigate whether using the difference between serum tryptase taken at the time of a reaction (T_{react}) and the patients own basal level (T_{basal}) can improve sensitivity
2) To describe the characteristics of serum tryptase in patients with suspected allergic reactions during anaesthesia

Methods

A total of 318 patients had T_{basal} measured during investigation at the Danish Anaesthesia Allergy Centre. Of these 199 had T_{react} measured. Sensitivity and specificity for serum tryptase was investigated for the intra-individual difference and for different absolute cut-off values. Relation between T_{basal} and age was explored using linear regression and the risk factors for severe reactions were investigated using multiple logistic regression.

Results

Using the intra-individual difference in serum tryptase marginally improved sensitivity of serum tryptase as a marker for anaphylaxis during anaesthesia, when compared to an upper limit of 11.4 μg/L. Sensitivity increased when only including severe reactions in the analysis. Linear regression showed a significant increase in T_{basal} with increasing age ($r=0.23$; $p<0.0001$) and 8.2% of patients had a T_{basal} >11.4 μg/L. The risk of severe reactions was increased with increasing age, male sex, T_{basal} >11.4 μg/L, positive investigation result and increasing ASA class (American Society of Anesthesiologist's physical status classification).

Conclusion

Serum tryptase is a widely used marker for anaphylaxis. This study sheds new light on the characteristics of serum tryptase in patients with suspected allergic reactions during anaesthesia.

Key Messages

- In patients with suspected allergic reactions during anaesthesia basal level serum tryptase increases with age and levels > 11.4 μg/l are associated with increased risk of severe reactions
- The sensitivity of serum tryptase as a diagnostic marker for allergic reactions during anaesthesia may be improved by utilising the difference between serum tryptase taken at the time of suspected anaphylaxis, and the patients own basal level serum tryptase

Keywords

Tryptase; basal levels; sensitivity; specificity; anaesthesia; anaphylaxis; allergy; age; drug allergy

Abbreviations

DAAC	Danish Anaesthesia Allergy Centre
ASA	American Society of Anesthesiologist's physical status classification
T_{basal}	Basal level serum tryptase
T_{react}	Serum tryptase taken at time of allergic reaction

Introduction

Tryptases are serine peptidases sharing enzymatic, structural and phylogenetic features with the trypsin family. Tryptases are found in most mast cells in the human body – regardless of type and tissue location. Basal level serum tryptase consists primarily of pro-α tryptase and pro-β tryptase[1] with pro-β tryptase probably predominating, as serum tryptase levels are not significantly lower in the 25% of the population who lack the gene for α-tryptase.[2] Protryptases are continously secreted from resting mast cells, and in subjects without signs or symptoms of anaphylaxis basal level serum tryptase likely reflects mast cell numbers in the body.[2]

In contrast, mature β-tryptase is stored in secretory granules of mast cells and only released during mast cell activation and degranulation.[3] The only other leukocyte containing tryptase is the basophil, containing levels of only 0.4% of that expressed in lung mast cells.[4]

During mast cell activation serum tryptase (mature β-tryptase) can therefore be used as a quite specific marker for anaphylaxis. A commercially available immunoassay measures total tryptase ie. the sum of pro-β tryptase, pro-α tryptase and mature β-tryptase (Phadia ImmunoCAP® Tryptase). Serum tryptase has proven to be useful in the investigation of suspected anaphylaxis during anaesthesia, where the diagnosis can be difficult to make as signs and symptoms are masked by the effects of anaesthesia and surgery. The use of serum tryptase has been recommended in suspected anaphylaxis during anaesthesia by several groups, but there are discrepancies regarding the upper limit of normal; some groups recommend values of 24-25 μg/l[4,5] others 13.5 μg/l.[7] In the American literature on anaphylaxis outside the operating room 15 μg/l is favoured.[3] Other groups recommend comparing with the patients own baseline without exact specifications of a relevant difference.[8,9] Recently it has been suggested that the difference between serum tryptase at the time of reaction (T_{react}) and the patients own basal level (T_{basal}) could be used diagnostically in suspected anaphylaxis both outside[10,11] and inside[12] the operating room. No studies have examined how using

this intraindividual difference in serum tryptase affects the performance of the test as a marker for allergic reactions during anesthesia.

Also, there are no studies describing T_{basal} in patients with suspected allergic reactions during anaesthesia. Studies in patients with insect venom allergy have shown that elevated T_{basal} is associated with severe clinical reactions.[13] Only few studies in venom allergy have investigated the effect of age on serum tryptase.[14,15] A preliminary study from our group previously suggested that T_{basal} increased with increasing age in patients with suspected allergic reactions during anaesthesia[16], but we have recently shown that there is no effect of age on T_{basal} in patients undergoing surgery and anaesthesia in the absence of allergic signs and symptoms.[12] Thus the aims of this study were

1) to retrospectively apply the intraindividual difference in serum tryptase diagnostically and investigate sensitivity and specificity of serum tryptase as a marker for anaphylaxis in patients with suspected allergic reactions during anaesthesia
2) to describe the characteristics of T_{basal} in the same population especially with regard to effect of patient age and risk factors for severe reactions

Materials and methods

Patients

In the period 1999-2009 a total of 319 adult patients (≥ 18 yrs) were referred to the Danish Anaesthesia Allergy Centre (DAAC) for investigation of suspected allergic reactions during anaesthesia. One patient with T_{basal} = 81.3 µg/l was excluded from the study pending investigations for mastocytosis.

In the period 1999-2003 investigations included serum tryptase, specific IgE, and skin testing. From 2004 Histamine Release testing and challenge tests (intravenous, subcutaneous and oral challenge tests) were added to the protocol.

Of 318 included patients 102 were investigated before, and 216 in, and after, 2004. Full investigations were planned for all patients, but for 4/102 and 15/216 respectively, investigations were either not completed yet or cancelled due to patients declining, not turning up for investigations or dying before testing. Thus full investigations were carried out in a total of 299 patients. All drugs and substances administered prior to the reported reaction were considered for investigation in each indvidual case. All patients were investigated for allergy to latex and chlorhexidine and since 2004 specific IgE for ethylene oxide was added, as all patients are exposed to these substances during anaesthesia and surgery in Denmark.

Data were obtained from the patients notes on 1) age 2) gender 3) American Society of Anaesthesiologists physical status classification (ASA) 4) surgery type 5) reaction class of the suspected reaction (C1 mild reactions resolving spontaneously; C2 moderate reactions; C3 severe reactions requiring treatment; C4 cardiac arrest) 6) result of subsequent allergy investigations in DAAC (positive or negative) and 7) allergen in case of positive investigation result.

Basal level serum tryptase (T_{basal})

Blood tests for T_{basal} were taken weeks-months after the suspected allergic reaction, when the patient had no signs or symptoms of allergic disease. All samples were analysed using the Phadia ImmunoCAP® Tryptase on the ImmunoCAP 100 analyzer with the manufacturers conjugates. It became evident that the antibody content of the conjugate was changed in May 2001 resulting in tryptase values analyzed after this time being

significantly lower than before (personal communication Bjarne Kristensen, Phadia, Allerød). This had, at the time, led Phadia to change the recommended upper limit of normal from 13.5 µg/l to the currently used upper limit of 11.4 µg/l. Consequently all samples taken before November 2001 were reanalyzed using the conjugate introduced in 2001 and this led to a significant decrease in serum tryptase values.

Serum tryptase at time of suspected allergic reaction during anaesthesia (T_{react})

The recommended sampling interval is 1-4 hours after the suspected reaction and the importance of noting both time of reaction and time of bloodsampling is emphazised. Samples were taken locally and transported by surface mail at room temperature. Samples received before November 2001 were re-analysed using the new conjugate as described above.

Statistics

Serum tryptase values are not normally distributed and all statistical analyses were carried out after logarithmic transformation to approximate a normal distribution. Due to small numbers reaction class 3 and 4 were combined for statistical analysis. Comparison of age in different groups was performed using unpaired Student t-test or Analysis Of Variance. Linear regression was performed looking for a relation between age and T_{basal}. Multiple logistic regression was used to determine the risk factors for severe reactions. P-values of < 0.05 were considered statistically significant. All analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Serum tryptase at the time of reaction (T_{react})

Samples for T_{react} were available for 191 (94M/97F) patients. Of those, 180 subsequently underwent full investigations with 93 (51.7%) testing positive and 87 (48.3%) testing negative. Information on timing of T_{react} samples was available for 89 and 83 patients respectively and timing of T_{react} sample in relation to the reaction is shown in figure 1. Median time to bloodsampling was 105 min (range 30-690 min) for patients testing positive and 120 min (range 25-1885) for patients testing negative. Only 12 patients in each group had samples taken later than four hours after the reaction; 11/12 of these were < 11.4 µg/l for patients testing negative vs. 5/12 < 11.4 µg/l in patients testing positive. The latest recorded elevated (>11.4 µg/l) T_{react} was taken at 570 minutes and was 30.8 µg/l (T_{basal} 2.93 µg/l) in a patient with a cardiac arrest, subsequently testing positive to a neuromuscular blocking agent. The earliest recorded elevated T_{react} was taken at 25 minutes and was 15.8 µg/l (T_{basal} 5.41 µg/l) in a patient with a C3 reaction where no allergen was found on investigation.

T_{react} values by reaction class can be seen in figure 2. There was a statistically significant difference in T_{react} values between patients testing positive and negative for all reaction classes with increasing significance with increasing reaction class (reaction class 1 $p= 0.0468$; reaction class 2 $p=0.0102$, reaction class 3+4 $p<0.0001$).

Serum tryptase appeared lower in patients testing positive for allergens with a suspected non-IgE mediated mechanism (colloids, opioids, oxytocin) than in patients testing positive for the remaining allergens (figure 3).

Difference between T_{react} and T_{basal} can be seen in figure 4. The median differences in serum tryptase were significantly higher for patients testing positive at 10.54 µg/l (IQ range 0.92-25.10) versus –0.59 µg/l (IQ range -1.63 to 0.84) for patients testing negative ($p<0.0001$).

A previous study of 120 surgical patients without allergic symptoms showed, that a perioperative serum tryptase increase of > 2.2 µg/l would only be expected in < 2.5% of patients.[12] Sensitivity and specificity for serum tryptase as a diagnostic marker for allergic reactions during anaesthesia were determined using this

intraindividual difference (T_{react} - T_{basal}) > 2.2 µg/l and upper limits of normal of 11.4 µg/l, 15.0 µg/l and 25 µg/l, respectively. Results are presented in table 1a for all four reaction classes, and table 1b for reaction classes 3 and 4 only.

The highest sensitivity was achieved using the intraindividual difference (T_{react} - T_{basal}) > 2.2 µg/l, both when looking at all reaction classes (sensitivity 66.7%) and reaction classes 3 and 4 only (sensitivity 82.8%). Sensitivity improved markedly for all diagnostic criteria when only including reaction classes 3 and 4 in the analysis, with only a limited decrease in specificity. As expected, sensitivity decreased with increasing cut-off value with a corresponding increase in specificity. The currently recommended cut-off value of 11.4 µg/l gave the highest sensitivity with values very close to those found for the intraindividual difference.

Basal levels of serum tryptase (T_{basal})

Samples for T_{basal} were available for 318 patients (139M/179F). There were 56.3% females and 31.8% of patients were aged 60 years or over. Table 2 shows patient characteristics with T_{basal} and age. Median T_{basal} was 4.56 (IQ range 3.14-6.60) for the whole population. Linear regression of T_{basal} vs age showed a highly statistically significant increase in T_{basal} with increasing age ($r=0.23$; $p<0.0001$). When dividing patients into 10 year groups significantly higher median values of T_{basal} were seen in patients aged 70 or over (figure 5). T_{basal} was significantly higher in males (5.44 µg/l vs 4.06 µg/l), however, males were significantly older than females. A significant increase in median T_{basal} with increasing ASA classification was mirrored by a significant increase in age. Similarly, a significant increase in median T_{basal} with increasing reaction class, was mirrored by a significant increase in age. A total of 149 (46.8%) patients had severe C3 and C4 reactions. Multiple logistic regression showed a significantly increased risk of severe reactions with increasing age, male sex, T_{basal} > 11.4 µg/l, higher ASA group and positive result on subsequent allergy investigation in DAAC (table 3).

A total of 299 patients had undergone subsequent investigations and 142 (47.5%) tested positive. There was no significant difference in median T_{basal} or agedistribution between patients testing positive and negative. An elevated T_{basal} (>11.4 µg/l) was seen in 26/318 (8.2%) of patients and there was no significant difference in agedistribution between patients with T_{basal} above and below 11.4 µg/l. Median T_{basal} varied with type of

surgery, and higher values were mirrored by higher age of patients in the individual surgery types. The highest median T_{basal} were seen in urological surgery, cardiovascular surgery and gut surgery. The lowest median T_{basal} were seen in organ surgery (primarily cholecystectomies) and gynaecological surgery (table 2).

Discussion

Serum tryptase at the time of reaction (T_{react})

At least three factors are thought to influence levels of T_{react}: 1) timing of bloodsample in relation to reaction 2) severity of reaction and 3) allergen.[3] Our results show, that most elevations in T_{react} are seen in samples taken within the recommended 1-4 hour interval[8], but elevated values can be found outside this interval. Other studies have found increases in T_{react} as early as 15 minutes after the reaction.[17,18]

In our study T_{react} values were significantly higher in patients testing positive on subsequent investigation. Also, an increase in T_{react} was seen with increasing reaction class especially in patients testing positive (figure 2). Previous studies have indicated that elevations in serum tryptase are closely related to hypotension[19], which is often the predominating symptom in severe reactions during anaesthesia.[20]

The immunological mechanism is important with tryptase release primarily occuring during IgE-mediated reactions. In our study allergens with a proven IgE-mediated mechanism such as antibiotics, chlorhexidine, latex, neuromuscular blocking agents, iv anaesthetics and patent blue elicited higher values of T_{react} than drugs with non-IgE mediated mechanisms eg opioids, colloids and oxytocin (figure 3). For opioids the majority of clinical reactions are caused by direct non-IgE mediated histamine release from basophils and/or mast cells and thus T_{react} is not always elevated.[21] Colloid reactions were mainly caused by dextrans, causing reactions mediated by IgG containing immune complexes, triggering the complement system and activating basophils and mast cells.[22] The fact that serum tryptase did not increase very much in our dextran positive patients (despite C3 reactions in 4/6 patients) may suggest, that activation of basophils predominate.[23] T_{react} does not increase in reactions to oxytocin as the mechanism is non-allergic and caused by an exaggerated physiological response to the drug.[24]

The literature reveals several different recommendations regarding sampling time and cut-off.[6,25] Some authors suggest more complicated diagnostic criteria of either cut-off > 24 µg/l or a 2hr sample of 3 times the basal level.[5] Several authors suggest that serial samples would increase sensitivity.[10,17] However, serial samples require high motivation from the attending anaesthesiologist. Many centres have problems getting just one sample taken within the required time. In France where a nationwide collaboration between multiple

anaesthesia allergy centres have existed for many years T_{react} was only taken during suspected reaction in 41% of cases.[25,26] In our centre advertising and teaching of anaesthetic departments and sending out anaphylaxis kits containing treatment algorithm and blood taking equipment has led to serum tryptase being taken in 68.7% of referred reactions. Thus recommendations on serial sampling may be difficult to implement in practice.

Our group has recently shown that serum tryptase showed minimal intraindividual variation in the absence of allergic symptoms in elective surgical patients.[12] The main advantage of using the intraindividual difference as a diagnostic criterion is to pick up discrete, but clinically relevant, increases in serum tryptase within the upper limit of normal, thereby increasing sensitivity. Only few studies have adressed the issue of sensitivity and specificity of serum tryptase when used during anaesthesia. Larger French multicenter studies[25,26] have reported high specificity and positive predictive value, but low sensitivity with cut-off of 25 µg/l. A recent smaller French study showed that sensitivity could be increased to 63% by using a ratio $T_{react}/T_{basal} > 3$.[27]

In our study using the intraindividual difference ($T_{react} - T_{basal}$) > 2.2 µg/l yielded the highest sensitivity of 66.7% vs. 62.4% for the currently recommended upper limit of normal of 11.4 µg/l. Using cut-off values of 15 µg/l and 25 µg/l gave relatively low sensitivity with a corresponding increase in specificity. For the safety of the patient during subsequent anaesthesia we suggest that high sensitivity is more important than high specificity in this patient population.

Sensitivity increased markedly when selectively looking at severe reactions (C3 and C4) but was still highest for ($T_{react} - T_{basal}$) > 2.2 µg/l.

There are several possible explanations for the sensitivity not exceeding 66.7% when looking at all reaction classes in our study:

Firstly, nearly 1/3 of investigated reactions were mild (C1), which are less likely to have led to an increased T_{react}.[19] Patients with positive investigation results in this group will thus account for some false negative tryptase tests. Also, our practice of including challenge testing in our investigation protocol means, that we have reproduced reactions with non-IgE mediated mechanisms, accounting for other ”false negative” results.

Specificity may be affected by the following: 1) Reactions caused by non-immunological or immunological mast cell degranulation with serum tryptase release, which we cannot diagnose with our investigation programme 2) Allergens we have overlooked despite careful consideration of each case and 3) Potential allergy to agents that cannot currently be investigated such as inhalational anaesthetic agents. All these factors could account for ”false positive” serum tryptase results in this study.

Another complicating factor is the administration of intravenous fluids which may cause dilution of T_{react} and potentially mask clinically relevant increases. This has been reported previously[12] and our study supports this as the median difference (T_{react} - T_{basal}) is actually negative in patients testing negative on subsequent investigations (figure 4). Thus many factors play a role when evaluating T_{react} results in a population of patients with suspected anaphylaxis during anaesthesia. The situation is likely to be less complicated in simple drug allergic reactions after administration of a single drug, and therefore extrapolation of results on sensitivity and specificity found in our study, to drug allergy in general, should be done with caution.

Basal levels serum tryptase T_{basal}

This is the first study examining T_{basal} in patients with suspected allergic reactions during anaesthesia. Males had significantly higher median T_{basal} than females, but were also significantly older. Only few studies have looked at serum tryptase and gender; one study found slightly higher values in females[28] and a study on children[29] found higher values in males. Our study showed a statistically significant increase in T_{basal} with increasing age $r=0.23$; $p<0.0001$. Interestingly, studies of serum tryptase in children[29] and in orthopedic surgery patients[12] were not able to find a relation between age and T_{basal}. To our knowledge only two other studies in venom allergic patients has shown a relation between age and T_{basal}.[13,15]

A recent large multicentre study of venom allergic patients found that 8.4% had a $T_{basal} > 11.4$ µg/l, and that male sex, older age and higher T_{basal} increased the risk of severe reactions to a field sting.[13] Our study finds the same three risk factors, and in addition higher ASA group and positive result on subsequent investigation are risk factors for severe reactions in our population (table 3).

Another study of venom allergic patients reported that 11.6% had T_{basal} >11.4 μg/l.[30] The same group recently found an elevated T_{basal} in 6.6% of patients in a combination of drug allergic and food allergic patients.[31] Our study shows that 8.2% of anaesthesia-allergy patients have a T_{basal} >11.4 μg/l. For comparison only 5.8% of 120 patients undergoing orthopaedic surgery in the absence of allergic symptoms had a T_{basal} >11.4 μg/l.[12]

This could indicate some similarities between patients with venom allergy and drug/anaesthesia allergy, which are not seen in patients with food allergy or in a randomly selected population of orthopaedic surgery patients.

Bonadonna et al found that bone marrow analysis showed signs of a monoclonal mast cell disorder in 30 of 34 venom allergic patients with T_{basal} >11.4 μg/l. Our patients were referred after suspected allergic reactions during anaesthesia and it may be speculated, that bone marrow analysis might reveal mast cell abnormalities in our patients with T_{basal} >11.4 μg/l. This could be a target for further studies when the full significance and consequenses of such findings are known.

The explanation for increasing T_{basal} with increasing age found in this study is not clear, but it is most pronounced in patients > 70 years. It is well known, that increasing age leads to co-morbidity and as mast cells and tryptase are implicated in a large number of disease processes in the body, it may be, that an elevated T_{basal} reflects an increased number of mast cells due to co-morbidity.

Reported causes of elevated T_{basal} include mastocytosis, AML, myelodysplastic syndromes and end stage renal disease.[3,32-34] Increased numbers of mast cells have been found in disease states as diverse as atherosclerotic cardiovascular disease[35], cancer[36] and autoimmune diseases[37]; all characterised by a combination of inflammatory processes and ischaemia leading to new vessel formation. Mast cells and tryptase have been implicated in angiogenesis[38,39] and inflammation[40], however, sofar no studies have investigated the possible relation between an increased number of mast cells and increased T_{basal} in these diseases in humans. A recent study showing an increased number of mast cells in vessel wall inflammation in aortic aneurysms suggested that serum tryptase may be a possible marker for the active inflammatory process.[41] If T_{basal} was increased in diseases with significant inflammation and/or angiogenesis this could in

part explain the higher T_{basal} observed with increasing age in our study. We have not examined the incidence of various co-morbidities in our population and this is a limitation to our study. However, when using the ASA classification as a crude expression of co-morbidity an increase in age and T_{basal} is seen with increasing ASA status (table 2).

Also a higher median T_{basal} was seen in patients undergoing cardiovascular surgery, for atherosclerotic disease, and in gut and urological surgery where cancer predominate, but this is based on small numbers and remains speculative.

This is the first study examining the characteristics of T_{basal} and the sensitivity and specificity of the intraindividual difference in serum tryptase in a population of patients with suspected allergic reactions during anaesthesia. We found a significant increase in T_{basal} with increasing age and further studies in other patient populations would be required to see if this finding applies to other groups of allergic patients.

We have also showed that sensitivity of serum tryptase as a marker for allergic reactions during anaesthesia is increased when applying the intraindividual difference in serum tryptase. Using the intraindividual difference also allows the determination of discrete elevations within the upper limit of normal, and identification of patients with elevated T_{basal}. If a T_{basal} sample is not available for comparison an upper limit of normal of 11.4 µg/l gives the highest sensitivity and specificity.

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References

1. Caughey GH. Tryptase genetics and anaphylaxis. J Allergy Clin Immunol 2006; 117:1411-4

2. Schwartz LB, Min HK, Ren S, Xia HZ, Hu J, Zhao W, Moxley G, Fukuoka Y. Tryptase precursors are preferentially and spontaneously released, whereas mature tryptase is retained by HMC-1 cells, Mono-Mac-6 cells, and human skin-derived mast cells. Journal of Immunology 2003;170:5667-73

3. Schwartz LB. Diagnostic value of tryptase in anaphylaxis and mastocytosis. Immunol Allergy Clin N Am 2006;26:451-63

4. Castells MC, Irani AM, Schwartz LB. Evaluation of human peripheral blood leukocytes for mast cell tryptase. Journal of Immunology 1987;138:2184-89

5. Harboe T, Guttormsen AB, Irgens A, Dybendal T, Florvaag E. Anaphylaxis during anaesthesia in Norway. A 6-year single-center follow-up study. Anesthesiology 2005;102:897-903

6. Mertes PM, Laxenaire MC, Lienhart A and the working group for the SFAR, Aberer W, Ring J, Pichler WJ, Demoly P for ENDA and the EAACI interest group on drug hypersensitivity. Reducing the risk of anaphylaxis during anaesthesia: guidelines for clinical practice. J Invest Allergol Clin Immunol 2005;15:91-101

7. Dybendal T, Guttormsen AB, Elsayed S, Askeland B, Harboe T, Florvaag E. Screening for mast cell tryptase and serum IgE antibodies in 18 patients with anaphylactic shock during general anaesthesia. Acta Anaesthesiol Scand 2003;47:1211-18

8. Krøigaard M, Garvey LH, Gillberg L, Johansson SGO, Mosbech H, Florvaag E, Harboe T, Eriksson LI, Dahlgren G, Seeman-Lodding H, Takala R, Wattwil M, Hirlekar G, Dahlén B, Guttormsen AB. Scandinavian Clinical Practice Guidelines on the diagnosis, management and follow-up of anaphylaxis during anaesthesia. Acta Anaesthesiol Scand 2007;51:655-70

9. Ebo DG, Fisher MM, Hagendorens MM, Bridts CH, Stevens WJ. Anaphylaxis during anaesthesia: diagnostic approach. Allergy 2007;62:471-87

10. Brown SGA, Blackman KE, Heddle RJ. Can serum mast cell tryptase help diagnose anaphylaxis? Emergency Medicine Australasia 2004;16:120-4

11. Stone SF, Cotterell C, Isbister GK, Holdgate A, Brown SGA for the emergency department anaphylaxis investigators. Elevated serum cytokines during human anaphylaxis: Identification of potential mediators of acute allergic reactions. J Allergy Clin Immunol 2009;124:786-92

12. Garvey LH, Bech BL, Mosbech H, Krøigaard M, Belhage B, Husum B, Poulsen LK. Effect of general anesthesia and orthopedic surgery on serum tryptase. Anesthesiology 2010; 112: 1184-9

13. Ruëff F, Przybilla B, Biló MB, Müller U, Scheipl F, Aberer W, Birnbaum J, Bodzenta-lukaszyk A et al. Predictors of severe systemic anaphylactic reactions in patients with hymenoptera venom allergy: Importance of baseline serum tryptase – a study of the European Academy of allergology and Clinical immunology Interest Group on insect venom hypersensitivity. J Allergy Clin Immunol 2009;124:1047-54.

14. Haeberli G, Brönnimann M, Hunziker T, Müller U. Elevated serum tryptase and hymenoptera venom allergy: relation to severity of sting reaction and to safety and efficacy of venom immunotherapy. Clin Exp Allergy 2003;33:1216-20

15. Kucharewicz I, Bodzenta-Lukaszyk A, Szymanski W, Mroczko B, Szmitkowski M. Basal serum tryptase level correlates with severity of hymenoptera sting and age. J Investig Allergol Clin Immunol 2007;17:65-9

16. Garvey LH, Roed-Petersen J, Menné T, Husum B. Danish Anaesthesia Allergy Centre – preliminary results. Acta Anaesthesiol Scand 2001;45:1204-9

17. Hunting AS, Nopp A, Johansson SGO, Andersen F, Wilhelmsen V, Guttormsen AB. Anaphylaxis to Patent Blue V. I. Clinical aspects. Allergy 2010;65:117-23

18. Laroche D, Aimone-Gastin I, Dubois F, Huet H, Gérard P, Vergnaud MC, Mouton-Faivre C, Guéant JL, Laxenarie MC, Bricard H. Radiology 1998; 209: 183-190

19. Van der Linden PW, Hack CE, Poortman J, Vivié-Kipp YC, Struyvenberg A, van der Zwan JK. Insect-sting challenge in 138 patients: relation between clinical severity of anaphylaxis and mast cell activation. J Allergy Clin Immunol 1992;90:110-18

20. Whittington T, Fisher MM. Anaphylactic and anaphylactoid reactions. Balliere's Clinical Anesthesiology 1998;12:301-21

21. Rook EJ, van Zanten AP, Van den Brink W, van Ree JM, Beijnen JH. Mast cell mediator tryptase levels after inhalation or intravenous administration of high doses pharmaceutically prepared heroin. Drug and Alcohol Dependence 2006;85:185-90.

22. Ljungström KG, Revenäs B, Smedegård G, Hedin H, Richter W, Saldeen T. Histopathological lung changes in immune complex mediated anaphylactic shock in humans elicited by dextran. Forensic Science International 1988;38:251-58

23. Simons FER, Frew AJ, Ansotegui IJ, Bochner BS, Golden DBK, Finkelman FD et al. Risk assessment in anaphylaxis: Current and future approaches. J Allergy Clin Immunol 2007;120:S2-24

24. Langesæter E, Rosseland LA, Stubhaug A. Haemodynamic effects of repeated doses of oxytocin during Caesarean delivery in healthy parturients. Br J Anaesth 2009;103:260-2

25. Mertes PM, Laxenaire MC, Les membres du GERAP. Anaphylactic and anaphylactoid reactions occuring during anaesthesia in France. Seventh epidemiologic survey (January 2001-December 2002). Annales Francaises d'Anesthésie et de Réanimation 2004;23:1133-43

26. Mertes PM, Laxenaire MC, Alla F, Groupe d'Etudes des Réactions Anaphylactoides peranesthésiques. Anaphylactic and anaphylactoid reactions occuring during anesthesia in France in 1999-2000. Anesthesiology 2003;99:536-45

27. Malinovsky JM, Decagny S, Wessel F, Guilloux L, Mertes PM. Systematic follow-up increases incidence of anaphylaxis during adverse reactions in anesthetized patients. Acta Anaesthesiol Scand 2008;52:175-81.

28. Min HK, Moxley G, Neale MC, Schwartz LB. Effect of sex and haplotype on plasma tryptase in healthy adults. J Allergy Clin Immunol 2004;114:48-51

29. Komarov HD, Hu Z, Brittain E, Uzzaman A, Gaskins D, Metcalfe DD. Serum tryptase levels in atopic an nonatopic children. J Allergy Clin Immunol 2009;124:845-48

30. Bonadonna P, Perbellini O, Passalacqua G, Caruso B, Colarossi S, Dal Fior D, Castellani L, Bonetto C, Frattini F, Dama A, Martinelli G, Chilosi M, Senna G, Pizzolo G, Zanotti R. Clonal mast cell disorders in patients with systemic reactions to Hymenoptera stings and increased serum tryptase levels. J Allergy Clin Immunol 2009;123:680-6.

31. Bonnadonna P, Zanotti R, Pagani M, Caruso B, Perbellini O, Colarossi S, Olivieri E, Dama A, Schiappoli M, Senna G, Antico A, Passalacqua G. How much specific is the association between hymenoptera venom allergy and mastocystosis? Allergy 2009;64:1379-82

32. Horny HP, Sotlar K, Valent P. Mastocytosis: State of the art. Pathobiology 2007;74:121-32

33. Sperr WR, El-Samahi A, Kundi M, Girschikofsky M, Winkler S, Lutz D, Endler G, Rumpold H, Agis H, Sillaber C, Jäger U, Valent P. Elevated tryptase levels selectively cluster in myeloid neoplasms: a novel diagnostic approach and screen marker in clinical haematology. Eur J Clin Invest 2009;39:914-23

34. Dugas-Breit S, Schöpf P, Dugas M, Schiffl H, Ruëff F, Przybilla B. Baseline serum levels of mast cell tryptase are raised in hemodialysis patients and associated with severity of pruritus. JDDG 2005;3:343-47

35. Kovanen P. Mast cells: multipotent local effector cells in atherothrombosis. Immunological reviews 2007;217:105-22

36. Kalesnikoff J, Galli SJ. New developments in mast cell biology. Nature Immunology 2008;9:1215-23

37. Hallgren J, Pejler G. Biology of mast cell tryptase. An inflammatory mediator. FEBS Journal 2006; 273:1871-95

38. Blair RJ, Meng H, Marchese MJ, Ren S, Schwartz LB, Tonnesen MG, Gruber BL. Human mast cells stimulate vascular tube formation. Tryptase is a novel, potent angiogenic factor. J Clin Invest 1997; 99: 2691-2700

39. Nico B, Mangieri D, Crivellato E, Vacca A, Ribatti D. Mast cells contribute to vasculogenic mimicry in multiple myeloma. Stem Cells Dev. 2008; 17 : 19-22

40. Libby P. Inflammation in atherosclerosis. Nature 2002; 402: 868-74

41. Mäyränpää MI, Trosien JA, Fontaine V, Folkesson M, Kazi M, Eriksson P, Swedenborg J, Hedin U. Mast cells associate with neovessels in the media and adventitia of abdominal aortic aneurysms. J Vasc Surg. 2009;50:388-95

Table 1a
Sensitivity, specificity and positive and negative predictive value for serum tryptase using different criteria for positivity in patients with suspected allergic reactions during anaesthesia (*all four reaction classes, n=180*).

Criteria for positive serum tryptase	**Sensitivity %**	**PPV %**	**Specificity %**	**NPV %**
(T_{react} - T_{basal}) > 2.2 µg/l	66.7	76.5	78.2	68.7
Treact > 11.4 µg/L	62.4	82.9	86.2	68.2
Treact > 15.0 µg/L	52.7	83.1	88.5	63.6
Treact > 25.0 µg/L	34.4	88.9	95.4	57.6

Table 1b
Sensitivity, specificity and positive and negative predictive value for serum tryptase using different criteria for positivity in patients with suspected allergic reactions during anaesthesia (*reaction class 3 and 4 only, n=102*).

Criteria for positive serum tryptase	**Sensitivity %**	**PPV %**	**Specificity %**	**NPV %**
(T_{react} - T_{basal}) > 2.2 µg/l	82.8	76.2	65.9	74.4
Treact > 11.4 µg/L	81.0	82.5	77.3	75.6
Treact > 15.0 µg/L	70.7	82.0	79.6	67.3
Treact > 25.0 µg/L	50.0	87.9	90.9	58.0

PPV positive predictive value
NPV negative predictive value
Treact serum tryptase taken at time of reaction
Tbasal basal level serum tryptase

Table 2
Patient characteristics and basal level serum tryptase for patients with suspected allergic reactions during anesthesia (n=318 unless other specified).

	Patients n (%)	Serum tryptase µg/L Median (IQ range)	p¤	Age Median (IQ range)	p§
Overall group	318 (100)	4.56 (3.14 - 6.60)		50 (36-62)	
Agegroup					
<60 yrs	217 (68.2)	4.13 (2.90 - 6.26)	0.0003	41 (32-51)	N/A
≥60 yrs	101 (31.8)	5.45 (3.53 - 8.76)		67 (63-72)	
Sex					
female	179 (56.3)	4.06 (2.92 - 5.99)	0.0007	43 (33-57)	<0.0001
male	139 (43.7)	5.44 (3.49 - 7.64)		58 (45-66)	
ASA classification (n=317)					
I	127 (40.1)	4.10 (2.62 - 6.18)	0.0093	38 (29-50)	<0.0001
II	147 (46.4)	4.68 (3.38 - 7.54)		57 (44-66)	
III	42 (13.2)	5.25 (3.48 - 7.63)		63 (56-69)	
IV	1 (0.3)	11.8		54	
Reaction class					
1	90 (28.3)	4.09 (2.64 - 5.95)	0.006	40 (30-56)	<0.0001
2	79 (24.9)	4.24 (2.70 -7.71)		46 (34-63)	
3	139 (43.7)	5.02 (3.51 - 7.21)		56 (46-66)	
4	10 (3.1)	5.70 (2.93-8.82)		55.5 (46-60)	
Basal level tryptase					
< 11.4 µg/L	292 (91.8)	4.23 (2.97 - 6.16)	N/A	49 (36-62)	0.12
≥ 11.4 µg/L	26 (8.2)	14.0 (11.9 - 17.4)		61 (33-70)	
Investigation result (n=299)					
positive	142 (47.5)	4.64 (3.05 - 6.50)	0.667	50 (37-62)	0.868
negative	157 (52.5)	4.35 (3.38 - 6.48)		50 (37-62)	
Surgery type (n=315)					
cardiovascular	36 (11.4)	5.43 (3.37 - 8.93)	0.0301	61.5 (55-66)	<0.0001
endocrine	23 (7.3)	4.06 (3.14 - 9.18)		56 (50-66)	
gut	44 (14)	5.25 (3.26 - 8.79)		51.5 (37-62.5)	
gynecological	51 (16.2)	3.99 (2.15 - 5.28)		35 (29-42)	
head and neck	29 (9.2)	4.64 (3.45 - 5.86)		46 (33-60)	
organ	15 (4.8)	3.58 (2.39 - 5.99)		43 (30-55)	
orthopedic	50 (15.9)	4.25 (2.92 - 6.60)		50 (40-60)	
urological	35 (11.1)	5.98 (3.79 - 7.76)		66 (43-72)	
other	32 (10.1)	4.99 (3.37 - 6.44)		48 (36.5-57)	

¤ unpaired t-test or ANOVA after logarithmic transformation
§ unpaired t-test or ANOVA
American Society of Anaesthesiologists physical status classification (ASA):
Class I healthy patient, no medical problems;
Class II mild systemic disease;
Class III severe systemic disease, but not incapacitating;
Class IV severe systemic disease that is a constant threat to life

Table 3
Risk factors for severe reactions in patients with suspected allergic reactions during anaesthesia using multiple logistic regression (n = 299)

Variable	p value	Odds ratio	95% CI
Tbasal > 11.4 μg/L	0.0079	4.815	1.509 - 15.367
Male sex	0.0328	1.709	1.045 - 2.793
Age (per year)	0.0131	1.022	1.005 - 1.039
Positive investigation result in DAAC	0.0055	1.917	1.211 - 3.036
ASA (ASA 3 vs ASA 1)	0.0194	3.690	1.477 - 9.259
ASA (ASA 3 vs ASA 2)		2.513	1.078 - 5.848

All variables were included in the model simultaneously.
Due to small numbers reaction ASA group 3 and 4 were added together.
DAAC Danish Anaesthesia Allergy Centre
ASA American Society of Anesthesiologist's physical status classification
T_{basal} Basal level serum tryptase

Figure legends

Figure 1. Time between serum tryptase (T_{react}) sample and reaction in patients with suspected allergic reaction during anaesthesia, testing positive (n=93) and negative on subsequent investigations (n=87).

Figure 2. Serum tryptase at the time of reaction (T_{react}) by reaction class and result of subsequent investigations.

Figure 3. Serum tryptase at the time of reaction (T_{react}) by allergen found on subsequent positive investigations.

Figure 4. Difference between serum tryptase at the time of reaction and basal level serum tryptase (T_{react} - T_{basal}) by age and result of subsequent investigations.

Figure 5. Basal level serum tryptase (T_{basal}) in 318 patients with suspected allergic reactions during anaesthesia divided into ten-year age groups.

Figure 1

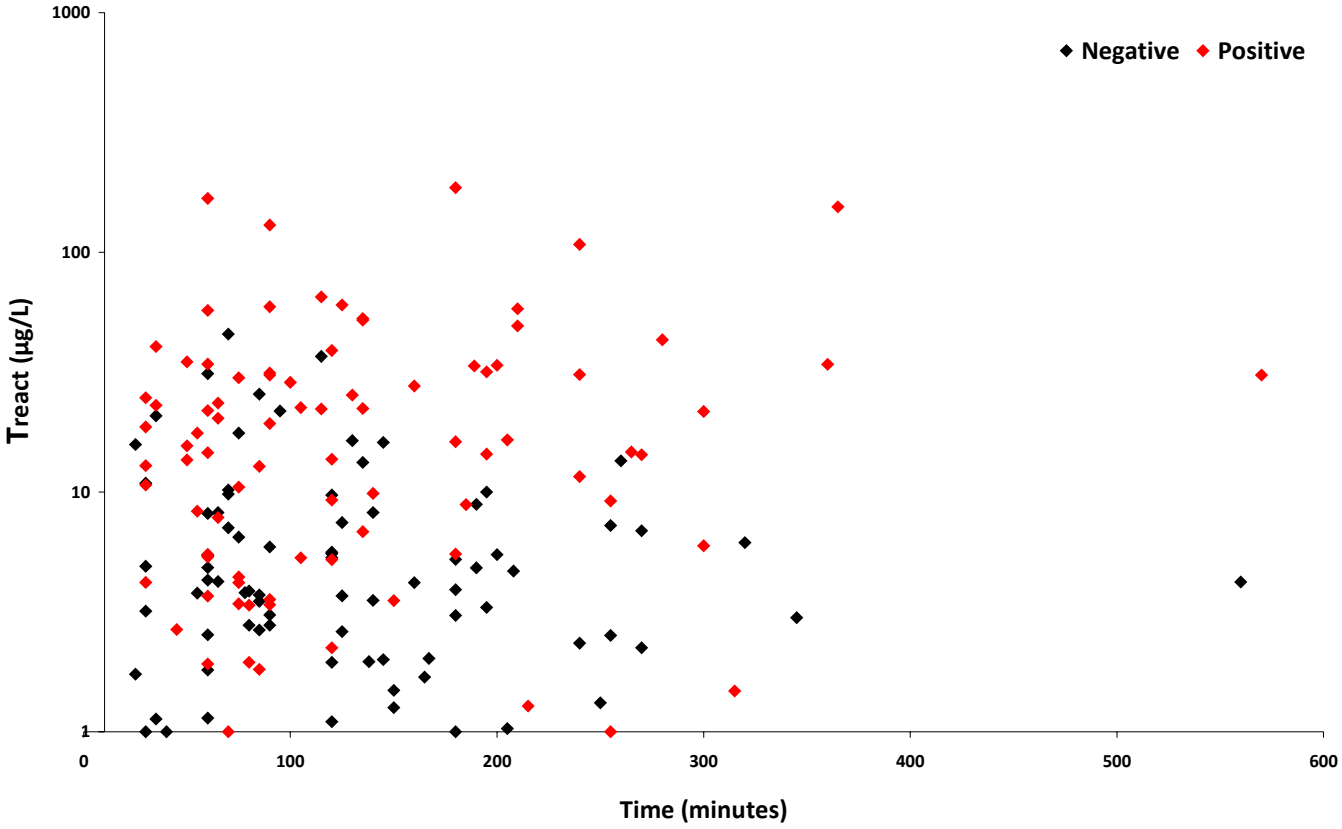

1000
100
10
1
Treact (µg/L)
Negative
Positive
0
100
200
300
400
500
600
Time (minutes)

Figure 2

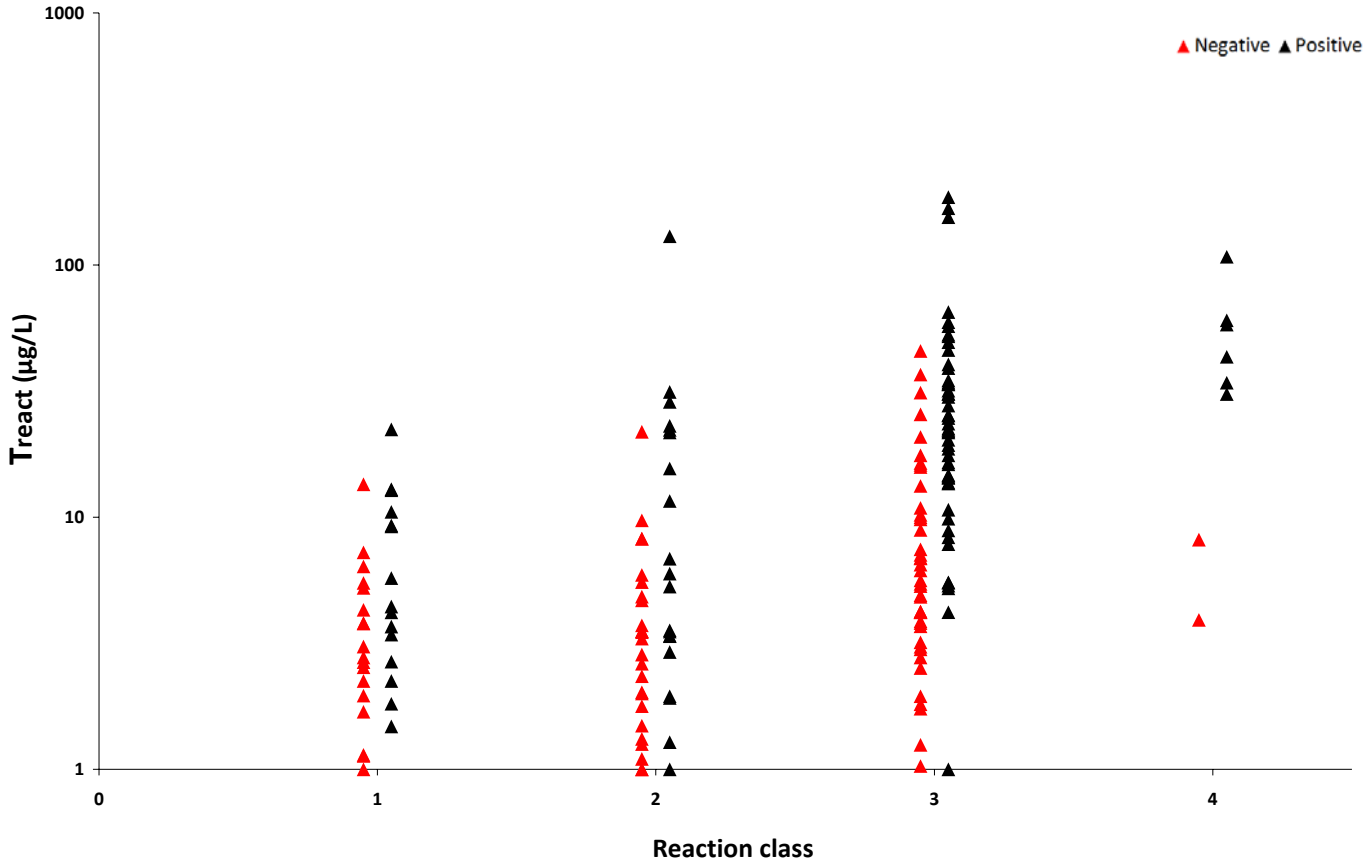

1000
100
10
1
Treact (µg/L)
Negative
Positive
0
1
2
3
4
Reaction class

Figure 3

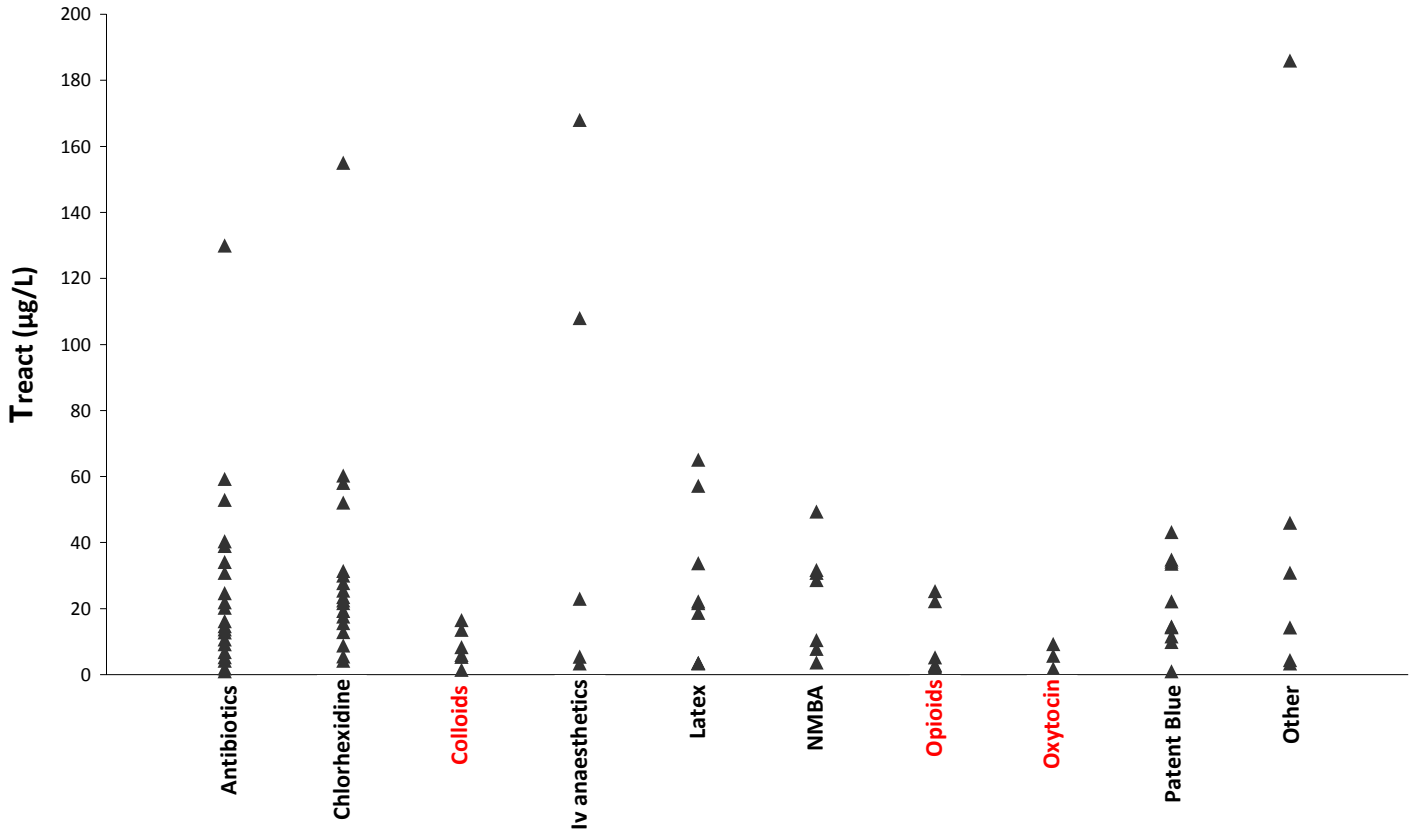

Treact (µg/L)
200
180
160
140
120
100
80
60
40
20
0
Antibiotics
Chlorhexidine
Colloids
Iv anaesthetics
Latex
NMBA
Opioids
Oxytocin
Patent Blue
Other

Figure 4

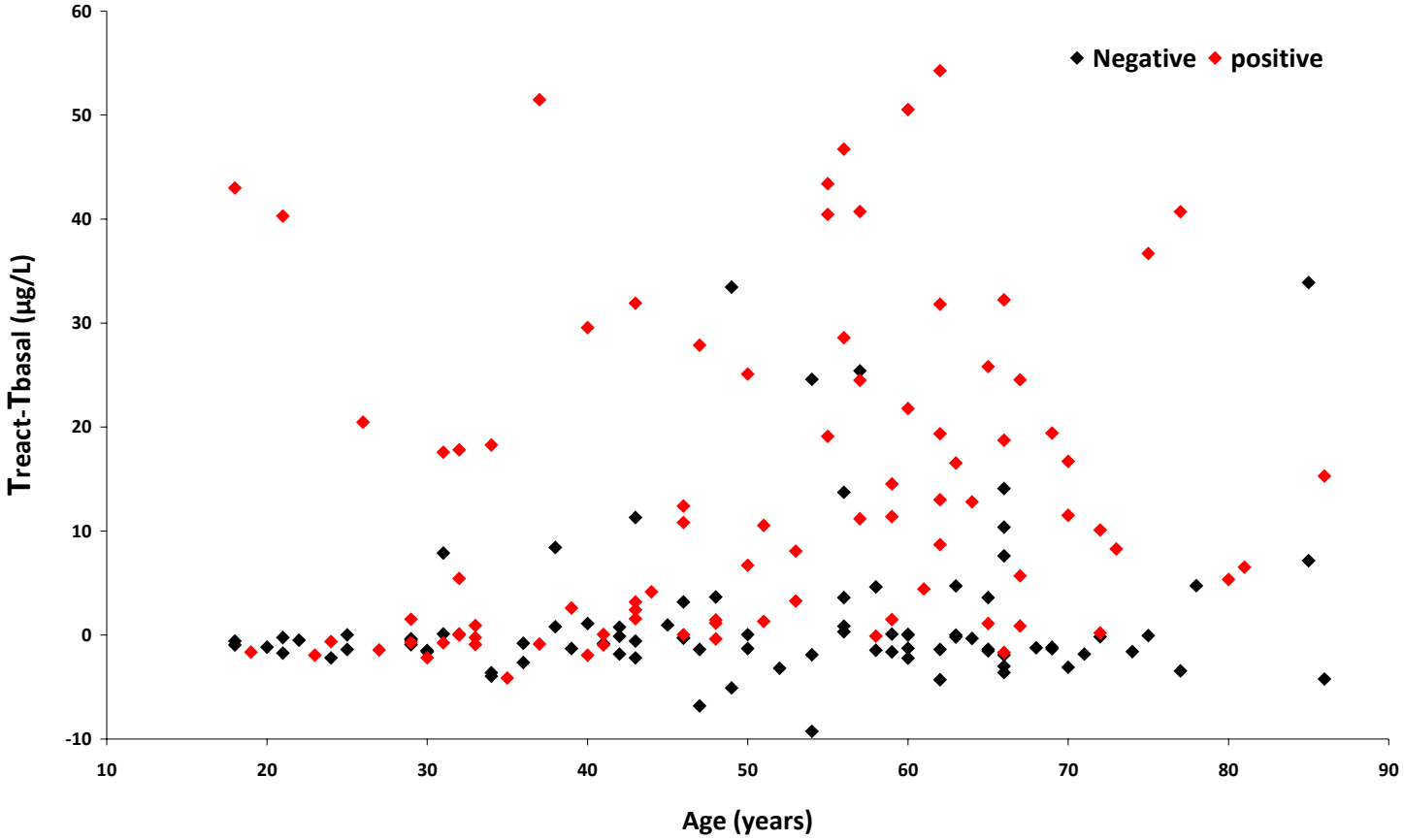
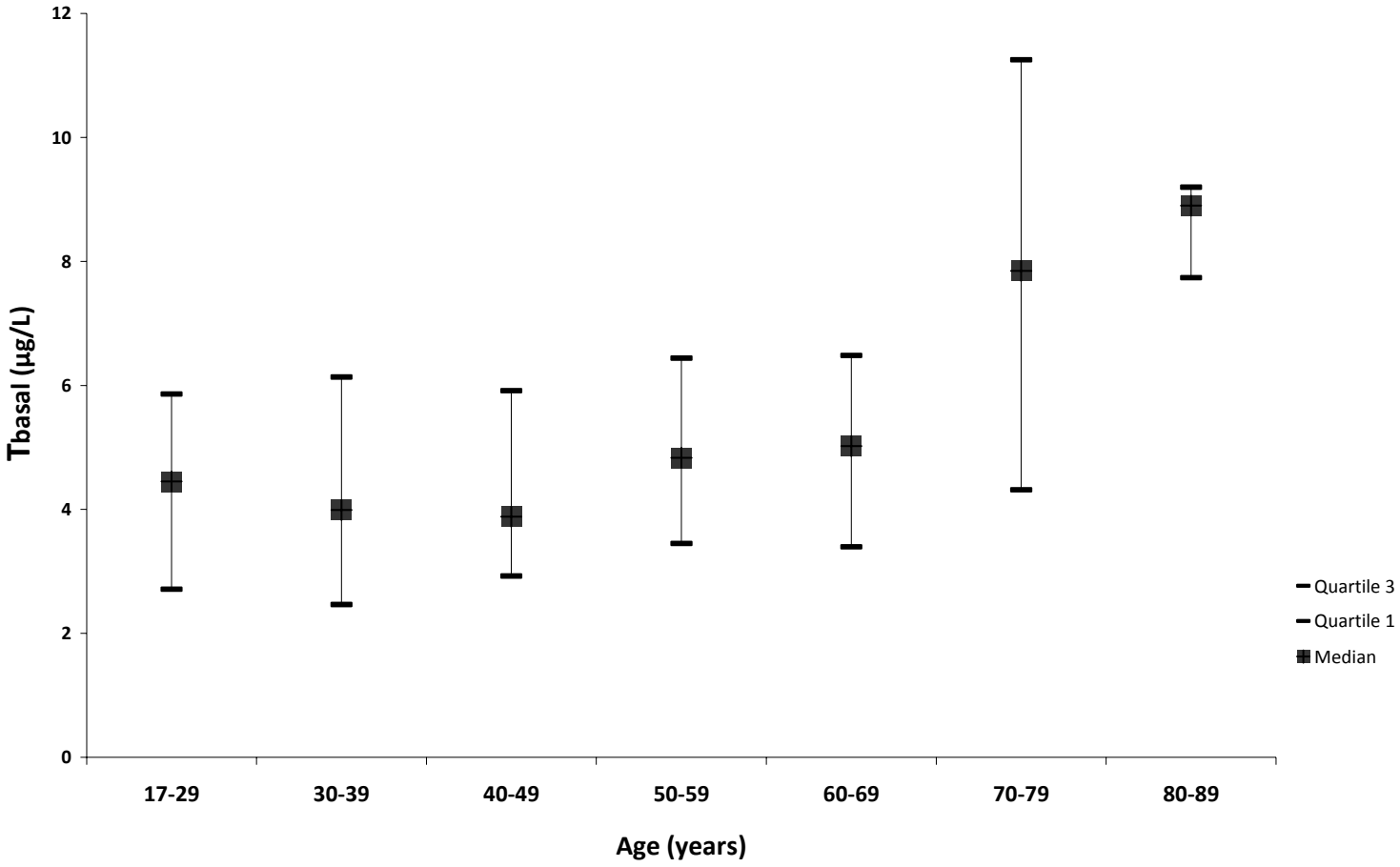

Negative
positive
Treact-Tbasal (µg/L)
Age (years)
60
50
40
30
20
10
0
-10
10
20
30
40
50
60
70
80
90

Figure 5


12
10
8
6
4
2
0
Tbasal (µg/L)
17-29
30-39
40-49
50-59
60-69
70-79
80-89
Age (years)
Quartile 3
Quartile 1
Median

Paper IV

Treatment with epinephrine (adrenaline) in suspected anaphylaxis during anesthesia in Denmark

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Abbreviated title: Use of epinephrine in anaphylaxis during anesthesia

Summary statement: Anaphylaxis during anesthesia is difficult to diagnose, potentially delaying treatment. First line treatment is intravenous epinephrine starting with bolus doses of 0.01mg. Early treatment (<10 min) seems to reduce the risk of needing prolonged treatment.

Abstract

Background

Literature on the use of epinephrine in the treatment of anaphylaxis during anesthesia is very limited. The objective of this study was to investigate how often epinephrine is used in the treatment of anaphylaxis during anesthesia in Denmark and whether timing of treatment is important.

Methods

Retrospective study of 270 patients investigated at the Danish Anaesthesia Allergy Centre after referral due to suspected anaphylaxis during anesthesia. Reactions had been graded by severity C1 mild reactions; C2 moderate reactions; C3 anaphylactic shock with circulatory instability; C4 cardiac arrest. Use of epinephrine, dosage, route of administration and time between onset of circulatory instability and epinephrine administration were noted.

Results

A total of 122 (45.2%) of referred patients had C3 or C4 reactions and of those 101 (82.8%) received epinephrine. Route of administration was iv in 95 (94%) patients. Median time from onset of reported hypotension to treatment with epinephrine was 10 minutes (range 1-70 minutes). Defining epinephrine treatment ≤ 10 minutes after onset of hypotension as early, and > 10 minutes as late, infusion was needed in 12 of 60 patients (20%) treated early vs. 12 of 35 patients (34.3%) treated late (OR 2.087).

Conclusions

Anaphylaxis is often difficult to diagnose during anesthesia and treatment with epinephrine can be delayed as a consequence. Early treatment (<10 min) seems to reduce the need for prolonged treatment with epinephrine. Anaphylaxis should be considered and treated in cases of circulatory instability during anesthesia of no apparent cause, not responding to the usual treatments.

Introduction

Anaphylaxis has been defined as a ”severe life-threatening generalised or systemic hypersensitivity reaction” [1], however there is no universally agreed definition and several other definitions have been proposed.[2-4] It can be triggered by a multitude of factors including foods, venoms and drugs and can also occur in the setting of surgery and anesthesia caused by drugs, latex, disinfectants or other substances used perioperatively.[5]

The incidence of anaphylaxis during anesthesia depends on the definition of anaphylaxis used and has been reported to be in the range of 1:3180 to 1:20.000 anesthetics.[6] Anaphylaxis in the perioperative setting differs from anaphylaxis outside the operating room in several ways. 1) Allergic signs and symptoms may be masked by the effect of anesthesia and surgery or hidden under surgical drapes 2) A large number of drugs and substances are administered simultaneously, making it very difficult to guess which substance caused the reaction[7] 3) The patient is usually fully monitored, has an iv access and is under observation by anesthetic personnel. Thus while the diagnosis of anaphylaxis during anesthesia is difficult to make and the cause will not be immediately obvious, the conditions for optimal management should be present once the diagnosis is made.

The recommended treatment guidelines for anaphylaxis, inside and outside the operating room, are all based on first line treatment with epinephrine.[4, 8-12] Not surprisingly, a recent Cochrane review could find no randomised or quasi-randomised trials of the use of epinephrine in anaphylaxis.[13] However, in anaphylaxis outside the operating room delayed injection of epinephrine has been reported to be associated with mortality.[14,15] Only limited information on the management of anaphylaxis is available in the literature. A recent systematic review of gaps in the management of anaphylaxis covering a large number of databases over a time period spanning 1966-2008 could only identify 59 relevant studies.[16] In addition, very little is known of the management of anaphylaxis by anesthetists. One Danish study from a full-scale anesthesia simulator concluded that in anaphylaxis scenarios the

diagnosis was made late by all teams and that no team had a structured plan for treatment.[17] Another study from the Australian Incident Monitoring Study concluded that there was a striking reluctance to administer epinephrine.[18]

In the Danish Anaesthesia Allergy Centre (DAAC) detailed information on the treatment of suspected anaphylaxis in patients referred for investigation has been collected since 1999.

The aim of this retrospective study was to describe the use of epinephrine in the treatment of suspected anaphylaxis during anesthesia in Denmark 1999-2008, with regard to dose, route of administration and timing of administration.

Materials and methods

Retrospective study of 270 adult patients investigated in DAAC after referral due to suspected anaphylaxis during anesthesia in the period 1999-2008. On referral, reactions were graded into reaction classes by one of two anesthesiologists using following classification: C1 - mild reactions, usually resolving spontaneously, C2 - more severe reactions, resolving within 10-20 min with/without treatment, C3 - anaphylactic shock usually requiring epinephrine to restore circulatory stability and C4 - cardiac arrest.

Details of treatment including use of epinephrine and other drugs, route of drug administration, dosage and time between onset of circulatory instability and epinephrine administration, were retrieved from referral papers filled in by the referring anesthetists, at the time of referral. Total doses of epinephrine were based on cumulated bolus doses only, as cumulated doses of infused epinephrine were not reported by referring anesthetists. It was also noted whether serum tryptase had been measured in connection with the reaction.

The need for epinephrine infusion was used as a surrogate parameter for a severe prolonged reaction. Epinephrine treatment ≤ 10 minutes after onset of hypotension was defined as early and epinephrine treatment > 10 minutes after onset of hypotension was defined as late. This definition was made on the presumption, that it takes up to 10 minutes to realise the lack of effect of the usual treatment modalities for hypotension during anesthesia such as ephedrine, phenylephrine, fluids, decrease in anesthetic dose etc and subsequently suspect the diagnosis of anaphylaxis.

Statistical analysis

Continuous data were reported as median and range and statistical analysis of differences in age within gender, ASA group and reaction class was performed using either unpaired Student t-test or Analysis

of Variance (ANOVA). Analysis of data on serum tryptase was performed after logarithmic transformation, to approximate a normal distribution.

P-values of < 0.05 were considered statistically significant. All analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

A total of 270 (158F/112M) patients with a median age of 49.5 yrs (range 17-86) were included in the study and patient characteristics can be seen in table 1. There were more females (58.5%) and median age was significantly lower in females than in males with a median of 43 yrs (range 17-85) vs 57.5 yrs (range 17-86).

The majority of patients (87.4%) belonged to American Society of Anesthesiologist's physical status classification (ASA) groups 1 and 2 and there was a statistically significant increase in age with increasing ASA classification.

Severe reactions (C3 and C4) were seen in a total of 122 patients (45.2%) and a statistically significant increase in age was seen with increasing reaction class.

Across all four reaction classes a total of 123 patients (45.6%) received epinephrine (table 2). There was an increase in the proportion of patients receiving epinephrine with increasing reaction class with 82.3% and 88.9% receiving epinephrine in C3 and C4 reactions, respectively. Median epinephrine doses by reaction class showed a trend towards increasing doses with increasing reaction class, but this did not reach statistical significance.

The preferred route of administration was the intravenous route and table 3 shows an overview of route of administration by reaction class. The inhalational route was primarily used in milder reaction classes (for cases of localised oedema of the airways) and the intravenous route was used in cases with circulatory symptoms C3 and C4 reactions. A total of 95 of 101 C3 and C4 reactions (94%) were treated with intravenous epinephrine. Median time to treatment with epinephrine for these 95 patients was 10 minutes (range 1-70 minutes). Treatment with epinephrine was given early ($\leq$ 10 minutes after onset of hypotension) in 60 of 95 patients (63.2%) and late (> 10 minutes after onset of hypotension) in 35 of 95 patients (36.8%). Median times to treatment with epinephrine were 5 minutes (range 1-10) and 20 minutes (range 15-70) for the early and late groups respectively.

Four-by four table of timing of treatment with epinephrine and the need for intravenous infusion of epinephrine is shown in table 4. An OR=2.087 (95% CI 0.814-5.353) was found for needing intravenous infusion when treatment with epinephrine was instigated > 10 minutes after onset of hypotension. There was no significant difference in the distribution of age and gender between groups treated early and late. A serum tryptase was taken in 70 of 95 cases (73.7%) and median serum tryptase was 16.5 µg/l (IQ range 5.8-32.8) in patients treated early vs 16.1µg/l (IQ range 4.8-25.6) in patients treated late (p=0.72), thus no difference was found.

In 33 out of 123 cases (26.8%) across all four reaction classes antihistamine and steroid treatment were given **before** treatment with epinephrine (table 5). For C3 and C4 reactions only, a total of 17 out of 101 patients (16.8%) with hypotension were treated with antihistamine and steroid **before** treatment with epinephrine. In most C3 reactions other vasoactive drugs such as ephedrine and phenylephrine were first line treatment (data not shown), except in 22 cases (23.7%) where epinephrine was the first vasoactive drug administered.

Discussion:

This is the first descriptive study of the use of epinephrine in the treatment of anaphylaxis during anesthesia. It is also the first study to include the timing aspect of epinephrine administration in the treatment of anaphylaxis. Our study indicates, that anaphylaxis is difficult to diagnose during anesthesia and that treatment with epinephrine can be delayed as a consequence. Late treatment (> 10 min) seems to increase the risk of needing prolonged treatment with epinephrine (OR 2.087), but this finding did not reach statistical significance (95% CI 0.814-5.353), probably due to the small number of patients studied. Other factors may contribute to the delay in treatment, such as a reluctance to administer epinephrine, even when the diagnosis of anaphylaxis has been made and antihistamine and steroids have been given. Epinephrine was administered in 82.1 % of C3 and C4 reactions and in 94% of these cases, it was given by the intravenous route. However, median doses used in C3 reactions of 0.2 mg (range 0.002-2.0mg) were high compared to current recommendations of an intravenous start dose of 0.01-0.05 mg.[8] In milder C1 and C2 reactions with eg. skin symptoms only or self-limiting tachycardia or bronchospasm a first line treatment with antihistamine and steroids would be acceptable. However, the patient should be observed for progression in symptoms and relevant doses of epinephrine should be made ready for use. Our study showed that in 16 out of 22 (72.7%) C1 and C2 reactions where epinephrine was needed, it was administered after antihistamines and steroids.

All patients in our study survived. In our study population we are only aware of one incidence of long-term sequelae in a 47-year old woman who developed hemiplegia following a prolonged resuscitation attempt. However, we have not looked at other consequences for patients such as prolonged admissions, unplanned admission to the intensive care unit, cancelled operations or changes to indications for surgery, which are likely to occur following anaphylaxis during anesthesia.

Post mortem studies of anaphylaxis conclude that severe reactions progress rapidly and are more likely to have a fatal outcome in older patients with comorbidity such as ischemic heart disease.[14] A post

mortem study from the UK reported that in cases where the allergen was administered intravenously (including anesthetic reactions) presentation was more likely to be as shock with a median time to cardiac arrest of 5 minutes from administration of the allergen.[15] The same study looked at treatment given during reactions and reported that in the 55 reactions to drugs, treatment with epinephrine was given before the cardiac arrest in only 16% of cases. In 73% of cases epinephrine was administered after cardiac arrest occurred and in the remaining 11% epinephrine was not administered at all. In contrast, a few cases of inappropriately high doses of epinephrine (1-2,5 mg given intravenously) administered for relatively mild reactions also had a fatal outcome, related to side effects of epinephrine. This has been reported before.[19]

The rare and unexpected occurrence of anaphylaxis combined with lack of knowledge of the correct treatment, especially with regards to dosage and route of administration of epinephrine thus, unfortunately, still leads to deaths. Only few studies have looked at the knowledge of treatment with epinephrine among doctors. A questionnaire study of 78 doctors starting in training posts in emergency medicine showed that 100% would use epinephrine in the treatment of anaphylaxis but only 5% identified the correct dose and route of administration.[20] Conclusions from another, more recent, questionnaire study of 91 doctors working in acute specialties in an Australian hospital were, that 92% would give epinephrine as first line treatment but only 20% knew the correct dose and route of administration. Interestingly, 20% of doctors would administer epinephrine in cardiac arrest doses to a conscious patient.[21]

Despite the fact that most doctors know that epinephrine is the first line treatment of anaphylaxis, retrospective studies of patients with anaphylaxis generally show a reluctance to administer epinephrine. This has been found in reactions to food[22], in emergency departments[23], in paediatric patients[24], in insect venom allergy[25] and in patients with mastocytosis at high risk of developing anaphylaxis.[26] This reluctance to administer epinephrine is even reflected in our study of the treatment

of anaphylaxis during anesthesia, where anesthesiologists chose to administer antihistamines and steroids before epinephrine in 16.8%, and did not administer epinephrine at all in 17.2% of C3 and C4 reactions with cardiovascular instability. As there is no proven benefit of antihistamines and steroids in anaphylactic shock[27,28] the administration of epinephrine should always precede other drug treatments once the diagnosis of anaphylaxis has been made.[8]

Only one other study could be found examining the management of anaphylaxis during anesthesia[18] and the authors reported that anesthetists showed a striking reluctance in administering epinephrine as an appropriate early intervention. This study also showed that antihistamines and steroids conferred no separate benefit in the acute phase. One of the main conclusions was, that anesthesiologists should always suspect anaphylaxis in cases of sudden, unexpected or severe hypotension.

Our study indicates, that in cases where the diagnosis is not made or treatment with epinephrine is delayed for other reasons, the risk of needing prolonged treatment seems to be increased. This is often mentioned in the literature but very little evidence can be found. The only studies come from experimental animal models of anaphylaxis, which have shown conflicting results. Some studies showed that a single bolus dose of epinephrine did not hasten recovery in dogs[29,30] and another study showed that early (< 5 min) bolus doses of epinephrine followed by titrated infusion of epinephrine provided the best survival in rats.[31] It could be speculated that in untreated cases or cases treated late, the immune response is magnified by the prolonged release of inflammatory mediators. In our study there was no difference between serum tryptase values in reactions treated early or late. This could indicate, that tryptase release is related to initiation of the allergic reaction and initial reaction severity, but not to the prolonged allergic response. However, this has never been reported before and remains speculative.

No human studies of timing of treatment of anaphylaxis could be found, but a study of litigations related to drug errors in anesthesia in the UK in the period 1995-2007 may be interpreted to show a worse outcome in patients treated late.[32] In this study anaphylaxis was reported in 31 cases and in 20 of these a drug was given despite a known allergy; there were no deaths or sequelae in this group. However, in the remaining 11 cases the cause of the reaction was unknown and thus the reaction was unexpected. In that group there were 5 deaths and 4 cardiac arrests with 2 resulting in severe neurological damage. One could speculate, that when a drug is given despite a known allergy it is either an oversight or a conscious decision on part of the anesthesiologist deciding to give a test dose. In both cases a rapid diagnosis of anaphylaxis is likely to be made due to a high index of suspicion and consequently treatment will be instigated promptly. On the other hand unexpected anaphylaxis occurring during anesthesia is difficult to diagnose and the delay in diagnosis and treatment might be speculated to be the cause of the poorer outcome in this group. Therefore, as previously suggested[18] in cases of sudden, severe hypotension during anesthesia, not responding to the usual treatment with ephedrine and fluids, anaphylaxis should be suspected and treatment with epinephrine considered. Intravenous bolus doses of epinephrine starting at 0.01-0.05 mg (10-50 µg) should be administered.[8] It is recommended, that the intravenous route for epinephrine treatment should be reserved for monitored patients and for specialists with experience in this treatment ie anesthetists/intensivists/physicians in emergency departments. In all other circumstances the recommendation is to use intramuscular epinephrine.[4,33]

In the UK post-mortem study 56 % of fatal reactions to drugs occurred in an operating theatre ie. with a fully monitored patient, with iv access and anesthetic personnel within reach.[15] There are no recent similar studies of deaths related to anaphylaxis in Denmark, but we are not aware of any fatalities caused by anaphylaxis during anesthesia in Denmark in our study period 1999-2008. A closed claims

study analysing deaths related to anesthesia in the period 1996-2004 in Denmark did not identify any cases of suspected perioperative anaphylaxis leading to a claim in the patient insurance association.[34] In conclusion, anaphylaxis during anesthesia is difficult to diagnose. As prompt and correct treatment seems a prerequisite for a good outcome it is imperative that anesthetic personnel are trained to diagnose and treat anaphylaxis in the difficult setting of surgery and anesthesia. As the first line treatment is epinephrine, which has a narrow therapeutic window, it is important that anesthetic personnel is familiar with correct dosing and route of administration. Although our study shows, that anesthetic personnel on the whole manage anaphylaxis satisfactorily there is room for improvement regarding the early use of titrated doses of intravenous epinephrine in suspected anaphylaxis during anesthesia.

References

1. Johansson SGO, Bieber T, Dahl R, Freidmann PS, Lanier BQ, Lockey RF, Motala C, Ortega Martell JA, Platts-Mills TAE, Ring J, Thien F, Van Cauwenberge P, Williams HC. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Comittee of the World Allergy Organization, October 2003. J Allergy Clin Immunol 2003; 113: 832-6

2. Sampson HA, Muñoz-Furlong A, Campbell RL, Adkinson NF, Bock SA, Branum A, Brown SGA, Camargo CA, Cydulka R, Galli SJ, Gidudu J, Gruchalla RS, Harlor AD, Hepner DL, Lewis LM, Lieberman PL, Metcalfe DD, O'Connor R, Muraro A, Rudman A, Schmitt C, Scherrer D, Simons FE, Thomas S, Wood JP, Decker WW. Second symposium on the definition and management of anaphylaxis: Summary report – Second National Institute of Allergy and infectious Disease/Food Allergy and Anaphylaxis Network Symposium. J Allergy Clin Immunol 2006; 117: 391-7

3. Rüggeberg JU, Gold MS, Bayas JM, Blum MD, Bonhoeffer J, Friedlander S, de Souza Brito G, Heininger U, Imuokhuede B, Khamesipour A, Erlewyn-Lajeunesse M, Martin S, Mäkelä M, Nell P, Pool V, Simpson N. The Brighton Collaboration Anaphylaxis Working Group. Anaphylaxis: Case definition and guidelines for data collection, analysis, and presentation of immunization safety data. Vaccine 2007; 25: 5675-84

4. Simons FER. Anaphylaxis. J Allergy Clin Immunol 2010; 125: S161-81

5. Mertes PM, Lambert M, Guéant-Rodriquez RM, Aimone-Gastin I, Mouton-Faivre C, Moneret-Vautrin DA, Guéant JL, Malinovsky JM, Demoly P. Perioperative anaphylaxis. Immunol Allergy Clin N Am 2009; 29: 429-451

6. Malinovsky JM, Decagny S, Wessel F, Guilloux L, Mertes PM. Systematic follow-up increases incidence of anaphylaxis during adverse reactions in anesthetized patients. Acta Anaesthesiol Scand 2008; 52: 175-81

7. Krøigaard M, Garvey LH, Menné T, Husum B. Allergic reactions in anaesthesia: are suspected causes confirmed on subsequent testing? Br J Anaesth 2005; 4: 468-71

8. Krøigaard M, Garvey LH, Gillberg L, Johansson SGO, Mosbech H, Florvaag E, Harboe T, Eriksson LI, Dahlgren G, Seeman-Lodding H, Takala R, Wattwil M, Hirlekar G, Dahlén B, Guttormsen AB. Scandinavian Clinical Practice Guidelines on the diagnosis, management and follow-up of anaphylaxis during anaesthesia. Acta Anaesthesiol Scand 2007; 51: 655-670

9. Mertes PM, Laxenaire MC, Lienhart A, Aberer W, Ring J, Pichler WJ, Demoly P, Working group for the SFAR, ENDA, EAACI interest group on drug hypersensitivity. Reducing the risk of anaphylaxis during anaesthesia: guidelines for clinical practice. J Investig Allergol Clin Immunol 2005; 15: 91-101.

10. The Association of Anaesthetists of Great Britain and Ireland and British Society for Allergy and Clinical Immunology; 2009. Suspected anaphylactic reactions associated with anaesthesia (revised edition 2009). Anaesthesia 2009; 64: 199-211

11. Kemp SF, Lockey RF, Simons FER, on behalf of the WAO ad hoc Committee on Epinephrine in Anaphylaxis. Position Paper. Epinephrine: the drug of choice for anaphylaxis. A statement of the World Allergy Organization. Allergy 2008; 63: 1061-70

12. Soar J, Pumphrey R, Cant A, Clarke S, Corbett A, Dawson P, Ewan P, Foëx B, Gabbott D, Griffiths M, Hall J, Harper N, Jewkes F, Maconochie I, Mitchell S, Nasser S, Nolan J, Rylance G, Sheikh A, Unsworth DJ, Warrell D Working Group of the Rescusitation Council UK. Statement paper. Emergency treatment of anaphylactic reactions – Guidelines for healthcare providers. Resuscitation 2008; 77: 157-9

13. Sheikh A, Shehata YA, Brown SGA, Simons FER. Adrenaline in the treatment of anaphylaxis: Cochrane systematic review. Allergy 2009; 64: 204-12

14. Greenberger PA, Rotskoff BD, Lifschultz B. Fatal Anaphylaxis: post mortem findings and associated comorbid diseases. Ann Allergy Asthma Immunol 2007; 98: 252-7

15. Pumphrey RSH. Lessons for the management of anaphylaxis from a study of fatal reactions. Clin Exp Allergy 2000; 30: 1144-50

16. Kastner M, Harada L, Waserman S. Gaps in anaphylaxis management at the level of physicians, patients and the community: a systematic review of the literature. Allergy 2010; 65: 435-44

17. Jacobsen J, Lindekær AL, Østergaard HT, Nielsen K, Østergaard D, Laub M, Jensen PF, Johannessen N. Management of anaphylactic shock evaluated using a full-scale anaesthesia simulator. Acta Anesthesiol Scand 2001; 45: 315-9

18. Currie M, Kerridge RK, Bacon AK, Williamson JA. Crisis management during anaesthesia: anaphylaxis and allergy. Qual saf Health Care 2005; 14:e19

19. Johnston SL, Unsworth J, Gompels MM. Adrenaline given outside the context of life threatening allergic reactions. BMJ 2003; 326: 589-90

20. Gompels LL, Bethune C, Johnston SL, Gompels MM. Proposed use of adrenaline (epinephrine) in anaphylaxis and related conditions: a study of senior house officers starting accident and emergency posts. Postgrad Med J 2002; 78: 416-18

21. Thain S, Rubython J. Treatment of anaphylaxis in adults: results of a survey of doctors a Dunedin Hospital, New Zealand. NZMJ 2007; 120: U2492

22. Le TM, van Hoffen E, Pasmans SG, Bruijnzeel-Koomen CAFM, Knulst AC. Suboptimal management of acute food-allergic reactions by patients, emergency departments and general practitioners. Allergy 2009; 64: 1226-35

23. Wang J, Sampson HA. Food anaphylaxis. Clin exp allergy 2007; 37: 651-60

24. Mehl A, Wahn U, Niggemann B. Anaphylactic reactions in children – a questionnaire-based survey in Germany. Allergy 2005; 60: 1440-5

25. Clark S, Long AA, Gaeta TJ, Camargo CA. Multicenter study of emergency department visits for insect allergies. J Allergy Clin Immunol 2005; 116: 643-9

26. Brockow K, Jofer C, Behrendt H, Ring J. Anaphylaxis in patients with mastocytosis: a study on history, clinical features and risk factors in 120 patients. Allergy 2008; 63:226-32

27. Sheikh A, ten Broek V, Brown SGA, Simons FER. H_1-antihistamines for the treatment of anaphylaxis: Cochrane systematic review. Allergy 2007; 62: 830-7

28. Andreae DA, Andreae MH. Should antihistamines be used to treat anaphylaxis? BMJ 2009; 339: b2489

29. Mink SN, Bands C, Becker A, Elkin J, Sharma S, Unruh H, Kepron W. Effect of bolus epinephrine on systemic hemodynamics in canine anaphylactic shock. Cardiovascular research 1998; 40: 546-56

30. Bautista E, Simons FER, Simons KJ, Becker AB, Duke K, Tillett M, Kepron W, Mink SN. Epinephrine fails to hasten hemodynamic recovery in fully developed canine anaphylactic shock. Int Arch Allergy Immunol 2002; 128: 151-64

31. Dewachter P, Raëth-Fries I, Jouan-Hureaux V, Menu P, Vigneron C, Longrois D, Mertes PM. A comparison of epinephrine only, arginine vasopressin only and epinephrine followed by arginine vasopressin on the survival rate in a rat model of anaphylactic shock. Anesthesiology 2007; 106: 977-83

32. Cranshaw J, Gupta KJ, Cook TM. Litigation related to drug errors in anaesthesia: an analysis of claims against the NHS in England 1995-2007. Anaesthesia 2009; 64: 1317-23

33. Mirakian R, Ewan PW, Durham SR, Youlten LJ, Dugué P, Friedmann PS, English JS, Huber PA, Nasser SM; BSACI. BSACI guidelines for the management of drug allergy. Clin exp allergy 2009; 39: 43-61.

34. Hove LD, Steinmetz J, Krogh Christoffersen J, Møller A, Nielsen J, Schmidt H. Analysis of deaths related to anesthesia in the period 1996-2004 from closed claims registered by the Danish Patient Insurance Association. Anesthesiology 2007; 106: 675-80

Table 1
Characteristics of included patients with suspected anaphylaxis during anesthesia.

	Patients n (%)	Age Median (range)	p¤
All patients	270 (100)	49.5 (17-86)	
Gender			
Female	158 (58.5)	43.0 (17-85)	< 0.0001
Male	112 (41.5)	57.5 (17-86)	
ASA classification			
I	105 (38.9)	38.0 (17-76)	< 0.0001
II	131 (48.5)	56.0 (18-86)	
III	34 (12.6)	62.5 (45-78)	
IV	0	0	
Reaction class			
1	83 (30.7)	40 (17-78)	< 0.0001*
2	65 (24.1)	46 (18-86)	
3	113 (41.9)	56 (18-86)	
4	9 (3.3)	57 (33-62)	

¤ unpaired t-test or ANOVA
* reaction class 3 and 4 added together for statistical analysis

Table 2
Number of cases and total dose of epinephrine administered in patients with anaphylaxis during anesthesia. Information on dose available for n=102 out of 270.

Reaction class	Epinephrine given	Total epinephrine dose in mg	
	n (%)	n	Median (range)
1	4/83 (4.8)	1	0.01
2	18/65 (27.7)	10	0.125 (0.03-1.0)
3	93/113 (82.3)	83	0.2 (0.002-2.0)
4	8/9 (88.9)	8	1.95 (0.6-2.0)
Total	123/270 (45.6)	102	

Table 3
Route of administration of epinephrine in suspected anaphylaxis during anesthesia and severity of reaction.

Route of administration	Reaction class				Total
	4	3	2	1	
Intravenous	8	87	10	1	106
Intramuscular	0	2	2	0	4
Subcutaneous	0	1	1	0	2
Inhalation	0	2	5	3	10
Not known	0	1	0	0	1
Total	8	93	18	4	123

Table 4
Timing of epinephrine treatment in relation to hypotension and need for epinephrine infusion in subgroup of patients with anaphylaxis, class 3 and 4, during anesthesia, treated with iv epinephrine (n=95).

	Need for iv infusion		
	Yes	**No**	**Total**
Timing of epinephrine			
Late (> 10 minutes)	12	23	35
Early (≤ 10 minutes)	12	48	60
Total	24	71	95

OR = 2.087 (95% CI 0.817 - 5.353)

Table 5
Sequence of drug administration in the treatment of anaphylaxis during anesthesia by reaction class (n=123).

Sequence of drug administration	Reaction class				Total
	4	3	2	1	
Epinephrine first drug administered*	4	22	5	0	31
Epinephrine administered ***before*** AH/S	1	45	0	0	46
Epinephrine administered ***after*** AH/S	1	16	12	4	33
AH/S not administered	2	10	1	0	13
Total	8	93	18	4	123

***** Epinephrine administered as first vasoactive drug (before efedrine, phenylephrine etc)
AH Antihistamine
S steroid