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Chlorhexidine Allergy in 4 Specialist Allergy Centres in the UK, 2009-2013: Clinical Features and Diagnostic Tests

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Abbreviations

CI Confidence Interval

NMBA Neuromuscular Blocking Agent

BAT Basophil Activation Test

SPT Skin Prick Test

IDT Intradermal Skin Test

sIgE Specific IgE

kUA/L kilounits (arbitrary) of sIgE per litre

SUMMARY

We describe an observational survey of diagnostic pathways in 104 patients attending four specialist allergy clinics in the UK following perioperative hypersensitivity reactions to chlorhexidine reactions. The majority were life threatening. Men undergoing urological or cardiothoracic surgery predominated. Skin prick testing and sIgE testing were the most common tests used for diagnosis. Fifty-three % of diagnoses were made on the basis of a single positive test. Where multiple tests were performed the sensitivity of intradermal, basophil activation and skin prick testing was 68% (50-86%), 50% (10-90%) and 35% (17-55%) respectively. Seven percent were negative on screening tests initially, and 12 cases were only positive for a single test despite multiple testing. Intradermal tests appeared most sensitive in this context.

Additional sensitisation to other substances used perioperatively, particularly neuromuscular blocking agents (NMBA), was found in 28 patients, emphasising the need to test for possible allergy to all drugs to which the patient was exposed even where chlorhexidine is positive.

Key Words

Chlorhexidine Allergy

Anaesthesia

Skin Tests

Specific IgE

Anaphylaxis

INTRODUCTION

Chlorhexidine is increasingly recognised as a significant allergen in the perioperative setting [1]. We aimed to describe and compare a larger series of cases from multi-centre British specialist allergy clinics [1] [2]. This increase is thought to be driven by increased use of chlorhexidine and increased awareness of allergy, even though there remains some evidence of under-diagnosis [2] [3] [4]. Unlike most perioperative reactions [5], the majority of reported patients have been men, frequently undergoing urological or cardiothoracic surgery in non-UK and single centre studies.

The performance of tests for chlorhexidine allergy has been estimated in single centres and there is published guidance on how to do tests for chlorhexidine allergy [6] [7], but it is not clear if these observations can be generalized to other clinic cohorts or countries [7]. We also set out to determine whether we could estimate sensitivity for the different tests available for diagnosing chlorhexidine allergy in a routine clinical setting and identify the most effective diagnostic strategy for determining sensitisation. Finally, multiple reactivity has been reported in some individuals with well documented chlorhexidine allergy [3]. We evaluate how frequently potentially misleading multiple sensitisation is noted in our clinics.

MATERIALS AND METHODS

Data collection

Data on all patients diagnosed with chlorhexidine allergy was retrospectively collected from records of four regional UK Allergy Centres (Sheffield Teaching Hospitals, Central Manchester University Hospitals NHS Foundation Trust, Southampton University Hospitals, and Leeds Teaching Hospitals) between 2009 and 2015. The patients were seen following routine referral into anaesthetic drug reaction clinics. The investigations carried out were not harmonised across clinics. Many of our series had only one test (most commonly SPT or sIgE) and the first positive test prevented further testing. The sequence of further testing differed between centres (only one offered BAT), between patients and across time (increasing use of IDT in patient who were negative in screening tests in some centres). As the data were collected as part of routine clinical audit, ethics committee approval was not required. Gender, chlorhexidine preparation used, the clinical setting, details of the reaction, investigations performed (skin prick and / or intradermal test, specific IgE and basophil activation test) and final clinical diagnosis were obtained.

Skin prick testing (SPT) was carried out using undiluted clear or pink Hydrex® (Chlorhexidine gluconate Solution 20% BP (Ph Eur) 2.5% v/v, denatured ethanol B 96%, purified water BP, Carmosine (E122)) with positive (histamine 10mg/ml) and negative (normal saline) controls: SPT was positive if a wheal ≥ 3mm than negative control was present at 15- 20 minutes, as reported previously and as per 2011 guidance [[7] [6]. All other drug skin prick tests were carried out in accordance with 2011 guidance. Both pink & clear Hydrex was used to exclude any possible reactions due reactors to the colourant in some centres.

Intradermal testing (IDT) was performed using 20 microlitre injections of chlorhexidine gluconate (clear or pink or both, as appropriate to the clinic) 1:1000 dilution and normal saline, administered on the volar aspect of the forearm. The results were interpreted as previously described [3] [7]. A positive IDT was defined as the mean of orthogonal weal diameters of at least 3mm greater than the negative control, in the presence of a flare [3] [7].

Chlorhexidine sIgE was measured by immunoassay (ImmunoCAP) on the Phadia ImmunoCap 1000 Analyser (Thermo Scientific, Loughborough, UK). A sIgE level >0.35 kUA/L was deemed positive in Sheffield, Leeds and Southampton and ≥ 0.4 kUA/L in Manchester (functionally equivalent to >0.35 as this laboratory reported measurement to a single decimal place only). All laboratories performed daily internal quality control and participated in the UK National External Quality Assurance Scheme for allergen specific IgE with satisfactory performance.

At Sheffield and Southampton, Basophil Activation Tests (BAT, Buhlmann FlowCast, Switzerland) were analysed on a Beckman Coulter EPICS XL flow cytometer. The chlorhexidine used to stimulate the basophils was from the same source as the skin prick tests. Chlorhexidine was used at concentrations of 0.05%, 0.005%, 0.0005% and 0.00005% for Hydrex® "clear" and 0.02%, 0.002% and 0.0002% for Hydrex® "pink". A wide range of concentrations were used to assess the strength of sensitisation and exclude potential irritant or toxic concentrations in patients and controls in view of the lack of experience, harmonisation and validation of this test.

Positive controls (Fc ε RI and fMLP), negative control (background) and a normal volunteer control were performed for each run. Fluorescently labelled antibody to CCR3 was used to identify basophils. Activated basophils were differentiated from resting basophils using a fluorescently labelled antibody to CD63, which only becomes expressed on the cell surface when basophils are activated [8]. A positive response was present if two or more

concentrations gave >5% basophil activation and a stimulation index >2. The stimulation index was calculated by dividing the % of activated basophils at each concentration by the % of activated basophils in the background tube.

Clinical reaction grading was in accordance with international guidance on reactions taking place in the perioperative setting (grade 1: Cutaneous signs, grade 2: Measurable but not life-threatening physiological abnormalities, grade 3: Life-threatening physiological abnormalities, grade 4: Cardiac and/or respiratory arrest) [6].

Patient inclusion criteria

The clinical history of Type I hypersensitivity required involvement of two or more systems with defined symptoms [9]. This diagnosis was made by the submitting clinician. The perioperative period was defined as admission for an invasive procedure, to their discharge or death. In the absence of an agreed diagnostic gold standard for establishing chlorhexidine allergy, or a recognised and harmonised provocation test, we accepted a diagnosis of chlorhexidine allergy when there was a consistent clinical history for Type I hypersensitivity along with one or more positive tests demonstrating sensitization; i.e. the potential for an IgE mediated mechanism had been demonstrated)[7].

Because each patient had different combinations of tests and test specificity was unknown we adopted a pragmatic strategy to assess test performance and compare with previous work. In the absence of a gold standard test, such as provocation, assessment of individual tests to estimate the sensitivity is challenging. Many of our series had only one test and the first positive test prevented further testing. For patients with multiple tests we required a very rigorous demonstration of sensitisation for each test, with at least two additional positive allergy tests, as has previously been reported for chlorhexidine and rocuronium [7] [10]. For

the purposes of estimating individual test sensitivity, the result of the test being assessed for performance was omitted from diagnostic decision-making and results of the remaining tests were used to determine sensitisation status for chlorhexidine. For example, when the sensitivity of SPT was being calculated, results of sIgE, IDT and BAT were used to determine allergy to chlorhexidine (where two confirmatory positive tests present). We were unable to estimate specificity using these data because we did not analyse a series of patients without allergy, to whom the same tests were applied.

RESULTS

Clinical features

134 patients were identified with a clinical diagnosis of chlorhexidine reaction; 18 patients had no positive tests (of whom, 11 had received only one test), one patient had not been tested and 12 patients had no evidence of perioperative reactions (referred because of occupational exposure or unexplained symptoms). These 30 patients were excluded from the analysis.

104 patients met our inclusion criteria having had a probable perioperative anaphylactic reaction to chlorhexidine. 66 patients were men. Details of surgical interventions were available for 70 patients, of whom 16 had cardiac procedures and 13 urological procedures. Other specialties appear to be under-represented; for example, Obstetrics and Gynaecology procedures had only been carried out in 2 patients.

The route of chlorhexidine exposure was reported in 53 patients, of whom 26 had only been exposed to chlorhexidine skin preparations. Three patients had been exposed to chlorhexidine coated central venous catheters (CVC); 3 to sterile lubricating gel (Instillagel^{TM}) and 3 to chlorhexidine mouth wash. One patient had been exposed to chlorhexidine mouth spray only, and the rest to a combination of these products. There were no clear relationships between the type of surgery and the chlorhexidine products used. For example, cardiac patients were

exposed to combinations of chlorhexidine skin preparation, lubricating gel and coated CVCs (data not shown).

The grade of reaction was available in 101 patients [6]. Most were severe grade 3 or 4; including grade 1 (9 patients), grade 2 (12 patients), grade 3 (72 patients) and grade 4 arrests (8 patients). Grade 4 reactions were not associated with any particular type of surgery. One of four patients in our analysis who was only exposed to chlorhexidine mouth wash/spray, experienced grade 4 anaphylaxis.

Hypotension was the commonest individual symptoms and was described in 75 patients. Generalised urticaria was seen in 64, bronchospasm in 33 and angioedema in 21 patients. Localised urticaria was present in 3 and generalised flushing in 11. There was no relationship between the presence of individual symptoms and different types of operation.

Details of the timing of reactions were only available in 19 patients: 15 were described as "immediate perioperative" (i.e. within 15 minutes") and only 4 were delayed at 30 mins (grade 1 reaction), 30, 45 and 90 minutes (all grade 3 reactions). Sequential mast cell tryptase results were available in 11 of these cases and showed a rise above baseline in 10 (not shown).

Test results for entire series

Because there was no harmonised testing pathway, different numbers and combinations of tests were used. Skin prick test (SPT) were most common, performed in 93/104 patients and positive in 72 (77%). Specific IgE (sIgE) was assayed in 78/104 patients and positive in 62 (80%). Intradermal testing (IDT) was performed in 23/104 and positive in 21 (91%). Basophil activation testing (BAT) was performed in 6 patients and positive in 3 (50%). The distribution of positive tests is shown in Figure 1. The mean sIgE levels and positive allergy tests for other substances for all 104 patients are shown in Table 1.

Results for patients who had three or more tests

Figure 2 shows the distribution of positive tests amongst the 25 patients who had three tests. Table 2 shows the mean sIgE levels and positive allergy tests for other substances for these patients.

For the analysis of the 25 patients who had three tests, we used 2 positive tests as a gold standard for making the diagnosis of chlorhexidine allergy in the presence of definitive sensitisation. Using this approach, we were able to estimate the sensitivity and demonstrate that all three test modalities should be used when the screening test is negative, as shown in Table 3.

Sensitisation to other potential triggers

28/104 chlorhexidine allergic patients had evidence of reactivity to other potentially relevant allergens, including neuromuscular blocking agents (NMBA, 17 patients), morphine (4 patients) and a small number of other agents (see Table 1).

Some patients had extreme multi-reactivity; for example, one patient who had two grade three anaphylactic reactions during orthopaedic procedures had positive SPT and sIgE to chlorhexidine (10.4 kUA/L) also had positive rocuronium IDT, and positive sIgE to suxamethonium, morphine and amoxicillin.

NMBA positive patients appeared more likely to be chlorhexidine sIgE positive than NMBA negative patients (13/15 v 49/63). This was not true for chlorhexidine IDT (6/7 v 15/16) or SPT (6/13 v 66/80); i.e. NMBA positivity correlated better with sIgE than IDT or SPT testing.

Multiple allergen reactivity was confirmed by the results of the patients who had 3 or more tests. 12 of the 25 patients who underwent three different chlorhexidine allergy tests, also showed evidence of allergy to other substances (Table 2), including seven patients with evidence of NMBA allergy. Of these seven patients with evidence of NMBA allergy, 6 had positive chlorhexidine sIgE or IDT, whilst only one had positive chlorhexidine SPT.

DISCUSSION

Our report describes the largest single series of patients with perioperative chlorhexidine allergy so far published from routine clinical assessment. It confirms and extends previous reports. Our observations are based on data from routine clinical practice; therefore not all

patients underwent the same tests. On the other hand, the data reflect existing clinical practice in the UK and should be less prone to bias than smaller reports of very specific types of reaction, for example those triggered by chlorhexidine coated CVCs.

It is notable that our clinical data are consistent with previous descriptions of perioperative chlorhexidine allergy outside the UK. For example, the majority of our patients were men and most reactions took place in Urology or Cardiothoracic surgery, as described previously [3] [4]. Severe reactions are common, but may be subject to selection bias since these cases have been selected to be referred for specialist assessment, as is true for most previous series. The explanation for the apparent underrepresentation of obstetric and gynaecological procedures is not clear. There is no clear reason why referral patterns for patients undergoing these procedures should differ from other surgical interventions as the majority of clinic referrals are made by anaesthetists.

The majority of the reactions experienced by our patients were severe grade 3 reactions, most commonly including hypotension, with cardiac arrest occurring in a significant minority, as previously described [1] [3] [4]. Hypotension is not a unique characteristic of reactions to chlorhexidine and has been shown to be a dominant feature of most perioperative allergic reactions [2] [11]. On the other hand, allergic reactions to penicillins or wasp venom appear to cause hypotension less frequently [1] [3] [4] [12] [13]

It is possible that perioperative allergic reactions, including those to chlorhexidine, tend to be more severe because the patient is unconscious and cannot respond to early symptoms. In addition, many patients undergoing surgery have cardiorespiratory co-morbidity.

Diverse sources of chlorhexidine were triggers, and it is noteworthy that chlorhexidine mouthwash caused cardiac arrest in one patient. Fatal reactions to topical chlorhexidine have been reported [14].

Hidden chlorhexidine exposure is a known problem; in a recent systematic review of published cases of perioperative chlorhexidine allergy, coated CVCs accounted for a third of cases, but infrequently caused cardiac arrest [4]. In our group of patients, CVC exposure to chlorhexidine was not particularly common, but frequently caused hypotension and cardiac arrest. It is not clear why our data on reactions triggered by chlorhexidine coated CVCs are different, but reporting bias may be relevant in small case series. In addition, protocols for using chlorhexidine coated CVCs may differ between centres. It is also possible that reactions to chlorhexidine coated CVCs are under-referred to our services. Anaphylaxis to chlorhexidine coated CVCs may be difficult to diagnose, particularly if hypotension is the main feature and may be mistaken for anaesthesia-induced hypotension, haemorrhage from arterial puncture or pneumothorax. Anaphylaxis induced by chlorhexidine has been confused with cardiogenic shock and sepsis [15] [16]. Interestingly, the efficacy of chlorhexidine coated CVCs in preventing infection outside ICU has also been questioned by a Cochrane review [17].

In an observational series such as this, and in the absence of a gold standard challenge procedure, only limited conclusions can be made about the performance of individual tests. Clearly the vast majority of diagnoses were supported utilising positivity in one of the two favoured tests (SPT or sIgE). As a result, those patients who had multiple tests were either negative in the screening test or were selected in some other way for multiple testing. True performance indices require unselected testing of all patients utilising all modalities. Where multiple tests were used the majority of cases were positive in at least 2 tests. However 9/104 (8.7%) of the whole cohort were only positive in a single test representing 9/25 (36%) of the

cases where multiple tests were applied. IDT appeared to be most sensitive as second line testing. We cannot estimate specificity in routine practice, as we have not included individuals who definitely do not have chlorhexidine allergy [7] [10]. However, high sensitivity in testing for chlorhexidine allergy is arguably more important than specificity, as a false positive will only result in chlorhexidine being avoided, whilst a false negative could lead to repeat exposure and anaphylaxis.

The basophil activation test was only ever positive in the presence of both sIgE and SPT, but is not available in most centres.

One possible explanation for differences in test positivity favouring IDT when multiple tests are used is that chlorhexidine sIgE reactivity is lost over time and sIgE and the tests reported here may have been performed several months after the clinical reaction [3] [18]. However there were cases where sIgE and SPT were positive in the absence of IDT. Table 3 clearly shows that IDT (and indeed SPT and sIgE) can be positive on its own and where screening tests are negative, thus further investigations should include IDT. We cannot address the issue of whether the isolated positive IDT (or SPT, or sIgE) might be “false positive”, nor can any previous series, as we have no definitive challenge data. UK clinics used 5mg/L chlorhexidine for IDT, whilst Opstrup used 2mg/L [7]. More prosaically, we will have excluded patients who had a positive SPT or sIgE and did not go on to have IDT in our sensitivity estimates in this subgroup analysis. Thus Table 3 more closely estimates the results of performing all test modalities where the screening test is negative, and shows that IDT clearly has a potential diagnostic advantage in this setting.

In addition, our IDT testing differed slightly from that previously used to validate IDT testing, in using the forearm rather than the back [7]. This variation is true of all skin tests in clinical practice, and argues strongly for adoption of harmonised approaches to skin testing.

A combination of SPT and sIgE has been recommended as a high sensitivity strategy for testing for chlorhexidine allergy [7], based on data from a cohort of patients in whom testing 'usually took place 2–4 months after the allergic reaction'. Our data may support this observation since SPT and sIgE dominate the positive investigations in the whole cohort, but IDT dominates once these single test positives have been screened out. It is noteworthy that 7 of our cohort of 104 patients, the only positive test was IDT. Had IDT not been performed after finding negative SPT and sIgE, sensitization to chlorhexidine in these patients may not have been revealed.

One logical approach would be to offer sIgE and SPT to all patients, but always to go on to do IDT if these tests are negative and there remains a high index of suspicion of chlorhexidine allergy.

Positivity to other potential culprits in a third of our cases is important. Multiple sensitisations to drugs were common. Twenty-eight of 104 patients had other positive allergy tests, confirming the finding of multiple reactivity in similar proportions to other cohorts of chlorhexidine allergic patients [3] [7]. However our patients mainly had reactivity to NMBAs, as opposed to the latex, opiates and beta lactams in the other reports.

In our series, multiple reactivity occurred in patients with most combinations of chlorhexidine allergy tests, but was most closely associated with a positive chlorhexidine sIgE test.
High total IgE (above 1500kUA/L) is a frequent cause of multiple reactivity in other settings. However, high total IgE is not thought to drive false positive chlorhexidine sIgE [18]. Until

neutralising and blocking experiments are reported, it remains unclear whether this multiple reactivity reflects cross reactivity, for example to quaternary amide groups.

Twelve of the 25 patients who underwent three different chlorhexidine allergy tests, also showed evidence of allergy to other substances (Table 2), including seven patients with evidence of NMBA allergy. Of these seven patients with evidence of NMBA allergy, 6 had positive chlorhexidine sIgE or IDT, whilst only one had positive chlorhexidine SPT. This suggests that extended panels of allergen testing may be routinely required to ensure all potential triggers are assessed for clinical relevance. It may also suggest that perioperative allergic reactions associate with multiple drug exposure or procedures.

In summary, we report on the largest series yet described of patients diagnosed with chlorhexidine allergy. We confirm that these reactions are frequently severe. Specific IgE and SPT are reasonable first line tests for chlorhexidine allergy, but IDT should be added if these are negative, particularly if referral to the allergy clinic is delayed or if the index of suspicion is high. False negativity in screening tests is not uncommon and may affect 7% of our series. Multiple sIgE reactivity is relatively common and, until further data are available on its cause and significance, should lead to specialist allergy assessment that looks for sensitization to all the potential drug triggers, and an imputibility assessment for each potential trigger, to avoid misdiagnosis. Hidden exposure to chlorhexidine is common in healthcare environments and we suggest that awareness of the potential allergenicity of chlorhexidine should be part of the training of all healthcare professionals. Chlorhexidine coated CVCs were not a common trigger of anaphylaxis in UK cohorts, but did appear to be associated with severe reactions.

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RS, WE, NH, TG, MH, SS, LS, EE, LN saw the patients and collected the data.

KS carried out in vitro BAT tests

MH and WE analysed the data and drafted the article.

NH, RS, WE, KS, EE, TG contributed to revising the article

CONFLICT OF INTEREST

No Conflict of Interest - WE, RS, KS, AN, NH, MH, TG, AW, EE, LS

Table 1: Combinations of test results in 104 Cases of Perioperative Chlorhexidine Allergy.

Positive tests	Number Patients (Pts)	Mean Chlorhexidine sIgE (kUA/L) (95% CI)	Pts with other positive allergy tests*	Pts with positive NMBA allergy tests	Pts with other positive allergy tests
Single pos IDT	7	0.34 (0-0.35)	3	1 x atracurium SPT 1 x vecuronium & atracurium IDT	1 x gelatine sIgE
Single pos IgE	16	4.34 (0-16.52)	6	1 x rocuronium SPT 1 x cisatracurium IDT 2 x NMBA IDT & sIgE	1 x QAM** sIgE 1 x morphine sIgE 1 x teicoplanin SPT
Single pos SPT	32	0.34 (0-0.35)	4	1x atracurium SPT	1 x carmosine SPT 1 x penicillin SPT 1 x latex SPT
Double pos IDT, sIgE	9	8.50 (0-21.82)	7	1 x vecuronium IDT & sIgE 1 x all NMBAs IDT & sIgE 1 x atracurium IDT 1 x atracurium sIgE	1 x teicoplanin IDT & SPT 1 x amoxicillin sIgE 1 x gentamycin IDT 1 x morphine IDT
Double pos SPT, IDT	3	0.34(0-0.34)	1	0	1 x gelatine IDT
Double pos SPT, sIgE	32	8.96 (0-27.92)	6	2 x all NMBA IDT 1 x Rocuronium SPT & suxamethonium sIgE 1x atracurium SPT 1 x suxamethonium IDT & sIgE	1 x QAM sIgE 1 x morphine, amoxicillin sIgE
Triple pos SPT, IDT, sIgE	2	1.08 (0.17-1.99)	1	1 x suxamethonium sIgE	1 x morphine SPT
Triple pos SPT, sIgE, BAT	3	6.18 (0-13.56)	0	0	0

Table 1 legend

There was no correlation between the number and type of positive tests or sIgE level and reaction grade for the cohort of 104 cases (not shown).

* Tests potentially relevant to the differential diagnosis of the reaction

**QAM = Quaternary Ammonium Moiety (e.g. Thiocholine or Suxamethonium sIgE)

Table 2: The mean sIgE levels and distribution of positive allergy tests for other substances for 25 patients who tested positive for 2 or more chlorhexidine tests

Patients who had three tests done	Number of patients	Mean Chlorhexidine SIgE (KUA/L) (95% CI)	Patients with other positive allergy tests	Patients with positive NMBA allergy tests	Patients with other positive allergy tests
Single pos IDT	5	0.34 (0-0.34)	2	1 x vecuronium & atracurium IDT	1 x gelatine sIgE
Single pos IgE	3	1.34 (0.59-2.09)	1	1 x NMBA IDT & sIgE1	0
Single pos SPT	1	0.34	1	0	1x carmosine SPT
Double pos IDT, sIgE	8	8.88 (0-23.07)	6	1x vecuronium IDT & sIgE 1 x all NMBAs IDT & sIgE 1x atracurium IDT 1 x atracurium sIgE	1 x teicoplanin IDT & SPT 1x amoxicillin sIgE 1 x gentamycin IDT 1 x morphine IDT
Double pos SPT, IDT	2	0.34 (0-0.35)	1	0	1 x gelatine IDT
Double pos SPT, sIgE	1	9.74	0	0	0
Triple pos SPT, sIgE, BAT	3	6.18 (0-20.88)	0	0	0
Triple pos SPT, IDT, sIgE	2	1.08 (0.17-1.99)	1	1 x suxamethonium sIgE	1 x morphine SPT

In the head to head comparison of SPT, sIgE and IDT, IDT was positive in 17/19 cases where 3 tests were performed, more frequently than any other test.

Table 3: Sensitivity of each test modality in the 25 patients with at least 2 positive chlorhexidine tests.

	SPT	sIgE	IDT	BAT
True positives	9	17	17	3
False negatives	16	8	2	3
Sensitivity in cases with at least 3 tests (95% CI)	36%* (17-55%)	68% (50-86%)	89% (75-100%)	50% (10-90%)
Published sensitivity [7]	95%	100%	68%	Not published

* the majority of cases were diagnosed on basis of SPT or sIgE and this subgroup represents cases where multiple tests were used, predominantly because the initial screening test was negative.

REFERENCES

1. Krishna MT, York M, Chin T, Gnanakumaran G, Heslegrave J, Derbridge C, Huissoon A, Diwakar L, et al. Multi-centre retrospective analysis of anaphylaxis during general anaesthesia in the United Kingdom: aetiology and diagnostic performance of acute serum tryptase. *Clin Exp Immunol* 2014; **178**:399-404.
2. Low AE, McEwan JC, Karanam S, North J, Kong KL. Anaesthesia-associated hypersensitivity reactions: seven years' data from a British bi-specialty clinic. *Anaesthesia* 2016; **71**:76-84.
3. Garvey LH, Kroigaard M, Poulsen LK, Skov PS, Mosbech H, Venemalm L, Degerbeck F, Husum B. IgE-mediated allergy to chlorhexidine. *J Allergy Clin Immunol* 2007; **120**:409-15.
4. Sharp G, Green S, Rose M. Chlorhexidine-induced anaphylaxis in surgical patients: a review of the literature. *Aust N Z J Surg* 2016; **86**:237-43.
5. Savic LC, Kaura V, Yusaf M, Hammond-Jones AM, Jackson R, Howell S, Savic S, Hopkins PM. Incidence of suspected perioperative anaphylaxis: A multicenter snapshot study. *J Allergy Clin Immunol Pract* 2015; **3**:454-5 e1.
6. Mertes PM, Malinovsky JM, Jouffroy L, Aberer W, Terreehorst I, Brockow K, Demoly P et al. Reducing the risk of anaphylaxis during anesthesia: 2011 updated guidelines for clinical practice. *J Investig Allergol Clin Immunol* 2011; **21**:442-53.
7. Opstrup MS, Malling HJ, Kroigaard M, Mosbech H, Skov PS, Poulsen LK, Garvey LH. Standardized testing with chlorhexidine in perioperative allergy--a large single-centre evaluation. *Allergy* 2014; **69**:1390-6.
8. McGowan EC, Saini S. Update on the performance and application of basophil activation tests. *Curr Allergy Asthma Rep* 2013; **13**:101-9.
9. Simons FE, Ardusso LR, Bilo MB, Dimov V, Ebisawa M, El-Gamal YM, Ledford DK, Lockey RF, et al. 2012 Update: World Allergy Organization Guidelines for the assessment and management of anaphylaxis. *Curr Opin Allergy Clin Immunol* 2012; **12**:389-99.
10. Leysen J, Bridts CH, De Clerck LS, Vercauteren M, Lambert J, Weyler JJ, Stevens WJ, Ebo DG. Allergy to rocuronium: from clinical suspicion to correct diagnosis. *Allergy* 2011; **66**:1014-9.
11. Egner W, Sargur R, Shrimpton A, York M, Green K. A 17-year experience in perioperative anaphylaxis 1998-2015: harmonizing optimal detection of mast cell mediator release. *Clin Exp Allergy* 2016; **46**:1465-73.
12. Bousquet PJ, Kvedariene V, Co-Minh HB, Martins P, Rongier M, Arnoux B, Demoly P. Clinical presentation and time course in hypersensitivity reactions to beta-lactams. *Allergy* 2007; **62**:872-6.
13. Rueff F, Przybilla B, Bilo MB, Muller 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. *J Allergy Clin Immunol* 2009; **124**:1047-54.

14. Pemberton MN, Gibson J. Chlorhexidine and hypersensitivity reactions in dentistry. *Br Dent J* 2012; **213**:547-50.
15. Rutkowski K, Wagner A. Chlorhexidine: a new latex? *Eur Urol* 2015; **68**:345-7.
16. Hong CC, Wang SM, Nather A, Tan JH, Tay SH, Poon KH. Chlorhexidine Anaphylaxis Masquerading as Septic Shock. *Int Arch Allergy Immunol 2015*; **167**:16-20.
17. Lai NM, Chaiyakunapruk N, Lai NA, O'Riordan E, Pau WS, Saint S. Catheter impregnation, coating or bonding for reducing central venous catheter-related infections in adults. *Cochrane Database Syst Rev* 2016; **3**:CD007878.
18. Opstrup MS, Poulsen LK, Malling HJ, Jensen BM, Garvey LH. Dynamics of plasma levels of specific IgE in chlorhexidine allergic patients with and without accidental re-exposure. *Clin Exp Allergy* 2016; **46**:1090-8.

Figure 1 Legend

The distribution of positive tests for all 104 patients.

Figure 2 legend

25 patients had three tests. All had SPT and sIgE. 6 patients also had BAT and 19 also had IDT.