An investigation on the use of baked milk foods in children with cow's milk allergy

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Abstract

Baked milk challenges and milk ladders have recently become a well-recognized part of cow's milk allergy management, used to determine the development of tolerance to baked milk. However, there is relatively limited research regarding current and appropriate practice in this area. Hence, the aims of this research were (i) to explore attitudes and practice of healthcare professional's use of baked milk challenges and milk ladders in a clinical setting (ii) to explore mothers' experiences of introducing baked milk into their child's diet and (iii) to evaluate if immune markers can identify IgE-mediated cow's milk allergic children able to pass milk challenges.

This research consisted of three separate studies: (i) a multi-national survey explored the current clinical practice of healthcare professionals using baked milk challenges and the milk ladder (ii) a qualitative study explored mothers' perspectives. (iii) a quantitative study explored if immune markers such as SPT and milk sIgE can predict milk challenge outcomes in IgE mediated CMA. This research found that while healthcare professionals largely considered the potential for severe reactions when making decisions about the appropriate venue for baked milk reintroduction there were inconsistencies regarding this, and the guidelines that were followed. Increased parental anxiety was reported for both baked milk challenges and milk ladders. Furthermore, mothers experienced confusion about the different versions of milk ladders and their practical implementation, and disappointment with healthcare support. They also expressed concerns regarding the lack of healthy and alternative food options in each stage of the milk ladder. Regarding the appropriate time of baked milk reintroduction, this research found that skin prick test and milk sIgE had poor value as predictive tools in the identification of milk challenge outcomes.

In the first in-depth exploration of this new area of practice in the management of cow's milk allergy, this research has provided novel results, which have important implications for healthcare professionals and researchers working with cow's milk allergy. Any changes to practice arising from these will benefit cow's milk allergic children and their families in the future.

Declaration

Whilst registered as a candidate for the above degree, I have not been registered for any other research award. The results and conclusions embodied in this thesis are the work of the named candidate and have not been submitted for any other academic award.

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Abbreviation list

AAF: Amino Acid Formula

AAAAI: American Academy of Allergy, Asthma, & Immunology

Abs: Antibodies

BM: Baked Milk

BMC: Baked Milk Challenge

BSACI: British Society of Allergy & Clinical Immunology

CCHMC: Cincinnati Children's Hospital Medical Centre

CMA: Cow's Milk Allergy

CM: Cow's Milk

EAACI: European Academy of Allergy & Clinical Immunology

EHF: Extensively Hydrolysed Formula

EoE: Eosinophilic Esophagitis

ESPGHAN: European Society for Paediatric Gastroenterology, Hepatology and Nutrition

FPIES: Food protein-induced enterocolitis syndrome

HCPs: Healthcare Professionals

ML: Milk Ladder

OIT: Oral Immunotherapy Treatment

SIgE: Specific Immunoglobulin E

SPT: Skin Prick Test

WAO: World Allergy Organisation

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- Journal Preparation

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- Poster Presentations

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Chapter 1. General Introduction

1.1 Background

Cow's milk allergy (CMA) is one of the most common food allergies in early childhood. It generally has a good prognosis and cow's milk (CM) is usually successfully re-introduced in the child's diet within the first 1- 5 years of life following a milk elimination diet (Fiocchi, Brozek, et al., 2010). Current research indicates that foods containing baked milk (BM) such as biscuits/cookies, cakes, muffins, waffles, could be introduced before uncooked CM re-introduction in children with CMA (Nowak-Wegrzyn et al., 2008). Re-introduction of BM utilises two different methods/protocols: baked milk challenge (BMC), in which a full portion of BM-product is introduced in increasing amounts over a day in a hospital/office, or a milk ladder (ML), in which case baked milk containing foods are re-introduced gradually (typically at home) over a period of days, weeks, months or years (+Venter, Brown, Shah, Walsh, & Fox, 2013). Successful re-introduction of BM-containing foods may accelerate milk allergy resolution and assist in establishing a normal diet, by avoiding unnecessary elimination diets which impair proper nutrient intake and affect socialisation (Meyer et al., 2017). This provides benefit not only to children's health status, but also to the quality of life of both children and parents (Dupont, 2013). However, in many cases children are following a strict milk free diet unnecessarily as they are unaware that they are baked milk tolerant.

CMA has an adverse nutritional impact on the children and negative psychosocial impact on the child and their families. The potential for re-introduction of baked milk and eventual tolerance of fresh milk is high. It is surprising therefore that so little is understood about the practice and impact of BMC and milk ladders from either a health care professional or parent perspective. Additionally, while immune markers such as Skin Prick Test (SPT) and milk specific Immunoglobulins E (sIgE) may provide some useful prognostic information for the appropriate timing for introduction of BM-containing food in IgE-mediated CMA (Bartnikas, Sheehan, Hoffman, et al., 2012), there are currently no clear clinical or laboratory criteria established that can predict which patients are likely to pass a BMC or certain steps on the milk ladder. Hence, this important field urgently needs further research to provide answers not only to healthcare professionals (HCPs) but also to parents on how to properly use these foods for the management of CMA.

1.2 Thesis layout

To inform the current thesis, Chapter 2 reviews literature relevant to the use of BM-containing foods in the management of CMA. It first sets out to define CMA and discuss its prevalence, natural history, diagnosis, and management, in children with this allergic disorder. The second section discusses the current research regarding the re-introduction of BM into children's diets and introduces the use of BMCs and gradual re-introduction of BM (milk ladder) in IgE-mediated CMA and mild to moderate non-IgE-mediated CMA. This thesis does not include milk challenge/reintroduction for Eosinophilic Esophagitis (EoE) and Food Protein Induced Enterocolitis (FPIES). Current guidelines in the treatment and management of CMA are discussed. The final part defines immune markers such as SPT and milk sIgE and discusses their role in the management of IgE-mediated CMA.

Current studies are reviewed examining whether immune markers can predict the outcome of re-introducing-BMC and the timing of tolerance development. Chapter 2 reviews the literature in order to provide the background and rationale to this thesis. The literature review suggests that for many reasons the re-introduction of BM-containing foods into the diet of milk allergic children before reintroducing "raw" milk is of interest not only to the United Kingdom (UK) but also internationally. Currently, the theory and practice of "how" to re-introduce BM-containing foods has not been well-described in literature. The overall aim of this research therefore is to understand current clinical practice in BM-reintroduction, the impact of BM-reintroduction on parents of children with CMA, and the extent to which biomarkers might be able to identify potential candidates for BM- reintroduction.

In line with the overall aim, Chapter 3 presents a survey that explores what guidelines and approaches are currently being used by HCPs across the world and what their experiences have been in introducing BM-containing foods in IgE-mediated CMA and mild to moderate non-IgE-mediated-CMA. This is the first published study that provides systematically collected clinical data on the use of BM-containing foods from HCPs' perspectives across the world.

Moving on from understanding HCPs' use and experience of BM re-introduction, Chapter 4 presents a qualitative study that explores mothers' experiences in using a milk ladder approach at home (gradual reintroduction of BM-containing foods) and the impact of these

foods in the management of their child's CMA. This is the first study that has investigated (using semi-structured individualised phone interviews) mothers' attitudes regarding the re-introduction of BM-containing foods at home and the outcomes of this approach in the management of IgE and mild to moderate non-IgE-mediated CMA. Thus, it has provided important information regarding the usefulness, appropriateness, acceptability, practicability, and safety of BM-reintroduction from mothers' perspectives. This valuable data could be used to improve the BM-reintroduction approach providing not only better patient care but also to help parents to feel less anxious and more confident during this process.

Moving on from understanding parents' use and experience of BM re-introduction, Chapter 5 presents a quantitative study that analyses a retrospective subset of data from a larger research project being coordinated by the Cincinnati Children's Hospital Medical Centre (CCHMC) in the USA to determine if immune markers such as SPTs and milk sIgE are appropriate tools in predicting oral milk challenge outcomes and hence help HCPs to identify children who will be optimal cow's milk allergic patients for baked milk or raw milk-reintroduction. Identifying valuable tools could provide useful information for the milk tolerant status of the patient and contribute to the avoidance of unnecessary exposure to the milk allergen through the challenge and thus reduce the risk of any reactions. In addition, milk challenges have a long waiting list for patients, are time consuming and expensive and if they can be replaced by other validated predictive tools this could benefit not only patients and parents but also allergy services.

Finally, Chapter 6 discusses the overall findings of the research, by collating the results of the three studies together. The implications for the gradual re-introduction of BM-containing foods in the management of IgE and mild to moderate non-IgE-mediated CMA are discussed. Strengths and limitations of the research are addressed and further research needs are outlined.

In summary, this PhD research represents the first in-depth exploration of the reintroduction of BM-containing foods into cow's milk allergic children's diets; a new area of practice in the management of cow's milk allergy. This research has provided novel results, which have important implications for healthcare professionals and researchers working with cow's milk allergy. Understanding HCPs and parents' perceptions and establishing reliable cut off values

for immune markers that can predict milk tolerance are of the utmost importance in terms of the validity of any future guidelines, and it could contribute to an effective CMA dietary management. Any changes to practice arising from these will therefore benefit cow's milk allergic children and their families in the future.

Chapter 2. Literature review of Cow's Milk Allergy & Immune Markers

2.1 Overview of chapter

This chapter reviews the current research related to the management of cow's milk allergy in children and the use of baked milk containing foods in the development of tolerance to cow's milk. The first section introduces the prevalence, prognosis, symptoms, diagnosis, and management, of CMA. Understanding of CMA treatment with the use of baked milk containing foods into the diet of children with IgE and mild to moderate non-IgE-mediated CMA is related to the worldwide survey (presented in Chapter 3) that provides information on the use of baked milk challenges and gradual reintroduction of milk in IgE and non-IgE-mediated CMA from healthcare professionals' perspectives. The second section discusses the role of baked milk containing foods in terms of their appropriateness and safety. It also presents the guidelines followed by HCPs before they decide to introduce baked milk containing foods into the diet of children with CMA. This section is related to the qualitative research study (presented in Chapter 4) that discusses data on the impact of introducing baked milk containing foods into children's diet from mothers' perspectives. The final section discusses the role of immune markers (SPT, milk specific IgE levels) for identifying potential IgE-mediated CM allergic patients able to tolerate milk containing foods. Understanding the role of immune markers in predicting milk challenges is related to the study presented in Chapter 5, which evaluates whether immune markers can predict oral milk challenges in children with IgE-mediated CMA. The protocol of the search strategy and methodology of this review is summarised in appendix 5.

2.2 Cow's Milk Allergy

2.2.1 Definition

Milk allergy is defined as a reproducible adverse immune reaction to one or more proteins of milk (Fiocchi, Schunemann, et al., 2010). Cow's milk is the usual cause of such an allergic reaction, but other kind of milk such as sheep, goat, buffalo or other mammals can also cause an abnormal immune response in human's (Host & Halken, 2014). Casein and whey are the major milk proteins that can cause an allergic reaction. Of the total milk protein, 80% is casein

protein fraction (alphaS1, alphaS2, beta and kappa-caseins) and 20% is whey (alpha-lactalbumin, beta lactoglobulin, bovine serum albumin) (Wal, 2002). It has been reported that casein is heat resistant and is responsible for persistent milk allergy i.e. milk allergy that is not outgrown during childhood (Järvinen et al., 2002). Whey proteins are heat labile and are commonly implicated in transient milk allergy i.e. milk allergy that is outgrown (Bloom et al., 2014). Based on the National Institute of Allergy and Infectious Disease (NIAID) guideline (Burks et al., 2011), cow's milk allergy is classified as:

1. *IgE-mediated CMA* that occurs immediately and up to 2 hours after exposure to cow's milk proteins and causes the rapid release of mediators from effector cells (mast cells, basophils), resulting in acute skin, airway and gastro-intestinal (GI) symptoms.

2. *Non-IgE-mediated CMA* that can take place up to 4 to 72 hours after ingestion of the offending food. Clinical symptoms are subacute or chronic in nature and usually present with isolated GI symptoms. There are different forms of non-IgE-mediated food allergies such as mild to moderate non-IgE-mediated CMA, eosinophilic esophagitis, eosinophilic gastritis and gastroenteritis, food protein-induced enterocolitis syndrome (FPIES), and allergic proctocolitis. Figure 2.1 shows the different types of CMA.

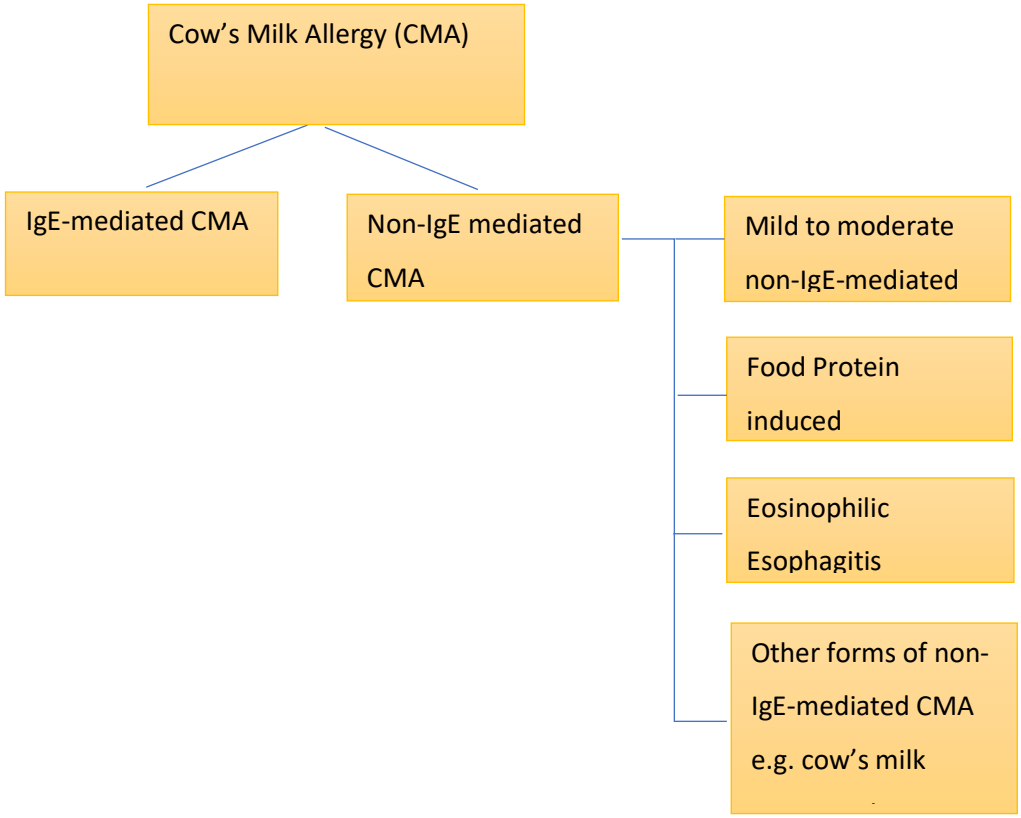

Figure 2.1: Different types of Cow's Milk Allergy

This PhD research project is focused on IgE mediated CMA and mild to moderate non-IgE mediated CMA.

2.2.2 Epidemiology and prognosis

The prevalence of CMA ranges from 1.9 to 4.9% in children, making this type of allergy the most common food allergy in the paediatric population (Fiocchi, et al., 2010). In the developed world, it is approximately 2 to 3% and these percentages are mainly referring to IgE-mediated CMA (Sicherer, 2011). The prevalence of non-IgE-mediated CMA is still not well known. UK data from 2008 estimated that the prevalence of CMA is 2-3% in young children aged 1-3 years old and that the majority of these children suffer from non-IgE mediated CMA (Venter et al., 2008). Further evidence from the UK showed that the majority of non-IgE-mediated CMA cases present with mild to moderate allergic reactions to cow's milk (Venter et al., 2013). However, conclusions from a 2015 systematic review meta-analysis indicate that it is difficult to estimate the actual prevalence of cow's milk allergy in children due to

the heterogeneity of paediatric population between the studies and the lack of uniformity of CMA diagnostic criteria (Schoemaker et al., 2015).

Cow's milk allergy prognosis is usually considered to be good and the majority of children outgrow their CMA by 3 years old (Venter et al., 2008). However, the actual time of resolution varies in infants and children, hence they are re-evaluated at 6 to 12 monthly intervals for the development of tolerance (Luyt, Ball, et al., 2014). In 2002, Host et al followed a cohort of 1740 new-borns and found a good prognosis of CMA with a total recovery of 56% at 1 year, 77% at 2 years, 87% at 3 years, 92% at age five years and 97% at age 15 years (Host et al., 2002). They reported that children with non-IgE-mediated-CMA had a better prognosis compared with young children with IgE-mediated CMA, who had a higher risk of persistent allergy, and of suffering from other food allergies and conditions such as asthma and rhinoconjunctivitis (Host et al., 2002). In 2014, Host et al reported that 45-50% of children tolerated milk by one year of age, 60-75% by two years, and 85-90% of participants by three years of age. Other studies reported that 15% of children with IgE-mediated CMA had persisting milk allergy at the age of eight, while those children with non-IgE mediated CMA had outgrown their allergy at age of five (Saarinen, Pelkonen, Mäkelä, & Savilahti, 2005). A United States study in IgE-mediated CMA children reported that the rates of acquired tolerance were 19% by 4 years, 42% by 8 years, 64% by 12 years, and 79% by 16 years (Skripak, Matsui, Mudd, & Wood, 2007). Hence, published data regarding the age at which children outgrow their milk allergy is inconclusive, due to different study populations, diagnostic criteria, and types of CMA. The exact mechanism of tolerance development is still unclear and depends on several factors including the type of CMA (IgE or non-IgE-mediated CMA), genetic predisposition, nature and dose of the allergen, age at first antigen exposure, and frequency of delivery of the food allergen (Nowak-Wegrzyn & Sampson, 2011).

2.2.3 Pathogenesis and symptoms

Cow's milk allergy usually presents in infancy and the most affected children develop symptoms before 6 months of age. The onset of symptoms starts rare after the 1st year of life (Sampson & Anderson, 2000). The lack of microbial exposure during the first year of life has been associated with risk of allergic sensitisation (Burbank, Sood, Kesic, Peden, & Hernandez, 2017). Most infants manifest CMA symptoms within 1 week after ingestion of milk-formula (Host & Halken, 2014). Cow's milk allergy may be developed in both breastfed

and cow's milk formula fed infants, and it usually involves two or more symptoms in two or more organ systems. The main systems that are affected are the skin (50-70% of individuals) gastrointestinal (50-60%) and respiratory systems (20-30%) (Host et al., 2014). The pathogenesis of CMA is complicated and depends on the genetic predisposition, environmental factors, conditions of exposure to milk allergen and the features of the causative allergen (Lee et al., 2017).

The immunological mechanism regarding the development of CMA is not very clear. It is known that CMA is caused by both IgE and non-IgE-mediated mechanisms and it results from either the failure to develop normal tolerogenic processes or their later breakdown (Vitaliti et al., 2012). Different factors may affect the development of IgE and non-IgE-mediated reactions to food in infancy and early childhood. In a large cohort of 1140 infants, eczema, rhinitis and dietary pattern were identified as risk factors in IgE-mediated-CMA, whilst in non-IgE-mediated CMA the risk factors were pets in the home, dietary pattern, maternal consumption of probiotics during breastfeeding and age at first solid food introduction (Grimshaw et al., 2015). The underlying mechanism of CMA also results in different symptom presentation between IgE and non-IgE-mediated CMA as follows:

IgE-mediated-reactions and symptoms

In IgE-mediated CMA, milk specific T helper cells type-2 (Th2) are activated and lead to the production of milk specific IgE. Patients release IgE antibodies (Abs) from B cells when milk proteins or peptides pass through the skin and penetrate the gastrointestinal or respiratory lining. The allergic response to antigen (e.g. milk protein) entrance takes place in two stages (Nakanishi, 2010):

1st stage - Sensitisation or antibody induction:

Mast cells are found on mucosal epithelia and their number is increased at sites of Th2 during the allergic response. IgE immunoglobulins are bound to mast cells, blanketing the plasma membranes of these immune cells. Half a million IgE molecules coat the surface of mast cells and bind to the high-affinity IgE receptors (Hong et al., 2016).

2nd stage: Elicitation or effector phase

An exposure to the same allergen cross-links the cell-bound IgE complex and leads to degranulation of mast cells within 5 to 16 minutes. The degranulation of mast cells induces the release and synthesis of important inflammatory mediators (vasodilation, bronchoconstriction, cellular localisation, eosinophil attraction) which are responsible for the characteristic symptoms of Type 1 hypersensitivity (Forsythe, 2016; Hong et al., 2016).

IgE-mediated-CMA presents with early onset symptoms that occur within minutes to 2 hours after ingestion of milk proteins. Histamine that is released in the circulation may cause oedema of the mouth and throat, rhinitis, red skin, angioedema, breathing difficulties, or an asthma attack (Fiocchi et al., 2010; Forsythe, 2016). Gastrointestinal symptoms such as diarrhoea, nausea, vomiting, and abdominal pain may be accompanied by symptoms of other organs (Eigenmann, 2007). IgE-mediated reactions are responsible for severe symptoms such as hypotension, vascular collapse, cardiac dysrhythmia and anaphylactic shock. Anaphylaxis is a life-threating reaction and its treatment is based on early administration of adrenaline. An untreated anaphylactic reaction may be fatal for the patient (Eigenmann, 2002). The severity of reactions cannot be predicted by the degree of past reactions and allergy tests such as the size of the SPT wheal and levels of milk specific IgE, due to their poor predictive value as a screening tool (Luyt, Ball, et al., 2014). The coexistence of other atopic conditions such as asthma, atopic eczema, the patient's age, and the degree of sensitisation may also influence the severity of the reaction (Burks et al., 2011). The symptoms of IgE-mediated CMA are usually manifested after the offending food intake and can be identified quite easily (Table 2.1).

Mild to moderate non-IgE-mediated reactions and symptoms

The exact mechanism of non-IgE-mediated CMA is unknown. Non-IgE-mediated CMA reactions are thought to be due to Th1-mediated inflammation. The Th1- cells secrete the cytokine interferon-γ and activate inflammatory pathways mainly via macrophage activation. Sensitisation of T-cells and production of cytokines may cause inflammation, tissue damage and formation of epithelial and giant cells (Vitaliti, 2012). T-cell mediated response to milk proteins may exacerbate conditions such as eczema, asthma, and rhinitis in children with CMA (Host et al., 2002). Non-IgE-mediated responses are characterised by delayed hypersensitivity reactions where the clinical symptoms may be manifested 2-48 hours after consumption of cow's milk. However, in some cases, non-IgE-mediated CMA symptoms may

occur within days/weeks after ingesting milk or milk products. These types of symptoms are usually accompanied by other conditions such as eczema or respiratory disorders (Sicherer & Leung, 2013). Clinical symptoms are subacute or chronic in nature and usually present with isolated GI symptoms. The severity of a reaction to a food allergen varies according to the form of the food (raw, cooked or processed), amount ingested, and the co-ingestion of other foods (Burks et al., 2011). The clinical features of IgE and mild to moderate non-IgE-mediated CMA are summarised in Table 2.1.

Table 2.1: Clinical features of CMA

Organ Systems	IgE-mediated-CMA	Non-IgE-mediated-CMA
Skin	• Pruritus • Erythema • Acute urticaria	• Pruritus • Erythema • Atopic eczema
Gastrointestinal	• Angioedema (lips, tongue, palate) • Oral pruritus • Diarrhoea • Nausea • Vomiting	• Gastro oesophageal reflux • Food reflux/aversion • Abdominal pain/infantile colic • Pallor and tiredness • Loose/frequent stools • Constipation • Perianal redness • Faltering growth in conjunction with at least one or more gastrointestinal symptoms above (with or without significant atopic eczema)
Respiratory **333**	• Nasal itching • Rhinorrhoea • Sneezing • Congestion • Chest tightness	

Organ Systems	IgE-mediated-CMA	Non-IgE-mediated-CMA
	• Wheezing	
Other	• Signs or symptoms of anaphylaxis or other systemic allergic reactions	

Differentiating CMA from lactose intolerance

In routine clinical practice, lactose intolerance may be confused with milk allergy, not only from a carer's point of view but also from that of GPs. However, the management of these conditions are completely different and an inappropriate recognition may lead to unnecessary dietary restriction. Lactose intolerance is a non-immunological response and it is caused by enzyme deficiencies, pharmacological agents or other substances (Fiocchi et al., 2010). This PhD research is not focused on lactose intolerance.

2.2.4. Diagnosis

There is a worldwide debate in the diagnosis and management of CMA, due to the lack of specific symptoms and reliable indicators and tests. Cow's milk allergy may be over-diagnosed or under-diagnosed in many cases by healthcare professionals, due to the confusion of presented symptoms. In 2015, Lozinsky et al published a study regarding the experience of GPs and parents on recognition and management of CMA. They reported a lack of awareness of guidelines and training of GPs in the diagnosis of CMA, whereas parents

had different perceptions from GPs on the presentation and improvement of CMA symptoms (Lozinsky et al., 2015). The findings above are supported by other studies that found CMA diagnosis was often delayed, taking four to nine weeks (Sladkevicius & Guest, 2010; Sladkevicius, Nagy, Lack, & Guest, 2010). A further study found that parents may incorrectly perceive their children to have experienced an allergic reaction to milk (Elizur, Cohen, Goldberg, Rajuan, & Katz, 2013). An early and correct diagnosis of CMA is important to prevent ongoing symptoms and avoid unnecessary dietary restrictions from the diet of infants or children. In clinical practice the patient's history and clinical examination is important to evaluate the different form of cow's milk allergy.

IgE-mediated CMA

The clinical diagnosis in IgE-mediated CMA is based on a combination of typical presenting symptoms and evidence of sensitisation by the results of immune markers such as SPT and sIgE levels:

- *Skin Prick Test (SPT)*

Is used to identify sensitisation to an allergen(s) by measuring the specific IgE bound to the mast cell. The location of each allergen is marked with a pen on the forearm. The distance between two tests should be ≥2cm to avoid contamination which may result in a false-positive reaction. A drop of either commercial or natural food solution is placed on the skin in identical order and it is immediately pricked. The wheal response is measured after transfer to paper from the skin with translucent tape. Measurement is undertaken in standard fashion, measuring the largest wheal diameter and the diameter orthogonal to it. The mean wheal diameter is calculated. Results are classified as positive if the mean diameter is 3 mm or more in the presence of a negative control (0.9% saline, Soluprick SQ allergens) and a positive histamine (10 mg/mL, Soluprick SQ allergens) reaction after 15 minutes (Bartnikas, Sheehan, Schneider, & Phipatanakul, 2012).

- *Milk Specific IgE immunoassay blood testing*

It has been used in CMA diagnosis for many years. It is a blood test that measures the amount of the circulating IgE antibody in the serum produced when the individual is exposed to a

specific food protein. Like SPT, blood testing can detect the presence of IgE, but a positive result does not in itself make a food allergy diagnosis. SPT and specific IgE testing can be used as a single test or in combination. A blood allergy test is used in cases of severe atopic dermatitis (eczema), extreme sensitivity, or the need to continue antihistamine therapy as SPT is not indicated. Blood samples are collected for measurement of specific IgE antibody levels to milk, casein and β-lactoglobulin. Serum specific IgE levels are considered positive at levels of ≥0.35 kUA/l. Clinical performance is expressed as sensitivity, ranging from 85-95% and specificity, ranging from 86%-94%. Sensitivity and specificity have been reported from multi-center studies including several hundred patients tested for a range of different allergens. Commercially available tests and reagents are used according to the instructions of the manufacturer (Bartnikas, Sheehan, Schneider, et al., 2012).

The individualised clinical assessment may include the patient's history, physical examination, trial elimination diet, diet diaries, oral food challenges, SPT with fresh milk or commercial reagents, and IgE measurements for determining milk-specific serum IgE against milk proteins such as casein, α-lactalbumin and β-lactoglobulin (Boyce et al., 2010).

- *Food challenges*

The double-blind placebo-controlled challenge is the gold standard in CMA diagnosis, but in practice this process cannot be used because it is expensive and time-consuming. Currently, in clinical practice a medically supervised oral (open) food challenge is conducted for the milk allergy diagnosis and it is often required to confirm the diagnosis of CMA because of the limitation in the diagnostic accuracy of the SPT and specific IgE testing, even in combination with the clinical history (Dambacher, de Kort, Blom, Houben, & de Vries, 2013). In oral food challenge fresh milk is usually used rather than baked milk for the CMA diagnosis. Oral milk challenges are carried out according to a hospital protocol in a well-equipped place in the clinic and supervised by a clinician (Fiocchi et al., 2010). A small and incremental amount of challenge food is administered within a period of 2-3 hours, followed by 2 hours of observation. In case of allergic reaction, the process is usually interrupted and necessary treatment is provided. Oral food challenge is called "positive" when the patient has an allergic reaction to food and "negative" when the milk challenge is completed without an allergic reaction. Oral milk challenges are used to determine if a patient has outgrown an existing milk allergy or to confirm CMA when the history or the allergy tests are unclear (Luyt,

et al., 2014). However, oral food challenges are time consuming and include the risk of anaphylaxis. SPT and sIgE cut-off values are being explored as they are cheaper, easier and less invasive (Hill, Heine, & Hosking, 2004; Sporik, Hill, & Hosking, 2000). A positive SPT or specific serum IgE test is not used to confirm the diagnosis because they indicate sensitisation to allergens (Calvani et al., 2007; Celik-Bilgili et al., 2005; Verstege et al., 2005). These tests should be combined with a clear history of an allergic reaction to confirm the diagnosis (Costa-Pinto & Basso, 2011).

Mild to moderate non-IgE-mediated CMA

The clinical diagnosis in non-IgE-mediated CMA considers the development of delayed gastrointestinal or cutaneous symptoms that improve or resolve with exclusion and reappear with reintroduction of cow's milk (Koletzko et al., 2012). Home milk-containing food reintroduction challenges are used to confirm the diagnosis and determine the tolerance to milk in mild to moderate non-IgE-mediated CMA (Venter, Laitinen, & Vlieg-Boerstra, 2012). In severe forms of non-IgE-mediated CMA, food challenges are not recommended at home and these patients are referred to secondary/tertiary care (Venter et al., 2013). Currently, the elimination and reintroduction of the milk/milk containing food is the only reliable diagnostic tool, due to the absence of validated diagnostic skin or blood tests (Luyt et al., 2014). Table 2.2 summarises the main characteristics of IgE and non IgE—mediated CMA.

Table 2.2: Main characteristics of IgE and non-IgE-mediated CMA

Characteristics	IgE-mediated CMA	Non-IgE-mediated CMA
Diagnosis	Allergy tests (IgE, SPT, food challenge taken in combination with clinical history)	Clinical history and Food challenge

Time of exposure to reaction	From minutes to 2 hours	From several hours to days
Severity of symptoms	Mild to anaphylaxis	Mild to moderate
Duration of CMA	May persist beyond 1 year of age	Usually resolved by 1 year

2.2.5. Management and treatment

The mainstay of CMA management is based on individualised avoidance of cow's milk and foods containing cow's milk from the patient's diet. The allergen avoidance helps in resolving the symptoms within approximately 2 weeks (Ludman, Shah, & Fox, 2013). Nutritional counselling and education regarding the management of allergic reactions are essential during CMA treatment (Venter et al., 2012). Children on prolonged milk free diets may be at risk of protein and energy malnutrition if specialised dietetic input is not provided (Meyer et al., 2013). Specialised allergy or paediatric dietitians play an important role in choosing milk formulas; monitoring of nutritional status; suggesting nutritional supplements; providing dietary advice for the breast-feeding mothers and infants; providing appropriate weaning advice; and recommending which foods should be omitted and which foods could be added to/tolerated in the diet of children with CMA (Groetch & Nowak-Wegrzyn, 2013; Mehta, Groetch, & Wang, 2013). Leaflets with written advice regarding suitable substitutes and recipes, online information and education regarding the food labels and lifestyle adjustment is an additional part of dietetic support (Venter et al., 2012). Children, who usually remain on the elimination diet for at least six months or one year, are individually assessed by the medical team for reintroduction of Cow's Milk (Burks et al., 2011). In formula-fed infants an extensively hydrolysed formula (EHF) and amino acid formula (AAF) are available for the management of CMA because they are less likely to trigger an allergic reaction. EHF consists of cow's milk proteins (casein or whey) that are broken down in smaller peptides while AAF are composed of pure synthetic amino acids (Host & Halken, 2004). Studies have shown that

AAF and EHF may provide a balanced nutrition with normal growth and development of infants suffering from CMA (Agostoni, Terracciano, Varin, & Fiocchi, 2016; Dupont, Hol, Nieuwenhuis, & group, 2015; Vandenplas, De Greef, Hauser, & Group, 2014).

Oral immunotherapy treatment (OIT) or oral tolerance induction is a possible option for IgE-mediated CMA treatment in the future. OIT involves the ingestion of increasing amounts of milk allergen on a regular basis to desensitize and help patients to develop tolerance. Some studies have used OIT and found that it provided effective sensitisation in young children with CMA (Chen & Land, 2017; Longo et al., 2008; Staden et al., 2007; Tripodi et al., 2013). However, immunotherapy for cow's milk is still under investigation and not considered as part of routine clinical practice because severe allergic events occur during oral immunotherapy (Pajno et al., 2017; Taniuchi, Takahashi, Soejima, Hatano, & Minami, 2017). Prospective control studies are required to investigate and compare the effectiveness of the development of tolerance to milk via introducing of natural baked milk containing foods or as a form of OIT.

2.2.6 Effects of CMA on patients and family - Impact of food challenges

Patients with a food allergy experience social and food restrictions and the constant vigilance required to manage their disease may impair their health-related quality of life. Research indicates that parents/caregivers of children with food allergies report that they and their children have a low quality of life compared with parents of healthy children or children with chronic diseases such as diabetes mellitus type 1, asthma, rheumatoid arthritis or other long-term conditions (Flokstra-de Blok et al., 2010). Another study demonstrated that parents or caregivers avoided including their child in social events, parties or any other family activities due to their concerns about any potential allergy food trigger exposure for their child (Bollinger et al., 2006). Having a child with food allergy can also impact the well-being of parents. Having a child who experiences unpredictable severe reactions and anaphylaxis has been associated with increased anxiety and stress in parents/caregivers (Lau et al, 2014). Additionally, there is evidence to suggest that parents of children with food allergy report increased anxiety, stress and depression compared with parents of children with no food allergy. Some parents have higher stress and anxiety due to the uncertainty of not having a medical diagnosis of their children's food allergy or lack of information related to the food allergy management from healthcare professionals (Birdi et al., 2016). Family

quality of life may be influenced by different factors such as age of the child, severity of reactions, food allergy trigger, previous life threatening reactions, having other concomitant allergy (eczema, asthma, other food allergy or multiple food allergy) (Antolín-Amérigo et al., 2016).

A well-conducted study has found that limited (or lack of) access to appropriate national health service primary and specialist care and inadequate support for effective long-term management of food allergy is associated with increased anxiety and stress for parents and their children (Akeson et al, 2011). Another study found that providing newly-diagnosed food allergic patients, and their families, with accurate and easily accessible (via a website) food allergy management information written by specialised healthcare professionals improved parents'/caregivers' and patients' quality of life (Vagras et al 2007). Family quality of life may be influenced by environmental factors such as lack of allergy awareness or lack of access to appropriate healthcare (Antolín-Amérigo et al., 2016). Hence, based on the evidence above, effective communication between parents/caregivers/patients and healthcare professionals, and access to accurate and comprehensive information related to health-decisions could benefit parents and patients to improve their quality of life and reduce stress and anxiety.

Interestingly, food challenge outcomes (both positive and negative) seem to have a beneficial role in the psychological condition of parents and children. Recent studies report that food challenge outcomes in peanut/egg allergic children reduced parental concerns and anxiety and improved their quality of life even when the outcome of a food challenge was positive (Howe, Franxman, Teich, & Greenhawt, 2014; Kemp, Allen, & Campbell, 2009; Zijlstra et al., 2010). Knibb et al (2012) observed that allergy patients who undertook a food challenge had improved health- related quality of life and reduced parental anxiety compared to food allergic children that did not undergo a food challenge. A recent meta-analysis assessed seven studies related to the effects of food challenges on food allergy health-related quality of life and found that food challenges are associated with improved food health-related quality of life of patients and reduced parental concerns and burden regarding the food allergy management of their children (Kansen et al., 2018).

Looking specifically at the impact of milk allergy on patient and family quality of life, there is limited evidence regarding its impact. However, one study has found that milk or egg allergy is associated with worse total and domain-specific caregiver QoL scores versus peanut or tree nut allergy (Howe et al., 2014). This is understandable given that food allergy triggers like milk and egg are hidden in a variety of foods and are mainly associated with severe reactions and anaphylaxis in children (Anagnostou, 2018). In children, most deaths that have been reported were caused by hidden food ingredients to which the patient was highly allergic. Interestingly, a recent review of studies demonstrates that cow's milk and seafood/fish are the main allergy food triggers that provoke fatal

reactions in many countries (Pouessel et al., 2018). The very severe nature of IgE- mediated milk allergy means that there is likely a great impact on QoL, although a limited number of studies have explicitly explored this.

Hence, parents' and children's quality of life is affected by the constant threat of exposure to food allergy triggers and the vigilance required to avoid any undesirable exposure or food contamination during daily social or family activities such as family and school meals, parties, eating out. Also, the potential difficulties regarding access to resources and information regarding how to cope with stress during CMA management, and uncertainty regarding reliability of the given information may add further stress for caregivers and patients. Despite the increased use of gradual allergen introduction as a potential treatment for patients with food allergy there is however a lack of studies that demonstrate the effects of long-term gradual allergy food introduction on children's and their parents' quality of life.

In particular, there is no data regarding the impact of home baked milk introduction on children and their families despite this being a staple of milk allergy management and treatment. Due to the research gap in this field, one of the purposes of this research is to listen carefully to parents that gradually introduce baked milk products into the diet of their milk allergic children and understand their experience, fears, concerns and needs during this process. Understanding of parents' concerns and needs can contribute to the improvement of resources such as clear and concise leaflets or a design of a comprehensive milk ladder plan, and any other educational materials that would likely reduce parental stress during the management and treatment of their children milk allergy.

2.3. Use of baked milk in the management of IgE and mild to moderate non- IgE-mediated CMA

The milk-free diet may help CM allergic children to prevent milk allergic reactions and inflammation, but may have a negative impact in the adequate intake of essential nutrients. Undernutrition may not only have consequences in children's development and growth, but also impair proper socialisation and family quality of life (Meyer et al., 2017). Children usually remain on the elimination diet for at least six months or one year and are individually assessed by the medical team for reintroduction of cow's milk at regular intervals (Burks et al., 2011). Current research indicates that tolerance to cow's milk is developed with time and this process can be helped with the gradual re-introduction of small amounts of baked milk or baked milk containing foods into the diet of children who are still allergic to milk (Dupont, 2013). However, the appropriate timing of baked milk reintroduction and place (hospital/clinical setting or home/outside the clinical setting) to conduct the first reintroduction of cow's milk are challenging questions for healthcare professionals and carers/patients. The process of assessment of the suitability of candidates for baked milk re-introduction depends on the type of CMA.

For IgE-mediated-CMA, clinically supervised oral food challenge (Jarvinen & Sicherer, 2012) and immune markers assessment including SPT and milk specific IgE (Boyce et al., 2010) in combination with the medical history are used to identify candidates' potential to tolerate CM. In IgE-mediated-CMA there is a risk of anaphylaxis and oral food challenges must be carried out under medical supervision in a well-equipped area suitable for treating severe reactions, close to an intensive care unit (Fiocchi et al., 2010). Hospitals follow a specifically designed protocol which includes criteria for determining allergic reactions, parental informed consent forms and information regarding the challenge food and process (e.g. dose). Milk challenges usually form the first step of a gradual re-introduction of milk or milk-containing foods. In recent years, CM allergic children are challenged using baked milk products (which are less allergenic) before moving on to challenges using fresh milk-containing products where appropriate. Baked milk challenge is a process of using baked milk or a milk-containing food (biscuits/muffin/pizza) in increasing doses over a period of one day (e.g. eating the same cake in increasing amounts) to perform a food challenge (Venter et al., 2013). Research is increasingly informing us that food processing such as baking or cooking at high temperatures, exposure to low pH or enzymatic digestion may destroy specific milk

proteins and help many children to tolerate baked milk-containing foods such as biscuits, breads, cakes, waffles and macaroni cheese (Nowak-Wegrzyn et al., 2008). However, there are children who may react to baked milk and an initial challenge to very small amounts of baked milk should be undertaken with support from a child's doctor or dietitian. Table 2.3 summarises the dietary management differences between IgE and mild to moderate non-IgE-mediated CMA.

Table 2.3: Differences between dietary management in IgE and non-IgE-mediated CMA

IgE- mediated CMA	Mild to moderate non-IgE-mediated CMA
Initial elimination/avoidance of cow's milk and milk containing foods in a period of 2- 4 weeks – In long: 6 months to 1 year	Initial elimination of cow's milk and milk containing foods in a period of 6 months to 1 year
Maternal avoidance of milk and milk products may be required in breastfed infants	Maternal avoidance of milk and milk products may be required in exclusively breastfed infants
Complete avoidance including "traces"	No need to avoid "traces"
Potential tolerance to small amounts of milk/milk containing foods	Potential tolerance to small amounts of milk/milk containing foods
Potential tolerance to baked milk/baked milk foods	Potential tolerance to baked milk/baked milk foods

2.3.1 Guidelines in the use of baked milk

This section will discuss what guidelines have been developed to aid healthcare professionals to decide who CMA patients are optimal candidates for baked milk introduction in the management of IgE and non- IgE mediated CMA. This section is not a part of the systematic review.

Guidelines in IgE-mediated CMA

Currently, the only guidelines that are focused on IgE-mediated cow's milk allergic patients and help healthcare professionals to select, monitor, and prepare appropriately, potential candidates for baked milk reintroduction are published by the British Society of Allergy & Clinical Immunology (BSACI) (Luyt et al., 2014). The BSACI guidelines for the diagnosis and management of CMA state that baked milk challenges could be used before fresh milk introduction because the reaction to baked milk is less likely to be severe during food challenges (Luyt et al., 2014). They suggest that baked milk reintroduction might be attempted in hospital or at home for children with IgE-mediated CMA according to the clinical criteria outlined in Table 2.4.

Table 2.4: Criteria taken into consideration in the baked milk reintroduction

Home -baked milk reintroduction/milk ladder	Hospital-baked milk reintroduction/baked milk challenge
• **Children that had only cutaneous symptoms on a mouthful milk allergen exposure** • **Reduction of milk specific IgE and Skin Prick Test** • **No reaction to milk in the past 6 months**	• Poorly controlled asthma and regular asthma preventative inhaler treatment • Multiple or complex allergy • Parents/carers who find it difficult to comprehend and comply with the protocol • Less severe reaction with trace of milk allergen exposure • Previous CMA symptoms affecting breathing, gut or circulation

Guidelines in mild to moderate non-IgE-mediated CMA

Currently, the only published guidelines related to baked milk introduction in mild and moderate non-IgE-mediated CMA have been introduced by the " MAP Guidelines (2013)" and its updated version "the iMAP Guidelines (2017)" (Venter et al., 2017; Venter et al., 2013). These guidelines not only enhance the knowledge of healthcare professionals in primary care and provide more help in recognising and differentiating between potential IgE-mediated CMA and non-IgE-mediated CMA but also confirm that all those remaining children diagnosed as mild to moderate non-IgE mediated CMA are suitable for home baked milk introduction (Venter et al., 2017). For the management of mild to moderate non IgE - mediated CMA the MAP and iMAP guidelines have suggested a baked milk food protocol that is also called a milk ladder (appendix 6) for gradual reintroduction of milk containing foods into the diet of non-IgE mediated CMA children at home. There are a variety of milk ladders that are distributed to patients or their caregivers by hospitals. However, none of these milk ladders have been validated by a clinical trial. A rationale for a milk ladder classification is presented in Figure 2.2. The scale starts in the first stage with less allergenic foods (lower dose of protein and more denatured) and completes gradually with more allergenic foods (higher protein dose and less denatured).

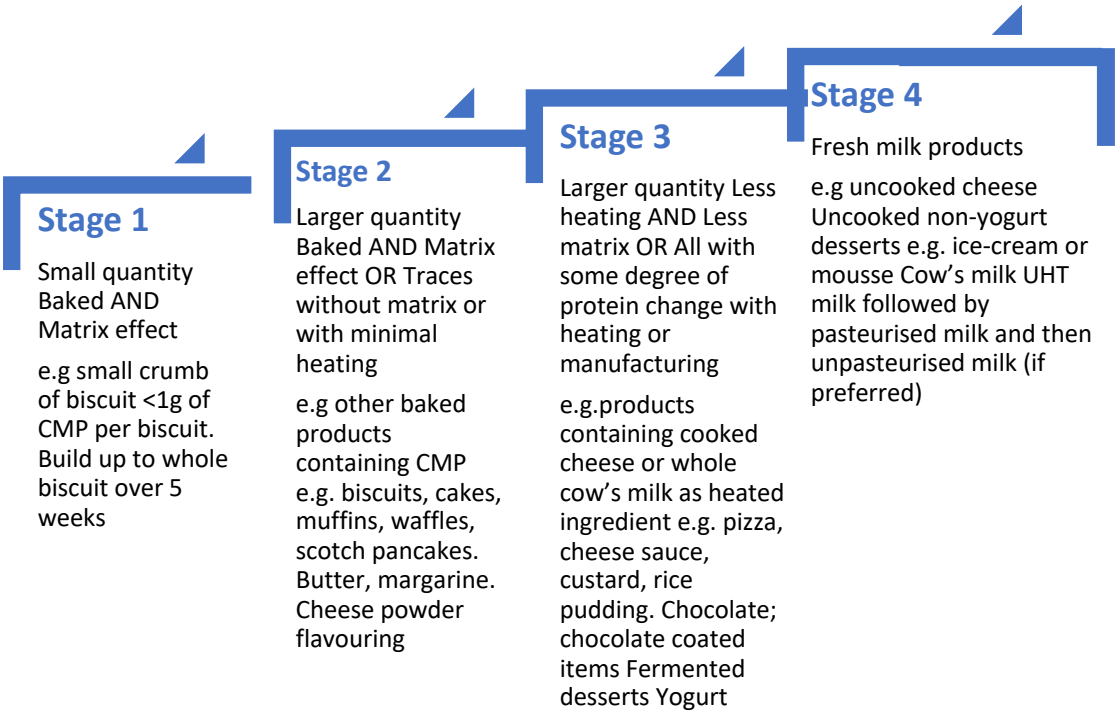

Figure 2.2: Classification of a milk ladder (Luyt, Ball, et al., 2014)

However, there are a variety of milk ladder versions available that are distributed by hospitals and there is not any milk ladder that has been validated by a clinical trial. In addition, hospitals follow their own baked milk challenge protocols based on different methods and guidelines (there is a lack of standardisation in the practice, process and dose regimes), and these different approaches may have different immunological effects (Upton & Nowak-Wegrzyn, 2018). In particular, baked milk tolerant children may react to milk and their reactivity could be unpredictable and severe (Bartnikas, Sheehan, Hoffman, et al., 2012; Nowak-Wegrzyn et al., 2008; Upton & Nowak-Wegrzyn, 2018). For this reason, many researchers suggest medical supervision for the initial baked milk introduction in children with IgE mediated CMA. The MAP guidelines (2013) suggest that no child with IgE-mediated food allergy should have a challenge in primary care or community settings (Venter et al., 2013).

European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines (2012) recommend to not prolong unnecessary dietary restrictions and suggest supervised CMP challenges (Koletzko et al., 2012). WAO Guidelines (2010) recommend that all interventions and avoidance strategies be re-evaluated on a yearly basis and oral food challenges should be carried out under medical supervision (Fiocchi et al., 2010). However, in contrast to these guidelines, as mentioned above, the BSACI milk allergy guidelines suggest home introduction of milk using a "milk ladder" in IgE-mediated CMA (Luyt et al., 2014; Luyt, Krishnan, Huber, & Clark, 2016). Hence, the lack of a national or international agreement regarding the baked milk containing food introduction could lead to different advice that could confuse caregivers and expose IgE-mediated cow's milk allergic children to the risk of an accidental reaction.

- Mild to moderate non-IgE-mediated CMA

The only guidelines related to BMC and ML in mild and moderate non-IgE-mediated CMA are the MAP Guidelines (2013) and its updated version "the iMAP Guidelines" (2017) (appendix

8) that suggest that all those remaining children diagnosed as mild-moderate non-IgE mediated CMA are suitable for home baked milk introduction (Venter et al., 2017; Venter et al., 2013). The international iMAP recommendations enhances the knowledge of healthcare professionals in primary care and provides more help in recognising and differentiating between potential IgE-mediated CMA and non-IgE-mediated CMA (Venter et al., 2017).

2.4. Role of baked milk in the development of milk tolerance in CMA

A literature review taking a systematic approach has been conducted to identify and critically evaluate studies that have investigated the effects of baked milk challenges on the development of milk tolerance in children with IgE and non-IgE mediated CMA and the role of immune markers (SPT and milk sIgE) in predicting oral milk challenge outcomes in IgE-mediated CMA. The purpose of this review is to explore what is known about the introduction of baked milk in milk allergic patients specifically regarding the usefulness and safety of this procedure, and which immune markers are currently used to identify the development of tolerance to milk containing foods in IgE-mediated CMA.

2.4.1 Methods of the systematic review

Data source - Search Strategy for identification of relevant studies

Published studies were identified by using Web of Science, PubMed (U.S National Library of Medicine & National Institutes of Health), CINAHL Heading and also Cochrane Central Register of Control Trial (CENTRAL) databases from 1995 until 2018. The search was performed by a combination of Medical Subject Heading (MeSH) algorithms (appendix 5) and the following keywords: Cow's milk allergy, baked milk challenges, heated milk foods, baked milk tolerance, milk ladder. Citation searching was also carried out using the search engine Google Scholar and Scopus citation database to identify peer-reviewed articles, abstracts and full-texts. The bibliographic software EndNote Reference Manager was used to manage and record references.

Study Design

Study selection including initial screening of titles and abstracts was performed to identify potentially relevant papers. The selection of included full papers was based on the following inclusion or exclusion criteria:

Inclusion criteria:

This review considered:

- All studies that used baked milk challenges and immune markers to diagnose CMA or identify tolerance to cow's milk protein or baked milk in children and adults with any study design (Observational case control - cross sectional – Prospective and retrospective cohorts and Randomized Control Trials).
- Published and unpublished articles and monographs,
- "Grey literature'' that includes organisational project papers and clinical guidelines {National Institute for Health and Clinical Excellence (NICE) guideline, British Society of Allergy and Clinical Immunology (BSACI) guidelines for the Diagnosis and Management of Milk Allergy, European Academy of Allergy and Clinical Immunology (EAACI) guidelines and American Academy of Allergy, Asthma and Clinical Immunology (AAAAI) recommendations in the management of food allergen introduction.
- Full reports of studies during the period 1990-2018.

Exclusion criteria

Studies which did not involve baked milk challenges/introduction of baked milk containing foods to diagnose or determine development of tolerance to cow's milk were not included in this review.

Study Selection

The identified references for which the title and abstract appeared to meet the predetermined inclusion criteria were obtained and their full papers were evaluated. Eligible studies were those relevant to the use of baked milk challenges and protocols in the diagnosis of CMA or in determining tolerance development to baked milk. Studies related to the role of immune markers (Specific milk IgE immunoglobulins and Skin Prick Test) in predicting food challenge outcomes in forms of baked milk were evaluated. Duplicate publications of research results were assessed. The search results were imported

into EndNote, a reference management software program. A flow diagram is provided below with the number of articles reviewed at each stage according to the PRISMA (Preferred Reporting Item for Systematic Review) group (reference needed here to the PRISMA guidelines).

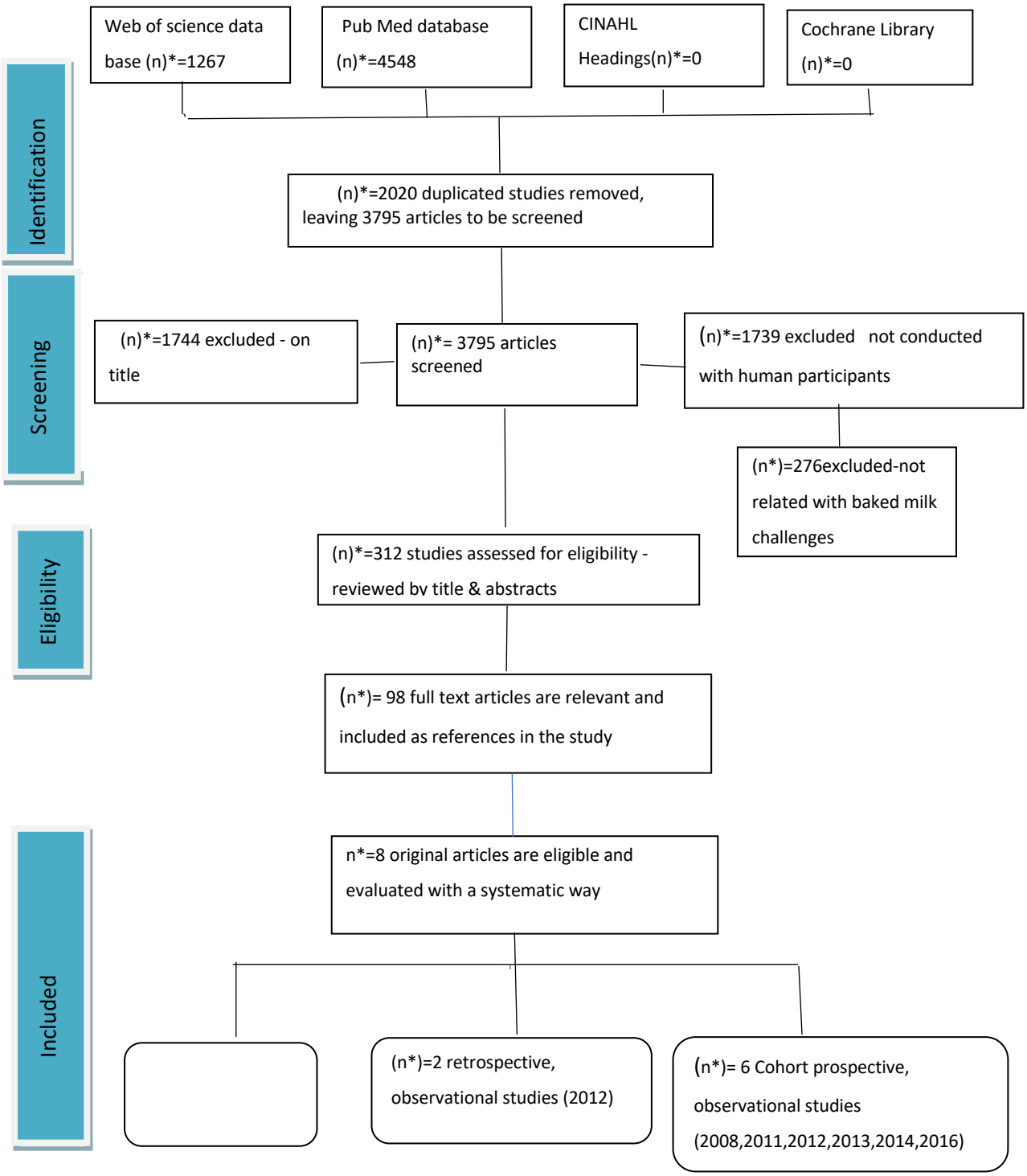

Figure 2.3: Flow chart of study selection

Data extraction – Quality assessment

The population characteristics and study design, exposure-outcome measurements, assessments and findings of the reviewed studies are presented in Table 2.5 and Table 2.6 of the next section 2.4.2. Quality assessment was carried out on each included study using an adaptation of the CASP (Critical Appraisal Skills Program) approach which involved an assessment of internal and external validity.("CASP Appraisal Tools Links with PHRU link. http:www.phru.nhs.uk/pages/PHD/resources.htm,"). Aspects, such as risk of bias caused by study design, conduct or analysis, statistical issues (odds ratio, P-value, confidence intervals etc) and choice of outcome measures were considered.

2.4.2 Discussion of findings

This section is divided into three parts and presents the findings of the studies that have been reviewed using a systematic approach. In the first part of the section, the baked milk challenge outcomes of children with CMA are discussed. In the second part of the section the role of immune markers in predicting baked milk challenge outcomes in IgE-mediated CMA is introduced. In the last part of the section the clinical symptoms that have been observed in CMA children who underwent a baked milk challenge in order to evaluate the safety of the baked milk dietary intake is presented.

Baked milk challenge outcome studies

All the studies that explored the impact of baked milk challenges on the development of milk tolerance in children with CMA have been reviewed and summarised in Table 2.5.

Table 2.5: Population characteristics, study design, exposure-outcome measurements, assessments and findings of the reviewed baked milk studies

References	Design-Population	Exposure &assessment measures	Preparation of baked milk products for testing	Findings
Nowak-Wegrzyn et al. 2008	Prospective-case control study N=100 children aged 2-17yrs (median age: 7.5 (2.1–17.3) 7yrs) Approval by IRB Mount Sinai, USA	SPT≥ wheal 8mm milk specific IgE: >5KUA/L if ≤2yrs or >15KUA/L if >2yrs	1.3 g of milk protein from non-fat dry milk powder in a muffin baked at 350 °F for 30 min. 1.3 g milk protein (cooked in a waffle maker at 500 F for 3 min)	68 (68%) participants were heated milk-tolerant, 9 (9%) participants tolerated heated & unheated milk, 23 (23%) participants showed reaction to heated milk
Kim et al.2011	Prospective-case control study, N=89 participants aged 0.5-21yrs (median: 6.6 (2.1–17.3) Comparison group:N=60 Approval by IRB Mount Sinai,USA (June 2004-Oct 2007)	SPT≥ wheal 8mm milk specific IgE: >5KUA/L if ≤2yrs or >15KUA/L if >2yrs	1.3 g of milk protein from non-fat dry milk powder in a muffin baked at 350 °F for 30 min. Cheese pizza containing 4.6 g of milk protein (baked at 425 F for 13 min or longer)	From 65 children initially tolerant to heated milk (HM), 39 (60 %) tolerated unheated milk. From the HM reactive group (n = 23), 2 (9 %) tolerated unheated milk, 3 (13 %) tolerated HM and baked cheese, whereas the majority (78 %) avoid milk strictly. Children initially tolerant to HM were more likely to become unheated milk tolerant compared with HM reactive children ($p < 0.001$) and those who incorporated dietary baked milk were more likely than the comparison group to become unheated milk tolerant ($p < 0.001$)
Bartnikas et al. 2012	Retrospective observational study N=35 children aged 3 -18yrs,	Medical records: Allergist-documented history of allergic reaction to milk	1.3 g of milk protein from non-fat dry milk powder in a muffin/cupcake	83 % (29/35) children passed BMC & 17%(6/35) failed BMC Most children allergic to cow's milk tolerated baked milk.

	Median age: 8.1 (3.1–18) Approval by IRB Boston Children's Hospital, USA (Sep 2009-Sep 2011)	and/or positive testing: SPT positive >3mm & 34(97.1%) out of ?? participants had SPT to casein -sIgE to milk lowest limit 35kUA/L& 33 (94%) out of ?? children had sIgE to casein, a-lactoalbumin &/b-lactoglobulin	baked at 350 °F for 30 min.	
Caubet et al. 2013	2 Cohorts – Prospective study N=97 children from Nowak-Wegrzyn et al (2008) study N=128 children from Nowak-Wegrzyn et al study Total N=225 from Kim et al (2011) study Median Age of HM tolerant group: 7.5 (4.0–11.0) Age of HM reactive group: 8.0 (4–10)	Allergic reaction to milk in past 6 months and positive testing (SPT or sIgE), or highly predictive testing (sIgE >5 kU_A/L if <2 years or >15 kU_A/L if >2 years, or SPT wheal 8 mm)	1.3 g of milk protein from non-fat dry milk powder in a muffin baked at 350 °F for 30 min. 1.3 g milk protein (cooked in a waffle maker at 500 F for 3 min)	69 % (83/121) passed baked milk challenges.
Mehr et al.2014	Prospective study N=70 HM tolerant median age: 4.5 (2.5–8) HM reactive median age: 7.3 (4.9- 7.3)	Allergic reaction to milk in past 12 months and positive testing (SPT or sIgE), or SPT wheal >7 mm if >2 years or >5 mm if <2 years	0.5 g of milk protein in a muffin baked at 180 °C for 20 min.	51 (73 %) passed the BMC and incorporated BM into their diet. 19 children (27 %) reacted to their challenge and 4 (21%) from 21 developed anaphylaxis and required intramuscular adrenalin. Predictors of clinical reactivity to BM were asthma and a history of CM anaphylaxis

Ford et al,2013	Prospective study N = 132, median age: 7.6 (4.0–11.0) Prospective	SPT≥ wheal 8mm milk specific IgE: >5KUA/L if ≤2yrs or >15KUA/L if >2yrs	1.3 g of milk protein from non-fat dry milk powder in a muffin baked at 350 °F for 30 min. Pizza (4 g of milk protein baked at 425 F for at least 13 min), rice pudding (7.7 g of milk protein baked at 325 F for 90 min)	95(72 %) CMA patients tolerated some forms of HM in their diets.
Kwan et al 2016	Prospective study N=30 Median age: median age: 7.5 (2-16)	SPT:8-14mm slurry muffin Included patients with SPT≥8mm - excluded SPT≥15mm Optimal casein specific IgE: 6KUA/I Negative decision point casein IgE:1KUA/I	1.3 g of milk protein from muffin baked at 350 °F for 30 min. The doses of one muffin:1/8, 1/8,1/4,1/4,1/2, and finally ¾, giving a total of two muffins (2.6grof milk protein)	18(60%) tolerated muffin challenges. Predictors of baked milk reactivity were asthma, asthma requiring preventer therapy, IgE-mediated clinical reactions to more than 3 food groups, and a history of CM anaphylaxis. The powder milk was not helpful

In 2008, Nowak-Wegrzyn et al. reported a study in which they food challenged 100 CMA children (average age 7.5 years, range 2.1-17.3 years) and found that the majority (75%) of participants became tolerant to baked/heated forms of CM such as muffin, cakes, breads and waffles before they became tolerant to pure/uncooked forms of CM. Growth and intestinal permeability were also monitored and no differences were observed in baked milk tolerant children (Nowak-Wegrzyn et al., 2008). In this prospective-case control study they excluded children with negative SPT, undetectable milk sIgE, unstable asthma, allergic rhinitis or atopic dermatitis, milk-induced eosinophilic gastroenteropathy, and patients with a recent reaction to baked milk.

Kim et al. (2011) conducted a follow up prospective - case control study and challenged 88 IgE mediated CMA children (average age 6.5 years, range 2.1-17.3 years) between 2004 and 2007. In 2011, they reported that among 88 children, 65 (74%) tolerated their initial muffin challenges, 39 (60%) of those children became tolerant to unheated milk within the 5-year

follow up period, 18 (12%) children tolerated a cheese-pizza challenge and 8 (12%) children chose to avoid milk. Interestingly, these researchers found that baked milk-tolerant children were more likely to become unheated milk tolerant compared with baked milk-reactive children (P<.001). They consequently argued that the inclusion of baked milk products in the children's diet at regular intervals may accelerate the development of tolerance to CMA compared to those children who follow milk exclusive diets.

In 2011 and 2013, Cubet et al. analysed data collected prospectively from 225 patients (average age 7 (2-17) years between 2004 and 2010. Two separate cohort studies were conducted in the same clinical research centre but in different periods. In the first cohort that was conducted between 2004 and 2007, among 97 children that undertook baked milk challenges, 23(24%) children reacted to baked milk, 66(68%) patients tolerated baked milk and 8(8.3%) children tolerated baked and unheated milk. In the second cohort that was conducted between 2008 and 2010, among 128 children that undertook baked milk challenges, 38(30%) children experienced an allergic reaction and failed the challenge, 83(65%) patients tolerated baked milk and passed the challenge, and 7(5.5%) children tolerated baked and unheated milk and passed both baked and unheated challenges.

In 2013, Ford et al conducted a prospective study and among 132 CMA children (average age 7.6 (4-11 years) that were challenged 95(72%) tolerated a variety of baked milk foods. This study confirms the findings of the studies above that the majority of patients with CMA are able to tolerate some baked milk containing foods and include them into their diet.

The four prospective studies above (all conducted in the USA) have similar and promising results regarding the development of baked milk tolerance in CM allergic children, but these studies need substantiation from randomised control trials that could compare different phenotypes of CMA or age groups, doses of the baked milk, and unheated food challenges. In these studies, the average age of participants was 7-8 years old and no sufficient data for infants and younger children were provided. The food doses that were administered during baked milk challenges were lower compared with the doses of unheated milk challenges and this may confound the findings of the studies. In addition, these studies did not determine whether the children outgrew their CMA because of either a repeated exposure to baked milk products or because they were suffering from a less severe type of CMA. Interestingly, none of these participants were challenged to fresh milk before they undertook a baked milk

challenge. Therefore, the high rates of baked milk tolerant children may include those who had already outgrown their CMA. Selection bias may present in data collection because the food challenges that underwent the children were not double blind placebo controlled challenges which are the gold standard in the allergy tests (Bock & Atkins, 1990).

In 2012, Bartnikas et al reported a study in which they challenged 35 CMA children (average age 8 (3-18) years) and observed that 29 (83%) children passed muffin/cupcake challenges and 6 (17%) children failed muffin/cupcake challenges. This retrospective chart review collected and analysed data from all patients who underwent hospital baked milk challenges between 2009 and 2011. Participants had a previous allergic reaction to baked or unheated milk and positive SPT or detectable milk sIgE. Children were challenged with home-made muffin/cupcake that was prepared by caregivers. The baked milk challenge protocol (food recipes and doses of challenge food) was based on previous method that was published by Nowak-Wegrzyn et al (2008).

Bartnikas et al is also a USA study and its results were similar to other USA studies. However, due to the retrospective design, longitudinal data was not available regarding the baked milk containing foods that children had at home and their progression to tolerance of fresh milk. The diagnosis of baked milk allergy was based on history and allergy tests, and it was not confirmed by an oral food challenge at entry of the study. Therefore, there is a possibility that some of the children who tolerated a muffin/pancake might also have been tolerant to unheated milk.

In 2014, Mehr et al challenged 70 CM allergic children (median age: 5.3 years) with muffin; 51 (73%) children tolerated baked milk at challenge and incorporated this baked milk food into their diet. An interesting finding of this study is that a large number of children (58%) with prior severe reaction to baked milk or anaphylaxis to unheated milk tolerated the baked milk challenge.

In 2016, Kwan et al challenged 30 children with CMA [average age 7 (2-16)] years and from those 8 (60 %) children were baked milk tolerant. Authors reported that gender, history of asthma or eczema, and history of anaphylaxis did not predict oral baked milk challenge in their sample (Kwan et al., 2016).

The USA studies described reported that those CMA children with the combination of multiple-IgE-mediated food allergies, asthma and prior anaphylaxis to milk were at risk of reacting to baked milk. However, the Australian and Canadian studies outlined above found that some children with a history of severe reaction to baked milk or anaphylaxis to unheated milk were tolerant to baked milk and a history of asthma/eczema/anaphylaxis was not a predictive factor for a reaction to baked milk. However, the main limitation for both studies was that CMA children were not challenged to cow's milk to confirm their milk allergy before they experienced a BMC.

In summary, the findings of this section of the review indicate that: the majority of children with CMA tolerate baked milk, although there are contradictory findings regarding the predictive indicators of a BMC outcome; there are few studies that have explored baked milk introduction in IgE mediated CMA and the majority of the studies were conducted in the USA; there are no studies that have investigated the effects of baked milk introduction in IgE and non-IgE mediated CMA at home; there are no studies that have evaluated the long-term effects of baked milk ingestion on growth and other atopic diseases in IgE and non-IgE mediated CMA.

The studies that have explored the effectiveness of baked milk introduction in IgE mediated CMA have found that the majority of CMA children passed the baked milk challenges and were able to tolerate baked milk. These studies provided baked milk challenge protocols in terms of food doses during a challenge and a practical guide with baked milk food recipes that may be used by other healthcare professionals and adapted in their research or clinical practice. They have provided evidence that CMA children who pass a baked milk challenge may be tolerant of a variety of products that contain baked milk and may outgrow their milk allergy quicker compared with those CMA children who react to baked milk or baked milk containing foods. However, further studies with a high-quality design are required to provide robust evidence to assess whether repeated exposures to baked milk products can help some children with CMA to outgrow their milk allergy quicker than other children and whether any effect observed can be explained by different phenotypes of milk allergy (persistent or transient cow's milk allergy). Interestingly, the findings of these studies indicate that baked milk introduction may expose some patients at risk of severe reactions or anaphylaxis. In the Mehr et al, (2014) study four children reacted sufficiently to require IM adrenaline and indeed all those who failed a BMC in these studies had by definition

experienced an allergic reaction. Further investigation is required to provide evidence regarding reliable indicators that could predict baked milk challenge outcomes. Furthermore, these studies have not provided data regarding the long-term effects of introducing baked milk containing foods in children at home and the type of baked milk containing food - protocols that were suggested to parents/ caregivers and continued at home need consideration and further investigation in term of their feasibility, efficacy and safety. All findings above need to be validated by multicentre clinical trials because they were conducted in a single centre (tertiary care) with a limited age range of participants. Food challenge protocols used in future studies also need to be standardised in terms of milk protein amounts, administered doses during challenge, and preparation and reasonable texture of challenge foods to avoid selection bias that may confound challenge outcomes. Additionally, diagnostic criteria need to be established (e.g confirmation of CMA diagnosis or milk tolerance status of a CMA child before starting the study) to avoid not only selection bias during the recruitment of participants but also observer bias that may be a result of the investigator's knowledge and expertise that may influence the way data is collected, measured or interpreted for each group of participants.

Safety of baked milk introduction: Symptoms associated with baked milk challenges

While it seems that staged introduction of baked milk may be successful in the development of milk tolerance, it is also important to consider the short and long-term safety of this treatment. Of the studies examined in the previous section, only four presented data about the range of clinical symptoms experienced during the baked milk challenges. Although reporting data indicated that there were participants who experienced adverse reactions (i.e. failed the baked milk challenges) in all the studies that have been outlined in the previous table, authors have not reported the specific symptoms experienced by patients. Clinical symptoms that have been reported in baked milk challenge studies are summarized in Table 2.6.

Table 2.6. Clinical symptoms observed during baked milk challenge

References	Symptoms	Baked milk challenge
Nowak-Wegrzyn et al. 2008	Oral pruritus, Atopic dermatitis flare, rash, hives or angioedema, sneezing, rhinoconjunctivitis, throat symptoms or cough, wheezing, shortness of breath or respiratory distress (gasping, cyanosis, decreased oxygen saturation), abdominal pain, nausea, vomiting or diarrhoea, dizziness, loss of consciousness or hypotension, anaphylactic shock	Baked milk, muffin, homemade breads &waffles
Kim et al.2011	Anaphylactic shock, oral symptoms, wheeze, cough	Cheese omellete waffle, pizza, muffin
Bartnikas et al. 2012	Rhinorrhea, hives, tongue itching, oral pruritus, 1 patient developed anaphylaxis at home with hives, lip swelling & vomiting & treated with epinephrine A late reaction to ongoing baked milk exposure at home occur	Muffin, cupcake
Mehr et al.2014	Anaphylactic shock Urticaria & angioedema (47%) Itchy mouth or tight throat (53%) Abdominal pain (20%) Vomiting and/or acute-onset diarrhoea (13%) 3 children developed symptoms at homme with ongoing exposure 1 week later (itch, abdominal pain, and flaring of eczema)	Muffin

In 2008, Nowak-Wegrzyn et al. reported that a child developed oral pruritus to homemade bread and waffle, and two other participants developed mild oral symptoms to homemade waffle and pizza during home reintroduction. They also reported anaphylaxis in both groups;

baked milk tolerant [3.2% (5 of 65 children)] and baked milk reactive children ranged from [17% (3 of 23 children)] to [35%(8 of 23 children)] during baked milk challenges in hospital. However, baked milk-reactive children experienced more severe reactions compared with baked milk-tolerant children during their challenge to fresh milk and received epinephrine. Children who reacted to baked milk challenges had a history of asthma or multiple food allergy. In this study has been reported that in IgE mediated CMA baked milk challenges need to be approached with caution and performed in a clinic with all safety measures and supervised by a clinician, due to the risk of severe reactions or anaphylaxis.

Hence, these findings indicate an association between reactions to baked milk challenges and a history of allergic diseases and highlight that baked milk reactivity and history of severe allergy disorders could be a predictor of a more severe and persistent CMA phenotype. Therefore, for patients with IgE mediated CMA, there is evidence to suggest that baked milk should be conducted in hospital under medical supervision.

Bartnikas et al (2012) reported that three children who passed baked milk challenges in clinic reacted to ongoing exposure to baked milk containing food at home. These finding are in line with the results of Nowak-Wegrzyn et al who also reported reactions to ongoing exposure to baked milk containing foods at home. However, it is difficult to determine the exact reason for this. In particular, standardisation is difficult to achieve in a home environment. It is not clear if parents followed the recipe exactly in terms of time and temperature of baking and the amount of milk protein content.

Kim et al, (2011) reported that overall 6 children had anaphylaxis during baked milk challenges in hospital. Regarding the safety of dietary baked milk intake at home, this is the only study that considered the long-term safety of baked milk introduction (at 12-month follow-up), and found that the incorporation of baked milk products into children's diets doesn't appear to cause any changes in underlying allergic diseases (no increase in the severity of chronic asthma, atopic dermatitis, or allergic rhinitis), intestinal permeability, or in the growth of patients. However, these are certainly plausible conclusions given the study findings and further investigation with longitudinal cohort studies is required to confirm the long-term effects of baked milk introduction in health-related quality of life of children with CMA.

Mehr et al.,(2014) reported that the children who reacted to baked milk were more atopic with multiple other food allergies, asthma and a higher rate of previous anaphylactic reactions to cow's milk compared with children who tolerated baked milk. The authors concluded that children with a multiple IgE-mediated food allergies, asthma and prior anaphylaxis to other foods appear to be at risk to experience an anaphylactic reaction to baked milk and recommended that the initial baked milk introduction should be conducted in hospital under a medical supervision. These results confirm Nowak-Wegrzyn et al findings that demonstrated an association between reactions to baked milk challenges and concurrent asthma or other foods allergies. Mehr et al reported also that a small proportion of children that had passed a baked milk challenge in hospital developed symptoms with ongoing exposure to the same doses of food that was tolerated in hospital. These results are in agreement with the findings that are reported by Bartnika et al study and indicate that healthcare professionals need to emphasise the improtance of accurate baking and quantity of milk proteins in the milk containing foods during the baked milk re-introduction at home and provide a detailed list of instructions to help parents or caregivers during this process.

Hence, it seems that adverse reactions are relatively common during the introduction of baked milk, particularly in those who have a history of reacting to baked milk prior to treatment and/or who have asthma or multiple food allergies. Thus, this procedure requires medical supervision and should only be conducted in an environment where any severe reactions could be managed. Even when a CMA child has passed a baked milk challenge this cannot guarantee future tolerance of baked milk containing foods or exclude the possibility of any allergic reaction to the same doses of this food. The majority of the baked milk challenge studies demonstrate that healthcare professionals and parents/caregivers need to be aware about the possibility of late reactions to ongoing baked milk exposure and monitor CMA children over time to ensure that baked milk tolerance is still maintained.

Role of immune markers in predicting food challenge outcomes

Milk proteins and development of milk tolerance

Looking at milk proteins, casein is implicated as the offending protein in persistent milk allergy whereas whey proteins, such as β-lactoglobulin and α-lactalbumin, are implicated in patients with transient milk allergy (Jarvinen et al., 2002). Cow's milk contains sequential/linear and conformational epitopes and cow's milk allergic individuals may produce specific IgE antibodies against both conformational and sequential epitopes (Vila et al., 2001). Children with persistent

IgE mediated CMA have shown significantly higher ratios of specific IgE to linear versus conformational epitopes compared with children who have achieved tolerance (Skripak et al., 2007). Nowak-Wegrzyn et al (2008) and Bartnikas et al (2012) reported that patients with transient IgE-mediated CMA produced milk specific-IgE-antibodies against conformational epitopes that are destroyed during heating of milk or milk containing foods.

The caseins and α-lactoalbumin are more heat stable compared with β-lactoglobulin and other whey proteins (Taheri-Kafrani et al., 2009). Bloom et al (2014) found that casein is heat-resistant while β-lactoglobulin and α-lactalbumin are heat labile. Heating of milk may affect protein conformational structure and modify conformational epitopes that leads to a change in the allergenicity of milk products. In addition, the heating of a complex food causes interaction of milk proteins with other food components such as wheat (e.g. muffin, cupcake) and may reduce the milk protein allergenicity (Nowak-Wegrzyn & Fiocchi, 2009). The assessment of milk allergy resolution differs between IgE-mediated-CMA and non-IgE-mediated CMA. In IgE-mediated CMA, the reduction of the SPT wheal size or specific IgE levels may indicate tolerance to baked or "raw" milk (Kido et al., 2016). Therefore, monitoring for the potential development of tolerance through evaluation of milk specific-IgE levels and wheal size of SPT may provide useful information regarding the most appropriate time at which to conduct a milk challenge. This review aimed to identify and present studies that evaluated the association between immune markers and milk containing food challenge outcomes.

Immune markers studies

This review identified few studies that have evaluated the predictive value of immune markers in helping to determine the development of milk tolerance in children with CMA. Table 2.7 summarises and presents the studies that investigated immune markers as predictors of baked milk challenge outcomes.

Table 2.7: Population characteristics, study design, exposure-outcome measurements, assessments and findings of the reviewed immune marker studies

Study	HM-tolerant-SPT (median)mm	HM-reactive- SPT (median)mm	HM-tolerant-Milk sIgE median (kU_A/L)	HM-reactive-Milk sIgE median (kU_A/L)	HM-tolerant-Casein sIgE median (kU_A/L)	HM-reactive-Casein sIgE median (kU_A/L)	Findings
Nowak-Wegrzyn et al. 2008 N=100 children	77 (77 %)-7 (2.5–19)	23 (23 %)-9.5 (5–24)	77 (77 %)-2.43(0-79)	11.6(0.69-101)	77 (77 %)-1.41(0-101	23 (23 %)-14.15(0.75-101)	HM reactive group had significantly larger SPT wheals and higher milk-specific and casein-IgE levels than other groups
Bartnikas et al.2012 N = 35	29 (83 %)-10 (0–20)	6 (17 %)-15 (7–20)	29 (83 %)-1.93 (<0.35–20.6)-	6 (17 %)-2.39 (<0.35–31.0)	29 (83 %)-1.05 (<0.35–10.3)-	6 (17 %)-1.07 (<0.35–31.5)	The levels of IgE to CM- casein and β-lactoglobulin were significantly higher in HM reactive group compared with HM tolerant group. Casein-specific IgE had the highest positive and negative predictive values compared with specific IgE to CM or b-lactoglobulin, and casein and b-lactoglobulin specific IgE/IgG4 ratios were significantly higher in HM-reactive group with compared with HM -tolerant group Milk protein SPT wheal may be more reliable than sIgE level in predicting outcomes of baked milk challenges.
Faraj et al.,2012 N=58	55(94.8%)	3(5.2%)	N/A	N/A	N/A	N/A	The majority of participants who tolerated the muffin challenge negative SPT (NPV=94.8%)

Caubet et al, 2013 N = 97, N = 128.	29 (83 %)-10 (0–20) 83 (64.8 %)-NA	6 (17 %)-15 (7–20) 38 (29.7 %)-NA	83 (64.8 %)-(0.2–42.3)	38 (29.7 %)-11.9 (0.8–50.5)	83 (64.8 %)-2.3 (0.2–30.5)	38 (29.7 %)-12.2 (0.5–67.0)	The levels of IgE to CM- casein and β-lactoglobulin were significantly higher in HM reactive group compared with HM tolerant group. Casein-specific IgE had the highest positive and negative predictive values compared with specific IgE to CM or b-lactoglobulin, and casein and b-lactoglobulin specific IgE/IgG4 ratios were significantly higher in HM-reactive group with compared with HM -tolerant group
Ford et al,2013 N = 132,	95 (72 %)-NA	37 (28 %)-NA	95 (72 %)-NA	37 (28 %)-2.4 (0.6–43.6)	95 (72 %)-NA	37 (28 %)-13.75 (0.36–49.9)	Casein- and milk-specific IgE level, milk-specific basophil reactivity, and milk SPT wheal diameter are all significantly greater among patients with milk allergy who react to HM than among those who tolerate it
Kwan et al 2016 N=30 median age: 7.5 (2-16)	18(60%) 3.08 (0.0–13.8)	12(40%) 6.33 (3.83–8.33)	18(60%) 6.91 (0.99–>100)	12(40%) 25.5 (1.82–>100)	18(60%) 4.5 (0.35–>100)	12(40%) 19.7 (1.08–>100)	All participants with negative SPT (>3mm) to baked milk tolerated muffin challenges

In a prospective study, **Nowak-Wegrzyn et al (2008)** reported that children who tolerated baked milk products and included these foods into their diet on a daily basis, had significantly smaller milk-SPT mean wheal diameters, and lower milk and casein-sIgE immunoglobulins compared with their baseline measurements after three months of baked milk products consumption.

In a prospective follow up study, **Kim et al (2011)** reported that children who tolerated baked milk foods had significantly ($p<.001$ and $p = .02$, respectively) reduced casein and β-lactoglobulin sIgE levels compared with baked milk-reactive children over time. However, no differences were observed in milk–sIgE levels between baked milk–tolerant children and baked milk-reactive children over time from their baseline characteristics to final follow up ($p = 0.07$). This study indicates that high levels of casein and β-lactoglobulin may predict reactivity to baked milk or unheated milk in CMA children while milk sIgE levels appear to be a poor predictive tool. However further investigation is required to confirm these results.

In a retrospective study, **Faraj et al (2012)** collected and analysed data from the records of an allergy clinic and evaluated if a negative SPT is a predictive value of an oral food challenge outcome. They found that the majority of CMA patients with a negative SPT to extensively-heated milk were able to tolerate a baked milk food (muffin) and concluded that baked milk-SPT may be a reliable predictor tool for a baked milk challenge outcome.

In a retrospective study, **Bartnikas et al (2012)** collected and analyse data from the records of an allergy research centre and evaluated if the immune markers, milk and casein -SPT, milk- sIgE are reliable predictive values and can identify children who are able to pass a baked milk challenge. They found that α-lactoalbumin, β-lactoglobulin sIgE measurements were poor predictors of baked milk challenges and milk-SPT wheal size was a better predictive value compared with casein-SPT wheal size and milk-sIgE measurements.

Cubet et al, (2013) combined the results of immune markers from both cohort studies and found that casein-sIgE measurement had a significantly greater accuracy for predicting baked milk reactivity in children compared with measurements of milk and β-lactoglobulin sIgE.

In a prospective study, **(Ford et al, 2013)** attempted to identify immune markers that could help to predict patients who may be able to tolerate baked milk containing foods by exploring

differences in the levels of these between children who tolerated or reacted to baked milk. These authors reported that milk and casein-sIgE, milk-SPT mean wheal size and milk-specific basophil reactivity were significantly ($p<.001$, $p<.005$, $p<.001$ respectively) higher in the baked milk – reactive children.

In a prospective study, **Kwan et al (2016)** conducted a prospective-case control study and challenged 30 CMA children (median age 7.3) to evaluate if a muffin slurry-SPT and immune markers such as milk and casein-sIgE measurements can predict a baked milk challenge outcome. They found that all CMA children [18(60%)] who had negative (wheal size<3mm) muffin-SPT successfully passed a baked milk food challenge to muffin and concluded that a slurry muffin-SPT is a reliable predictive value in helping to identify milk allergic children that are able to tolerate a baked milk food. Authors also suggested that casein-sIgE measurement had a significantly greater accuracy for predicting a baked milk challenge outcome compared with the measurement of milk-sIgE.

The majority of the studies above had a prospective design and concluded that SPT, milk and casein-sIgE measurements appear to predict milk containing-food challenge outcomes, whilst a-lactalbumin-sIgE and b-lactoglobulin-sIgE seem to be poor predictor markers in determining the development of baked or milk tolerance. However, there are differences between the results of the studies above and it is difficult to compare their findings due to variation of the study design, inclusion criteria, population, and methods regarding the preparation of foods for challenges or SPT.

Bartinkas et al (2012) and **Faraj et al (2012**) studies have a retrospective design and the data were not collected for the purpose of their research. In both studies, CMA diagnosis was not confirmed by milk challenges and they included participants with relatively small measurements of SPT (wheal size <5mm). The above parameters may confound the findings of the studies because it is possible some participants were not truly allergic to cow's milk. Faraj et al did not challenge milk allergic patients who had positive muffin-SPT and therefore the specificity of the muffin-SPT was not well established. In addition, the sample size was not homogenous because Faraj et al included a larger cohort of egg allergic patients as well. In both studies, the challenge foods were prepared by caregivers and therefore there was no control to ensure that there was equal amounts of milk proteins and temperature of baking of the challenge food. The reliability of the SPT and sIgE measurements is also

questionable because they were not obtained close to the time of the performance of food challenges.

All studies collected data only by one centre and this reduces the generalizability of their findings. A multi-centre trial is required to include a larger number of participants that come from different geographic locations with a wider range of population groups and compare the findings among the centres. No clear diagnostic criteria were defined in the majority of studies and most participants were identified as milk allergic based on history and laboratory tests and their diagnosis was not confirmed by failed unheated milk challenges. Thus, some participants who passed the baked milk challenges may be tolerant to unheated milk as well. There was a lack of blinding of any party.

Except from both cohorts that were conducted by Cubet et al (2013), all other studies evaluated a relatively small sample size for each group of participants and that possibly did not ensure sufficient power to allow validated calculations of positive predictive values, sensitivity and specificity of immune markers. Another consideration is related with the similar age of participants in the studies. The majority of studies provided data for a population with median age from 7 to 9 years. This data is not representative for younger children with CMA and this should be considered when generalising these results in community practice.

According to the studies above, reliable predictors of a successful baked milk challenge are still not well established and oral food challenges should not therefore be replaced by these allergy tests. Immune markers such as SPT and casein-sIgE values may be able to predict baked milk tolerance or reactivity, although these findings have not been validated by large-scale clinical trials examining a greater range of group ages and across different medical centres.

Inconsistencies in baked milk introduction

The review of baked milk challenge studies demonstrates that there is a lack of consensus in the use of food protocols during baked milk challenges in research setting (Kwan et al., 2016; Nowak-Węgrzyn, 2016). In clinical routine hospitals and specialist allergy healthcare professionals follow their own baked milk challenge protocols based on different methods and guidelines due to the lack of standardisation in the practice, process and dose regimes.

These different approaches regarding the type of foods or quantities, frequency of consumption and the variety of protocols may have different immunological effects on the rate of allergy resolution and uncertain impact on reaction during an exposure to baked milk food (Upton & Nowak-Wegrzyn, 2018). The review of immune markers studies in the previous section showed that SPT and milk sIgE measurements can help stratify risk of allergic reactions to a milk challenge, although there is limited data available to their predictive value regarding baked milk challenge outcome (Bartnikas, Sheehan, Schneider, & Phipatanakul, 2012; Ford et al., 2013). Food challenges still remain the only allergy test to evaluate the tolerance to baked milk but they carry the risk of inducing life-threatening anaphylaxis in children with IgE-mediated CMA. Therefore, according to the findings of the review of baked milk studies discussed in Section 2.4.2 , due to the current lack of reliable predictive indicators that could identify optimal candidates for baked milk challenges, baked milk challenge should be untertaken in a clinical setting where any allergic reactions could be managed in patients with IgE mediated CMA.

However, there is still a debate regarding the place (hospital or home) of baked milk introduction in IgE-mediated CMA (Leonard, Caubet, Kim, Groetch, & Nowak-Węgrzyn, 2015). As mentioned in Section 2.4.2 above (table 2.5), baked milk introduction is associated with unpredictable allergic reactions that include skin, gastrointestinal, respiratory, and cardiovascular symptoms that in some cases may be life-threatening for milk allergic patients (Bartnikas, Sheehan, Schneider, et al., 2012; Mehr et al., 2014; Nowak-Wegrzyn et al., 2008). For this reason, many researchers suggest medical supervision for the initial baked milk introduction in children with IgE mediated CMA (Bartnikas, Sheehan, Hoffman, et al., 2012; Mehr et al., 2014; Nowak-Wegrzyn et al., 2008). An update of nutritional guidelines in the management of CMA reports that a baked milk challenge should be performed in a clinic and supervised by a medical staff (Dupont et al., 2018). In addition, the MAP guidelines (2013) and iMAP guidelines (2017) suggest that no child with IgE-mediated food allergy should have a challenge in primary care or community settings (Venter et al., 2013)(Venter, Mazzocchi, Maslin, & Agostoni, 2017). European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines (2012) recommend to not prolong unnecessary dietary restrictions and suggest supervised milk challenges (Koletzko et al., 2012). The World Allergy Organization (WAO), the Adverse Reactions to Food Committee of the American Academy of Allergy, Asthma and Immunology (AAAAI) and European Academy of Allergy and Clinical Immunology (EAACI) have published guidelines regarding the unheated milk re-introduction

in IgE-mediated CMA. They recommend that milk challenges should occur under medical supervision in an environment where severe reactions can be appropriately managed, but the guidelines are not yet focussed on the introduction of baked milk.

In contrast to the guidelines and recommendations mentioned above, the BSACI milk allergy guidelines suggest home introduction of baked milk using a “milk ladder” in IgE-mediated CMA patients who had only cutaneous symptoms on a mouthful milk allergen exposure, reducing milk sIgE and SPT, and no reaction to milk in the past 6 months (table 2.6,). However, Kawn et al reported that asthma and previous anaphylaxis did not predict milk challenge outcomes. Mehr et al found that a child with a previous anaphylaxis passed a baked milk challenge. Bartnikas et al and Mehr et al also found that children who passed a baked milk challenge in hospital reacted at home when they continued to consume the same doses of baked milk food that was tolerated in hospital. In addition, baked milk challenge studies have shown that a minority of children with reducing IgE and SPT measurements reacted during a baked milk challenge (Kwan et al., 2016). Therefore, allergic reactions to baked milk appear to be unpredictable and even though current research indicates that a small proportion of children may react, health care professionals and parents need to act with caution during baked milk introduction because in IgE mediated CMA there is a risk of severe reactions and anaphylaxis.

Hence, the lack of agreement regarding the appropriate place (hospital or home) of baked milk containing food introduction and the use of a standardised gradual milk introduction protocol could lead to different advice that could confuse not only healthcare professionals but also patients/caregivers and expose IgE-mediated cow’s milk allergic children to the risk of an accidental reaction. More robust evidence is required to update guidelines regarding the appropriate place of baked milk introduction, an optimal standardised and validated gradual milk introduction protocol and the optimal age of CMA children for this approach.

According to this literature review there is robust evidence that the majority of CMA children can tolerate baked milk during a baked milk challenge. However a number of questions have been raised regarding the use of baked milk products in the management of CMA: What guidelines finally inform healthcare professionals decision to introduce gradually baked milk products into the diet of their CMA patients? “Where” (hospital/home), “when” (appropriate time of baked milk introduction) and “how” (baked milk challenge or milk ladder) healthcare

professionals introduce these foods? Is baked milk introduction a safe process in clinical practice? Is a graded milk introduction protocol such as a milk ladder appropriate, acceptable, practicable, and safe? Are SPT and milk sIgE measurements valuable predictor tools to help healthcare professionals to identify the appropriate time of baked milk introduction and replace a baked milk challenge? This PhD research has attempted to provide answers to the important questions above.

2.4.2. Safety of the baked milk containing foods

While it seems that staged introduction of baked milk may be successful in the development of milk tolerance, it is also important to consider the short and long-term safety of this treatment. In mild to moderate non-IgE-mediated CMA, there is a lack of studies that have evaluated the impact of the introduction of baked milk food into the children's diet in terms of appropriateness, acceptability, suitability and safety. In IgE-mediated CMA, only one study has considered the long-term safety of this treatment (at 12-month follow-up), and found that the incorporation of baked milk products into children's diets doesn't appear to cause any changes in underlying allergic diseases (no increase in the severity of chronic asthma, atopic dermatitis, or allergic rhinitis), intestinal permeability, or in the growth of patients (Kim et al, 2011). However, in a cohort study, adverse reactions during the introduction of baked milk have been reported. Clinical symptoms that have been reported in baked milk challenge studies are summarized in Table 2.8.

In one study, a child developed oral pruritus to homemade bread and waffle, and two other participants developed mild oral symptoms to homemade waffle and pizza during home reintroduction (Nowak-Wegrzyn et al., 2008). In another study, three children developed symptoms at home a week later, after eating a muffin that was the same as they had been exposed to in a hospital-baked milk challenge in terms of milk protein and temperature of baking (Mehr et al., 2014). In addition, several studies have reported severe reactions and treatment with epinephrine during baked milk challenge. Anaphylaxis has been reported in both groups; baked milk tolerant [3.2% (5 of 65 children)] and baked milk reactive children ranged from [17% (3 of 23 children)] to [35%(8 of 23 children)] during baked milk challenges in hospital (Nowak-Wegrzyn et al., 2008). However, baked milk-reactive children experienced more severe reactions compared with baked milk-tolerant children during their challenge to fresh milk and received epinephrine (Nowak-Wegrzyn et al., 2008). According

to these findings the researchers of the cohort above suggested that baked milk reactivity could be a predictor of a more severe and persistent CMA phenotype. In addition, they reported that disorders such as asthma or multiple food allergy could be also a predictor of severe and persistent CMA because they found an association between reactions to baked milk challenges and asthma or multiple food allergy (Nowak-Wegrzyn et al., 2008). These are certainly plausible conclusions given the study findings. Hence, it seems that adverse reactions are relatively common during the introduction of milk, particularly in those who have a history of reacting to baked milk prior to treatment and/or who have asthma or multiple food allergies. Thus, this is a procedure that requires medical supervision and should only be conducted in an environment where any severe reactions could be managed.

Table 2.8: Clinical symptoms during baked milk challenges

References	Symptoms	Baked milk challenge
Nowak-Wegrzyn et al. 2008	Oral pruritus, Atopic dermatitis flare, rash, hives or angioedema, sneezing, rhinoconjunctivitis, throat symptoms or cough, wheezing, shortness of breath or respiratory distress (gasping, cyanosis, decreased oxygen saturation), abdominal pain, nausea, vomiting or diarrhoea, dizziness, loss of consciousness or hypotension, anaphylactic shock	Baked milk, muffin, homemade breads &waffles
Kim et al.2011	Anaphylactic shock, oral symptoms, wheeze, cough	Cheese omellete waffle, pizza, muffin
Bartnikas et al. 2012	Rhinorrhea, hives, tongue itching, oral pruritus, 1 patient developed anaphylaxis at home with hives, lip swelling & vomiting & treated with epinephrine A late reaction to ongoing baked milk exposure at home occur	Muffin, cupcake
Mehr et al.2014	Anaphylactic shock Urticaria & angioedema (47%) Itchy mouth or tight throat (53%) Abdominal pain (20%) Vomiting and/or acute-onset diarrhoea (13%) 3 children developed symptoms at homme with ongoing exposure 1 week later (itch, abdominal pain, and flaring of eczema)	Muffin

2.6 Summary

Cow's milk allergy is the most prevalent type of food allergy in children, with an estimated prevalence of 0.2-4.9% in the worldwide paediatric population. Allergic reactions may present with cutaneous, respiratory and gastrointestinal symptoms that occur immediately or after many hours/days. Cow's milk allergy is classified severe or mild to moderate according to clinical expressions of IgE and non-IgE-mediated reactions. This research has focussed on IgE and mild to moderate non-mediated IgE CMA. The diagnosis of IgE-mediated CMA is based on a combination of patient's history and confirmation of allergy tests like SPT, serum milk specific - IgE values and oral milk challenge. However, the gold standard for CMA diagnosis is the oral milk challenge that should be conducted under medical supervision in an environment with available resuscitation facilities, due to the high risk of severe reactions/ anaphylaxis. For mild to moderate non-IgE-mediated CMA diagnosis there are no validated tests and diagnosis is based on a good clinical history of symptoms and avoidance followed by reintroduction of milk containing foods to determine whether the symptoms improved on avoidance. Early and reliable diagnosis of CMA is very important to initiate appropriate dietary treatment, avoiding unnecessary diet restriction and alleviating symptoms.

The mainstay of CMA management is the elimination of cow's milk and milk containing foods. However, there is evidence that many children who react to fresh milk, cheese and yoghurt may tolerate baked milk containing foods such as cakes, biscuits, muffins, waffles, pizza. Baked milk, especially when it is mixed with flour and fat makes the milk less likely to cause allergic reactions. Processing of milk proteins such as through baking may reduce their allergenicity and enhance tolerance to baked milk. Several studies have shown that the majority of CM allergic children may tolerate milk in baked forms before they become tolerant to fresh milk and milk products. The incorporation of baked milk products into children's diets appears therefore to accelerate the development of milk tolerance, which may also improve children's nutritional status and quality of life.

The oral milk challenge remains the best method to determine development of tolerance to milk. In IgE-mediated-CMA a hospital based baked or unheated milk/milk containing food challenge is usually offered and in mild to moderate non-IgE-mediated-CMA, tolerance is usually assessed by a gradual reintroduction plan (milk ladder) at home. However, there is

limited data regarding the appropriate time for baked milk reintroduction in IgE and mild to moderate non-IgE mediated CMA. Additionally, there is a lack of evidence by which to determine best practice in home baked milk introduction in IgE mediated CMA.

Hence, in current clinical practice, the decision regarding baked milk reintroduction is based on an individual clinical assessment and depends on the type of CMA. Current data also indicates that reliable predictors such as SPT or milk sIgE of a successful baked milk challenge are still not well established. Some studies have reported anaphylaxis and treatment with epinephrine during baked milk challenge in IgE-mediated CMA; it is not therefore a risk-free procedure. Hence, at present, the decision regarding baked milk reintroduction is based on an individual clinical assessment and depends on the type of CMA. Hence, there is a gap in the literature that needs to be addressed regarding the guidelines that are followed before baked milk containing foods are introduced into the diet of milk allergic children. What protocols are used during baked milk reintroduction and where a baked milk challenge or milk ladder is conducted.

In brief, the findings of this literature review indicate that there is a paucity of evidence regarding the practice and impact of BMC and milk ladders from either a health care professional or parent perspective. Additionally, further investigation is required to assess if immune markers such as Skin Prick Test and milk sIgE can provide some useful prognostic information for the appropriate timing for introduction of BM-containing food in IgE-mediated CMA.

2.7 Aim & Objectives

The overall aim of this PhD is therefore to investigate three key aspects of this important, and currently under-researched, area of paediatric food allergy. Firstly, to explore current clinical practices of HCPs regarding the use of BMCs and gradual re-introduction of BM-containing foods (milk ladders) and what guidelines they use before they decide upon a baked milk re-introduction. Secondly, to explore parents' perspectives regarding the use of baked milk containing foods into their child's diet. Finally, to evaluate if immune markers such as SPT and milk sIgE can predict milk challenge outcomes in children with IgE mediated CMA and help HCPs to identify those who might benefit from baked milk re-introduction.

Objectives

1. To evaluate the attitudes and practices of HCPs on the conduct of BMCs and graded re-introduction of BM/milk ladder in IgE and mild to moderate-non-IgE-mediated CMA.
2. To explore parents' experience of the re-introduction of BM-milk containing foods into the diet of their child who has been diagnosed with IgE and non-IgE-mediated CMA.
3. To assess immune markers (SPT, milk sIgE) prior to baked or unheated milk challenge and evaluate if there is an association between these immune markers and milk challenge outcomes in children with IgE-mediated CMA.

These were addressed as follows:

1. A multi-national survey explored the current clinical practice of healthcare professionals using baked milk challenges and the milk ladder
2. A qualitative study using semi-structured interviews were conducted in mothers with children diagnosed with IgE or non-IgE mediated CMA
3. As a part of a larger quantitative study, data was extracted and analysed regarding the immune markers and milk challenge outcomes of children diagnosed with IgE-mediated-
4. CMA

2.8 Possible implications of the findings of this research

The findings of this research could potentially have clinical implications regarding the need to standardise and validate BMC and milk ladder protocols in IgE and non-IgE-mediated CMA and -provide clear guidance and information regarding the most appropriate place (home or hospital) to conduct baked milk challenges or milk ladders for IgE-mediated CMA. Moreover, the findings could also help to determine *when* is the appropriate time to recommend BMC and gradual BM-re-introduction based on immune markers such as SPT versus milk sIgE, in IgE-mediated CMA. "Standardised" and "validated" prognostic indicators such as BMC and immune markers can be very useful in clinical practice: improving allergy services, providing high quality personalised and specialised care in children, and avoiding unnecessary

restriction of milk containing foods. Additionally, understanding parents' needs during the re-introduction process of BM-containing foods can improve the communication between parents and HCPs, facilitating this approach and improving the process of BMC and gradual re-introduction of milk (milk ladder) in terms of its appropriateness, acceptability, practicability and safety.

Chapter 3: Methodology

3.1 Overview

The overall aim of this chapter is to outline the methodological approach taken to guide the collection and analysis of data for the purpose of the PhD research. The epistemological beliefs that guide and justify the methodological approach of the research are presented. The quantitative and qualitative methods that were used to design and plan the studies of this research are also discussed. The individual design and analytical techniques of participants' recruitment, data collection and analysis are presented in each relevant chapter.

3.2 Epistemological position of the research

Understanding the relationship between the epistemology and the methodology and methods used for this research was fundamental to ensure a coherent rationale underpinning the design of the studies conducted for the purpose of this project. Epistemology refers to the assumptions that are made related to how knowledge is viewed, how we can communicate knowledge to others, and how the researcher's epistemological approach can influence the research outcome (Burrell & Morgan,1979). The epistemological position of the researcher guides and influences the methodological approach of the research in terms of the choice of tools/techniques and protocols that are used to design and frame the research (King & Horrocks, 2010). Many authors suggest that researchers should reveal the paradigms that guide their thinking and planning process and their perceptions of what is real and what can be known{Kuper, 2008, An introduction to reading and appraising qualitative research} (Kuper, Reeves & Levinson, 2008; Tavallaei & Abu Talib, 2010).

Positivist and interpretivist paradigms guide this research and they have been used complementarily to address the research questions using a multi-method approach. The ontological position of positivist epistemology is that we can discover knowledge related to an objective reality and can attempt to identify causes that influence the outcomes. The

scientific method is in line with a positivist standpoint, and as such, seeks to test predictions and make generalizations (Neuman, 2011). In this paradigm, the assumption is that, since there is one objective reality, research results can be replicated from different researchers (if they collect, analyse and record the same data in the same way) and can be generalised to other populations (Scotland, 2012). The positivist paradigm typically uses quantitative methods and aims to gather objective and precise data.

The ontological position of interpretive epistemology is relativism i.e. that reality is individually constructed and differs from person to person. The interpretive epistemological standpoint is that, in contrast to positivism, reality is subjective and therefore different people may construct meaning and interpret the same phenomena in different ways (Black, 2006). Interpretivism is an approach that uses qualitative methods to explore and understand the reality of individuals or groups or cultures through their experiences and perceptions (Creswell, 2009). Qualitative research attempts to understand and explain actions from participants' perspectives and can utilise a number of methods of data collection such as survey, observational studies, case study with data collection tools such as focus groups, interviews, and open-ended questionnaires (Nind &Todd, 2011). According to the interpretivist paradigm, researchers assert their beliefs when they select what and how to research and how to interpret their data (Kalos, 2010). They study the social reality from the perspective of the participants and prefer to work with qualitative data which provides rich descriptions of social constructs and use a narrative form of analysis to describe with details the social reality (Neuman 2011). The interpretivist paradigm allows the researcher to seek answers, construct and interpret his/her understanding from the gathering data and explore the world by interpreting the understanding of individuals. Thus, research conducted within an interpretivist paradigm produces highly contextualized data, and interpretations of this data involve subjective individual constructions (Scotland, 2012).

This research presents a multi-method approach, deriving from the different epistemological perspectives outlined above (positivist paradigm and interpretive paradigm) and methodological approaches that complement one another: quantitative data analysis regarding the feasibility and safety of baked milk challenges and milk ladders from healthcare professionals perspectives'; qualitative data regarding the practicability, acceptability, suitability and safety of milk ladders from parents' perspectives; and a quantitative study

attempting to inform the appropriate time of baked milk introduction by identifying valuable tools that could provide useful information about the milk tolerant status of milk allergic children.

Four dominant paradigms have been associated with multi- and mixed-methods research, namely positivism/post-positivism, constructivism/interpretivism, transformative, and pragmatic . While mixed methods research can prove challenging from a paradigmatic standpoint (because the aim is to integrate the findings of qualitative and quantitative studies) it has been argued that this is not as difficult in multi-methods research because it can utilise the appropriate paradigm in line with the single type of data being collected (Morse,2003). Multi-methods can therefore involve a different combination of methodological and philosophical components:

- Single method and single paradigm
- Multiple methods within a single paradigm
- Multiple methods within multiple paradigms

This multi-method research programme accommodates three single studies and each single study uses a single method within a single paradigm. The qualitative study used semi-structured interviews analysed using thematic analysis within the interpretivist paradigm. The survey involved a quantitative questionnaire and inferential statistical analysis within positivist paradigm to collect and analyse data; and in the third study quantitative secondary data was analysed using inferential statistics again within the positivist paradigm. Further details regarding the multimethod approach of this research are referred in the next sections 3.3 and 3.4.

This research approach provides a holistic view regarding the usefulness and safety of baked milk introduction in clinical and home settings. As a practicing dietitian in food allergy I am familiar with the use of baked milk products in routine clinical practice, although there is limited knowledge based on evidence regarding the safety of this process in terms of the appropriate time and place (hospital/home) of the baked milk introduction and its feasibility in terms of acceptability, practicability, and safety at home. Furthermore, there is a lack of specific recommendations, based on evidence, that guide the baked milk introduction process. Therefore, exploring other healthcare professionals' perspectives could provide

information in a better understanding of baked milk challenge and milk ladder approach. In addition, due to the complexity of this process, parents' experiences and perspectives were explored to offer a deeper knowledge on the usefulness and impact of baked milk products into milk allergic children's diets at home.

3.3 Using a multi-method design for research

Using more than one method of data collection and analysis is well-established in social science research (Alexander et al, 2008). Several authors became interested in the use of multi-method or mixed-method research in healthcare services because this approach offers a deeper understanding and a more holistic view examining different aspects of the same question or problem (Creswell and Plano Clark, 2011). In mixed-methods research both qualitative and quantitative research strategies are applied to the same research question(s). Investigators collect and analyse the data and integrate their findings using both qualitative and quantitative methods in a single study or a programme of research (Tashakkori and Creswell, 2007). In a mixed-method approach, different research designs can lead to different understandings of the problem and provide alternative findings and explanations of the research question(s) (Blackman and Benson, 2004).

Multi-method research design, known also as multiple method design, involves combining any different methods and collects data from several resources. For instance, it can include two or more exclusively qualitative approaches, two or more quantitative approaches, or a combination of qualitative and quantitative approaches, hence multi-methods research. It has therefore been argued that mixed method research is in fact one category of multi-method research (Creswell & Plano Clark, 2007; Dunning, 2016). In multi-method studies multiple types of qualitative or quantitative data are collected while in mixed method studies both qualitative and quantitative data are incorporated (Creswell & Plano Clark, 2007). However, the main difference between mixed-method and multi-method approaches is that in mixed-methods the findings of quantitative and qualitative research should be integrated and evident throughout the presentation and interpretation of the results while there is no analytic integration of the findings of qualitative and quantitative in multi-methods studies (Creswell & Plano Clark, 2007).

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Proponents of a multi-method approach have argued that using more than one method to address the research question(s) produces results that are more robust and compelling than single method studies, and enhance the validity of the research (Jamieson, 2011). The usefulness of multi-method research has also been recognised as a way to capture the complexity or different aspects of a phenomenon (Mason, 2006). The strength of a multi-method research design is that it offers a way of making the research more meaningful, complete and purposeful than is the case when using either a singular qualitative or quantitative approach. In addition, weaknesses in one method can be counter-balanced by strengths in another (Boyer, 2008).

3.4. Utilising a multi-method design for this PhD research project

As outlined in Section 3.3, a multi-method design was chosen for this PhD research project because, while baked milk introduction is widely practiced with milk allergic children, the topic has been the subject of surprisingly little research. There are some important questions about its practice which have not yet been explored by research, and these must be considered as a matter of urgency. Hence, a multi-methods approach will help us to develop a more comprehensive understanding of a range of research questions related to the introduction of baked milk in terms of its usefulness, safety, appropriateness, compliance, acceptability, and practicability from healthcare professionals' and parents' perception and indications of immune markers and food challenge outcomes:

- What are healthcare professional's perceptions about the usefulness of a baked milk challenge and a milk ladder as a dietary management of children with CMA?
- Where (hospital/home setting) and how to introduce a baked milk containing food into the diet of children with CMA?
- What are parents' perceptions about the usefulness of a milk ladder plan as a dietary management of their children's CMA?
- When is the appropriate time of baked milk introduction? Are there reliable tools to identify optimal candidates for baked milk introduction?

In line with a multi-method design, three different methods were employed to answering three objectives independently in terms of data collection, analysis and interpretation of this research:

1. A survey of healthcare professionals utilising an online questionnaire to collect quantitative data regarding the healthcare professionals' attitudes and views in the use of baked milk introduction to understand "where" (hospital/home) and "how" the baked milk introduction (in the form of food challenges or a milk ladder approach) is conducted and also "what" guidelines are followed before healthcare professionals decide to introduce baked milk containing foods.
2. A qualitative study utilising semi-structured telephone interviews to understand parents' experiences in introducing baked milk containing foods into the diet of their children who are still allergic to cow's milk.
3. A retrospective study utilising secondary quantitative data collected from electronic patient records to evaluate validated tools that could be able to predict the outcome of the baked milk introduction and hence, they identify potential candidates for baked milk challenges or milk ladders.

These three complementary studies utilising a multi-method approach were designed to provide a holistic approach to gaining an understanding of the introduction of baked milk products into the diet of children with CMA.

Healthcare professionals involved in the management and treatment of CMA and adult mothers of children with CMA were recruited to participate in this research. Ethical considerations informed the planning of the

methods for this research. All participants were provided with information about the purpose of the study and their involvement in the research and their right to withdraw from the study was clearly explained by the participant information sheet. All participants were encouraged to ask questions about the study and were given time to consider their participation. Participants' details were kept confidential and that any names collected (e.g. via informed consent forms for the qualitative study) were kept separately from the data. Further details regarding the ethics have been provided in the chapter of each study.

3.5 Introduction to data collection and data generation methods for this PhD research project

3.5.1 Quantitative study: Survey

Quantitative research generally aims to gather information from a relatively large number of participants and focuses on generating numerical data. In the social sciences, quantitative methods such as surveys are used to quantify attitudes, opinions, behaviours, and other defined variables in a given aspect. Numerical data can be collected through questionnaires, and surveys, or by manipulating pre-existing statistical data using computational techniques (Schmied {Schmied, 2012, Effect of Heat-Killed Escherichia coli`, Lipopolysaccharide`, and Muramyl Dipeptide Treatments on the Immune Response Phenotype and Allergy in Neonatal Pigs Sensitized to the Egg White Protein Ovomucoid}, 1993).

This quantitative study used a survey to gather information from a pre-defined group of respondents that were familiar with the content of questions and able to provide answers. In surveys, questionnaires are the most widely used data collection method (Ponto,2015). In this study data was collected by using an online questionnaire. A self-completed questionnaire was constructed by the researcher and all respondents were asked the same set of questions. Respondents accessed the questionnaire through their web browser by using a hyperlink. The questionnaire was designed to address the objectives of the study (Chapter 4) The questionnaire was constructed with the help of healthcare professionals (dietitians, paediatricians, allergists/immunologists and nurses) who were involved in the management of CMA in primary/secondary/tertiary care. The design and content of the

questionnaire was based on a careful review of the literature discussion with healthcare professionals working in this field. The majority of questions were closed questions for ease of completion and thus to facilitate a higher response rate. The questionnaire could be completed within 20 minutes. A broad range of data were collected regarding the opinions, attitudes and beliefs of healthcare professionals about the re-introduction of baked milk products in the management of IgE and non-IgE CMA. Numerous questions were asked about the baked milk challenges and milk ladder. The content validity of questions was assessed by healthcare professionals located in different regions of the world who assessed whether each question was essential, necessary, and useful.

In the first page of the online questionnaire was a welcome section which explained to respondents the purpose of the research, their voluntary and anonymised participation, and guidance for questionnaire completion. The questions of the survey were classified in five sections. The first section collected information about the background and characteristics of healthcare professionals such as speciality, country and place of their practice. The second and third sections collected data related to the use of baked milk challenges and milk ladders (such as guidelines considered before the baked milk reintroduction), and place (clinic/home) of baked milk introduction. The fourth section collected data regarding any potential allergic reactions during a baked milk challenge or milk ladder such as what type of symptoms were observed and how frequent they were. The last section collected data regarding the safety of baked milk challenges and the milk ladder process at home, and whether, according to the perspective of the healthcare professionals, parents were anxious during the reintroduction of baked milk products. Finally, respondents were thanked for completing the questionnaire.

Strengths and limitations

Through the survey, a broad range of data were collected regarding the opinions, attitudes and beliefs of healthcare professionals about the use of baked milk products in the management of IgE and non-IgE CMA. Bias derived from the researcher's subjectivity was eliminated because all participants were provided with the same standardised questionnaire. Written definitions were provided with any questions that required further explanation to ensure that participants had a consistent understanding of these questions

and to thereby enhance the rigour of the data collected. The respondents had the chance to use a text answer if they wanted to provide further information or clarification.

Before distributing the questionnaire, its content validity was ensured by submitting the questions to careful review (evaluating whether each question was essential, necessary and useful) by healthcare professionals located in different regions of the world. Their feedback was used to revise the questions where necessary. The questionnaire was pilot tested to ensure that the respondents could answer the questions without any problems and data could be effectively reported. The questionnaire was tested in terms of the time taken for completion, clarity of instructions, questions and layout, and omissions of respondents' answers.

In general, a major limitation of surveys is that the number of respondents who choose to respond may be different from those who chose not to respond and data bias may be derived from non-responses of the questionnaire. Even though the questions of the questionnaire are very well-formulated, there is still a chance that some respondents may interpret differently or incorrectly some questions and provide unclear data biasing the results. Another limitation is that respondents may not be fully aware of their reasons for all their answers due to the lack of expertise or memory on a subject, and data errors can thus occur. Another weakness of a survey is that it is inflexible compared to interview questions because it is not possible to follow up further on individual respondent's answers.

3.5.2 Qualitative study: Semi – structured interviews & thematic analysis

In the last few decades qualitative methods have gained an important place in the health sciences. As discussed in Section 3.2, the main difference between quantitative and qualitative studies is that quantitative research collects numerical data and generates statistics while the qualitative methods collect qualitative data related, for example, to experiences or feelings, and analyse these at a conceptual level typically with a focus on understanding the richness of the data (Rhodes, 2014). In addition, qualitative research is more flexible compared to quantitative research because it allows greater adaptation of the interaction between the researcher and participants, and participants are free to express themselves in their own words and provide responses in greater detail. Qualitative research

involves an examination of variables or phenomenon with a naturalistic and interpretative approach that seeks to provide an in-depth and complex understanding of how people see and interpret their social world (Snape & Spencer ,2003). Qualitative research can be conducted in various ways (methods) that have been categorised into five groups: ethnography, narrative, phenomenological, grounded theory, and case study (Tong, Sainsbury, & Craig, 2007). The most common qualitative data collection tools are:

- Participant observation that is appropriate for collecting data on naturally occurring behaviours of participants
- In-depth interviews that are appropriate for collecting data on individuals' personal histories, perspectives, and experiences, particularly when sensitive topics are being explored.
- Focus groups that are effective in eliciting data on the cultural norms of a group and in generating broad overviews of issues of concern to the cultural groups or subgroups represented.

Qualitative research typically generates data such as field notes, audio or video recordings and associated transcripts. Exploratory, qualitative, semi-structured in-depth interviews were used in this study to explore parents' experiences and perspectives regarding the introduction of baked milk products into the diet of their children with IgE and non-IgE mediated CMA. These interviews provided the opportunity to understand parents' attitudes, opinions and experiences related to the use of baked milk products at home. The interview schedule included a list of topics and some key questions to give a rough guide to follow in the interview; however, their exact use varied from interview to interview. In addition, the order of questions depended on the flow of conversation and the exact questions varied from person to person (Rubin 1995). The interviews were conducted on a one to one basis by telephone or a call via the Internet between the researcher and a single participant. According to ethical considerations, participants were informed about the length of time needed for the interview and had understood the content and purpose of the study before they consent to be interviewed. Interviews were arranged at a time that mothers were under least pressure. Mothers had the right to decline to answer any questions.

Data Analysis: Thematic Analysis

There are different methods of data analysis in qualitative research such as Grounded Theory (GT), Thematic Analysis (TA), and Interpretative Phenomenological Analysis (IPA). This

research used thematic analysis to analyse the qualitative data because there is no need of detailed theoretical and technical knowledge and this form of analysis is suitable for new researcher in a qualitative research career. Primarily, however, thematic analysis was chosen as it is a method that offers an accessible, useful and theoretically flexible approach to identify patterns or themes and analyse qualitative data without being bound to a particular epistemological or theoretical perspective (Braun & Clarke 2006). It has the advantage of providing a usable and clear framework on how to analyse qualitative data. The goal of thematic analysis is to identify important and interesting patterns (themes) in the data and use these themes to address the research question of the study. Braun & Clarke (2006) provide a six-phase guide which is a very useful framework for conducting thematic analysis. Further details for each step is provided in Chapter 5.

Strengths and limitations

A key strength of qualitative research is that it provides rich data and an in-depth understanding of an experience. A semi structured interview method can provide complex textual descriptions of how people experience a given research issue and help the researcher to interpret and understand the complex reality of a given situation. For instance, researchers can gain information related to contradictory behaviours, beliefs, opinions, emotions, and relationships of participants. Interviewees have the opportunity to ask if some questions are not very clear and discuss their experiences without needing to write anything down and they also receive feedback and personal assurance on how the researcher will use the information received.

In the manner in which they react and ask questions the interviewer may have an impact on the data collection. Bias may be derived from the interviewer's comments, tone or non-verbal behaviour and the way that participants' responses were interpreted by the researcher. However, these issues were overcome in this research due to the fact that the researcher was an allergy dietitian with interview skills and therefore able to manage the interaction between interviewer and interviewee. It is also important to note that, in this study, participants' willingness to participate in an interview may have been reduced due to their high family and work commitments, and this may cause data biases because the researcher might be more likely to gather the perspectives of mothers who have fewer commitments. It was a fact that the majority of mothers had limited available time for the interview due to their work and family commitments, although this issue was overcome by

arranging a suitable time according to mothers' availability. Sometimes it was required to interrupt the interview and complete it at a later time. The interview did not include questions related to sensitive information. To increase the validity of the semi-structured interviews, the clarity of the topics and questions had been reviewed by colleagues and parents before conducting the interviews. In addition, data entry was performed by the PhD student who is an experienced dietitian and reviewed from colleagues to prevent any bias at the coding stage.

3.5.3. Quantitative study: Secondary data analysis

According to the National Institute of Health (NIH) in the United States, "'primary data analysis is limited to the analysis of data by members of the research team that collected the data, which are conducted to answer the original hypotheses proposed in the study. All other analyses of data collected for specific research studies or analyses of data collected for other purposes are considered as secondary data, whether or not the persons conducting the analyses participated in the collection of the data" (Cheng HG, 2014). Thus, secondary data analysis is a research method that analyses an existing dataset that was collected initially for some other purpose. Further analysis of existing data may provide additional knowledge, interpretation or conclusions (Bulmer et al 2009).

To meet the third objective of this research (Chapter 2, section 2. 7), a secondary analysis of an existing dataset that was obtained for a larger research project was carried out. The data was collected by the allergy team of the Children's Hospital Medical Centre in Cincinnati for a larger high-quality research project conducted in the USA. This data included electronic patients' records related to measurements of immune markers' in milk allergic children and their food challenge outcomes. The data that was sent to the University of Portsmouth was coded and anonymous and it was provided in an encrypted flowsheet database email. Frequency tables and cross-tabulation were run for all variables that were included in the main analysis of data to identify potential missing values of each variable in the dataset. Further analysis of this data could provide additional knowledge to healthcare professionals and parents about the milk tolerant status of milk allergic children and help them to decide when is the appropriate time and what form of milk containing food to re-introduce into the diet of children with CMA.

Strengths and limitations

The major advantages of a secondary analysis of existing data is that it can be developed in less time with a relatively low cost compared with other methods of data collection. It is also a sensible and ethical use of research data, as similar datasets are not duplicated, and thus additional time and resources not invested in collecting data that already exists. It was a great opportunity for this PhD research to obtain access to such dataset from a large-scale population research in the USA.

However, one limitation of the secondary analysis of existing data is that the researcher who is analysing the data has not been involved in the data collection and therefore cannot control or influence the data collection, and specific information regarding the data collection may not have been reported. In addition, the data has not typically been collected to address the research question of the particular study and some important variables may not be available for the analysis. Furthermore, some specific variables such as age, race or ethnicity of participants may not be available due to confidentiality.

3.6 Summary

A multi-method design was used to achieve the overall aim of this research and thereby provide a holistic view on the use of milk-containing foods from healthcare professionals', mothers' and milk allergy tools' perspective. Two quantitative studies and a qualitative study were separately used to explore current practice according to healthcare professionals' opinions, the impact of baked milk introduction at home according to mothers' experience, and if immune markers such as SPT and milk sIgE can predict the milk tolerant status of children with CMA and help HPC to identify the appropriate time of baked milk containing food introduction. A survey with a mix of closed-ended and semi-closed questions was used to explore healthcare professionals' attitudes and experience. Semi-structured individualised phone interviews were used to explore mothers' experiences and opinions. A secondary analysis of existing data that was collected from a larger research carried out to determine if immune markers such as SPT and milk sIgE can identify children who will be optimal cow's milk allergic patients for milk containing food

reintroduction. The key strength of this research is that it considers important questions about the guidance and practice of baked milk introduction from clinical and parental point of view; a common area of practice in the management of cow's milk allergy for which there has been little previous research.

Chapter 4: Use of baked milk challenges and milk ladders in clinical practice: a multinational survey of healthcare professionals

4.1 Overview

According to the systematic review in chapter 2, there is a need to understand how HCPs use a BMC or ML in clinical practice, when and where these challenges are performed and what guidelines are followed by HCPs before they decide to recommend the baked milk introduction in the management of IgE and mild to moderate non-IgE CMA.

The overall aim of this chapter is to provide information regarding the baked milk challenge and gradual re-introduction of milk (or milk ladder) from healthcare professionals' perspectives. According to the findings of the literature review in the previous chapter, current research suggests that graded introduction of cow's milk, starting with baked milk-containing foods such as biscuits/cookies, cakes and waffles, may be used as a prognostic indicator for outgrowing cow's milk allergy. This chapter examines the experiences and opinions of HCPs and what guidelines they follow before deciding to proceed to a BMC and/or milk ladder. This was achieved by administering an electronic questionnaire to HCPs across the world. Health organisations and associations such as the British Society for Allergy and Clinical Immunology and the World Allergy Organisation identified HCPs involved in CMA management and distributed the survey questionnaire via emails. The main questions related to: participants' characteristics; where the BMC and/or ML were conducted; symptoms observed during challenges; guidelines followed by HCPs before deciding to proceed to a BMC and/or a milk ladder; and HCPs' perspectives regarding the safety of a BMC/ML and parental feelings during BM re-introduction. The findings are discussed in terms of their contribution to an agreement for universal guidance on the use of BM-containing foods for the management of IgE and non-IgE-mediated CMA and clinical implementation related to the development of safe BM-reintroduction plans. The findings of this study were peer-reviewed and published (appendix 1).

4.2 Background

4.2.1 Rationale for the study

In the past, the cornerstone of CMA treatment was strict elimination of cow's milk and foods containing CM from the patient's diet (Fiocchi et al., 2010). However, the appropriate prevention and management of CMA is still debated. There is a more recent theory that strict avoidance of milk proteins may contribute to the persistence of CMA and the recommendation for strict milk avoidance due to a lack of effective and approved treatment of CMA (Kim & Sicherer, 2010; Vandenplas, 2017). Hence, some in paediatric allergy research and clinical practice argue for a modification of the existing milk-restricted diet in favour of an individualised approached based on the tolerance to milk in the forms of baked milk products (Kim et al., 2011; Sampson et al., 2013). Indeed, the importance of BM-reintroduction into the diet of children with CMA has become well-recognized as a part of CMA management (Nowak-Węgrzyn, 2016). Current research regarding the use of BM-containing foods has been reviewed and reported in Chapter 2, Section 2.2.1**.** Interestingly, it has been demonstrated that the majority (75%) of children who participated in baked milk studies tolerated the BM- containing foods, and, of particular note, were 28 times more likely to become tolerant to "raw" cow's milk compared to those children who were not able to tolerate these baked milk products (Kim et al., 2011; Nowak-Wegrzyn et al., 2008). Furthermore, the ingestion and incorporation of BM-containing foods into the children's diet seemed to accelerate the resolution of CMA without any adverse effects on children's growth, intestinal permeability, or the severity of coexisting diseases such as asthma, atopic dermatitis and allergic rhinitis (Bartnikas et al., 2012). The use of BMC and ML may therefore help to avoid an unnecessary restriction of BM-containing foods or to prevent a severe reaction that could be provoked with uncooked milk; children reactive to BM appear to be at higher risk of systemic reaction than those children that tolerate BM but still remain allergic to uncooked milk (Nowak-Wegrzynet et al., 2008) and perhaps help to induce tolerance.

In the United Kingdom, CM is one of the most common foods responsible for fatal anaphylactic reactions in children less than 16 years of age, and food allergy is the main cause of a fatal anaphylactic reaction outside the hospital setting (Turner et al., 2015; Wang & Sampson, 2007). It is difficult to estimate how many people die each year from food

anaphylaxis and to confirm the trigger that caused these tragedies. In the past two years, three children (two boys, who were 3 and 13 years old, and a 9-year-old girl) died due to a fatal anaphylactic reaction following a bite of a milk-containing food outside of healthcare settings in the United Kingdom (UK). (Fleicher, 2017; O'Çarroll 2017; Robinson, 2017). In the USA, a 3-year-old boy has tragically died following a severe anaphylactic reaction during a routine baked milk challenge (Smith,2017). These tragic events emphasize that the safest procedure should be in place during food challenge in terms of set-up, staffing, supervision, and protocols. Importantly, the decision to challenge at home should not be taken lightly as there is a risk of severe reactions, even anaphylaxis, in IgE-mediated CMA.

At the time of completion of this survey, few guidelines were available on BM-reintroduction. In the UK, the MAP Milk Allergy guidelines for mild to moderate non-IgE-mediated CMA and the BSACI guidelines for home introduction of BM-containing foods in IgE-mediated CMA were first published at the end of the survey period (details on the descriptions of these guidelines are referred to Chapter 2, Section 2.2.3) (Luyt et al., 2014; Venter et al., 2017; Venter et al., 2013).

However, there are no studies indicating which patients are optimal candidates for home introduction of BM. Additionally, there is no universal agreement for the criteria used to classify the severity of allergy symptoms as mild, moderate, or severe and no reliable biomarkers have been determined which could be used to indicate the safety of home introduction of milk-containing foods. In the absence of universally agreed guidelines, it was not clear "how", "when" and "where" BM-reintroduction was being conducted in practice. This is a novel study which explored what guidelines and approaches are currently being used by HCPs across the world and what their experiences have been in introducing a full portion of a BM product as a challenge (BMC) over 1 day or as a more gradual introduction over a number of days/weeks before moving on to other baked milk foods, as per a ML approach.

4.2.2 Aims & Objectives

The aim and objective of this study were as follow:

Aim: To evaluate the attitudes and practices of HCPs on the conduct of BMCs and graded re-introduction of BM/milk ladder in IgE and mild to moderate-non-IgE mediated CMA.

Objective: A multinational survey explored the current clinical practice of healthcare professionals using baked milk challenges and the milk ladder

4.3 Methods

4.3.1 Study design

A web-based global survey was conducted to explore and capture the current clinical practice and views of healthcare professionals, using a baked milk challenge and/or a milk ladder in the management of IgE and non-IgE-mediated CMA. An online questionnaire (appendix 7) was developed consisting of 23 short questions which could be completed within approximately 15 minutes.

The main sections of the questionnaire were:

- Characteristics of HCPs including: professional background and level of allergy training, practice setting (private/hospital-primary/secondary/tertiary care), proportion of HCPs working time devoted to seeing patients with food allergy amount of time spent consulting patients with food allergies, country of residence, guidelines that HCPs considered before they made the decision about the setting of BMC/ML.
- Were these challenges used and where were these challenges performed?
- What was the HCPs' opinion on the safety of home-BMC and ML?
- What was the HCPs' opinion on parental anxiety in BMC/ML process?
- What symptoms were observed?

The literature was reviewed carefully prior to designing the questionnaire and the questions were discussed with healthcare professionals such as allergists, dietitians, and allergy nurses who shared their experience and queries on baked milk introduction. The questions were designed to collect data that answer the research question and address the objectives of this research. It was designed to collect demographic variables that included data related to characteristics of respondents such as education, occupation, place of work and workload that can be used to check if the data collected is representative for the general population. It was also designed to collect attitudes and

opinions variables related to the clinical practice of healthcare professionals. The validity and reliability of the questionnaire were assessed by healthcare professionals and colleagues. CMA experts reviewed the questionnaire and provided feedback on the representativeness and suitability of the questions.

The questionnaire included a combination of open and closed questions. Closed questions are usually quicker and easier to both answer and analyse. Open questions gave respondents the opportunity to provide answers in their own way and give context to their answers. In terms of closed questions, the questionnaire included: a list of items and respondents could select any of these items; a category of responses where the respondent could select only one response; and a matrix, where responses to two or more questions could be reported using the same grid. Each person was asked the same set of questions in a predetermined order.

To increase the validity, reliability and response rate of the questionnaire (i) a clear explanation about the purpose of questionnaire was provided (ii) the questions were carefully designed and presented (iii) the delivery and return of the completed questionnaire was carefully planned (iv) a covering email that summarised the research was sent by the Health organisations and associations to healthcare professionals to ensure a high level of responses and (v) a pilot test was conducted before the questionnaire was distributed to participants. The questionnaire was self-completed by the respondents in their own time and they accessed it through their web browser using a hyperlink sent by email. The first part of the questionnaire had a welcome screen and explained the purpose of the survey and why it is important the respondent to complete the questionnaire. At the end of the questionnaire, respondents were thanked for completing the questionnaire.

An initial pilot testing of the survey was carried out on a group of HCPs practising in different parts of the world to ensure the clarity of questions. Although related to healthcare, this study did not recruit NHS patients and did not therefore require review by an NHS research ethics committee. Advice received at the start of this research study indicated that no ethical review was required by the University of Portsmouth Science Faculty Ethics Committee,

however, it has since become apparent that such review should have been sought. This has been discussed with the current Science Faculty Ethics Committee Chair and Members, who agreed that this was a genuine oversight/misunderstanding, and issued the letter attached in appendix 8.

4.3.2 Justification of the online questionnaire

Electronically administered questionnaires are an inexpensive way to collect data for research purposes (Kelley, Clark, Brown, & Sitzia, 2003). However, the main benefit of using online questionnaires is that this data collection method is less time consuming compared to a paper questionnaire or telephone interview approach. Participants' responses are processed automatically, and the results are accessible at any time. It is also particularly suited to collecting data from across the world, as posting questionnaires worldwide would be very expensive and coordinating a series of telephone interviews across time zones while also taking account of participants' other commitments could be logistically problematic. The Bristol Online Survey software was a convenient tool with which to develop the questionnaire and the participant had the choice to skip optional questions that were not suitable for them and provide answers to mandatory questions. Data was automatically analysed using descriptive statistics and it was instantly available to be transferred into the SPSS statistical software or spreadsheets for further statistical analysis. The margin of error was reduced with the online survey because HCPs could enter their responses directly into the system. Participants were able to complete the online survey at a time convenient to them. This survey was designed to be sent to HCPs whose current practice involves the management of CM patients. To summarise, the survey approach by means of online questionnaire was chosen for this study primarily because it was the most practical approach for a worldwide survey, but also because the results could be produced quickly while minimising the risk of human error; the data could be transferred and analysed in various applications to appropriately answer the research question; it could be achieved with low budget; and, finally, it required less time compared with other quantitative methods.

4.3.3 Recruitment of Participants

HCPs involved in the diagnosis and management of IgE and non-IgE-mediated CMA were invited to complete the online questionnaire. The participants were identified through the following international professional organisations:

- Food Allergy and Intolerance Specialist Group of British Dietetic Association (FAISG)
- British Society for Allergy and Clinical Immunology (BSACI)
- American Academy of Asthma Allergy and Immunology (AAAAI)
- American Dietetic Association (ADA)
- International Network for Diet and Nutrition in Allergy (INDANA)
- Allergy Society of South Africa (ALLSA)
- Dietitians Association of Australia (DAA)
- Australasian Society of Clinical Immunology and Allergy (ASCIA)
- World Allergy Organisation (WAO)

The above-mentioned associations and organisations were conducted and asked to distribute to their members who involved in the management of CMA an email with the questionnaire link. A reminder email was sent 4 weeks later. An example of a contact invitation email is attached in appendix 9.

Inclusion Criteria

All healthcare professionals that were involved in the diagnosis and management of IgE and non-IgE-mediated Cow's Milk Allergy, using baked milk challenges in clinical practice and/or gradual re-introduction of milk (milk ladder) were eligible to complete the online questionnaire.

4.3.4 Data safety and monitoring

At the beginning of the online questionnaire, there was a section that informed respondents about the purpose of this survey and provided directions for the completion of the questionnaire. HCPs were aware that the data will be anonymous and confidential and stored according to Data Protection Act 1998. Respondents could save their responses and

complete the questionnaire at a later date. Each respondent was allocated a study number for data analysis and dissemination of outcomes. All computer files/electronic records were password protected on a secure drive. Only the researcher and academic supervisors had access to the encryption key. No sensitive data or patients' data were collected by this study.

4.3.5 Data Analysis

The Bristol Online Survey software was used to analyse and describe the results. Descriptive statistics were used to summarise data using a combination of tabulation and graphical description. For further statistical analysis data were entered and analysed using IBM SPSS Statistics for Windows version 22.0. Pearson's chi-square test was used: a) to determine whether or not there was a statistically significant association between the use of BMC and ML; b) to test whether or not a statistically significant association exists between the settings (clinical/home) in which BMC/ML were performed and the types of CMA (IgE and non-IgE-mediated CMA) being evaluated. A p-value less than 0.05 was considered statistically significant.

4.4 Results

4.4.1 Characteristics of participating HCPs

A total of 114 HCPs completed the questionnaire and provided data on their clinical practice regarding using either a BMC and/or a ML in both IgE- and non-IgE-mediated CMA. The largest groups of respondents were dietitians with an interest in allergy (52 (46%)) followed by paediatric allergists/immunologists (46 (40%)). The majority of participants (106 (93%)) indicated that they were involved in the management of IgE- and non-IgE mediated CMA in infancy and childhood. Most of the participants were based in the United Kingdom (56 (49%)), followed by the United States (20 (18%)), and were practicing in secondary care/hospital (52 (39%)) followed by tertiary care/specialist centres (42 (37%)). The majority of HCPs reported that they based their decision regarding BM introduction on an individualized clinical assessment (history of symptoms, SPTs, laboratory tests) and a smaller number of HCPs reported that this decision was based on national/regional/international guidelines. Demographic features of all respondents are shown in Table 4.1.

Table 4.1: Characteristics of participating HCPs

Characteristics	Options	Respondents (n=114) (%)
Professional background	Dietitian Paediatric Allergist/Allergist/Immunologist Paediatrician with Allergy interest Other*	52(46) 32(28) 14(12) 16(14)
Practice Settings	Secondary Care/Hospital Tertiary Care/Specialist Centre Private Practice Primary Care/Community Other**	52(39) 42(37) 19(14) 16(12) 4(3)
Allergy training	Work-based experiential learning Speciality in Allergology/Immunology Postgraduate Diploma in Allergy MSc in Allergy PhD in Allergy Postgraduate Certificate in Allergy Other***	50(38) 36(27) 10(8) 8(6) 8(6) 4(3) 16(12)
Food allergy patients as proportion of weekly workload	>50% ≤50%	62 (54) 52 (46)
CMA patients seen by HCPs	Infants/children Adults	106(93) 37 (32)
Country	United Kingdom North & South America Oceania, Africa, Asia Europe	56(49) 24 (21) 20(18) 14(12)
Guidelines for hospital -BMC	Medical history/SPT/sIgE Regional/National International Hospital policy	40(35) 24(21) 12(11) 9(8)
Guidelines for hospital -BMC	Medical history/SPT/sIgE Regional/National International Hospital policy	40(35) 24(21) 12(11) 9(8)
Guidelines used to direct home-BMC	Medical history/SPT/sIgE Regional/National International Hospital policy	39(34) 26(23) 6(5) 5(4)
Guidelines used to direct hospital-ML	Medical history/SPT/sIgE Regional/National International Hospital policy	24(21) 26(23) 4(4) 3(3)
Guidelines used to direct home-ML	Medical history/SPT/sIgE Regional/National International Hospital policy	22(19) 30(26) 4(3) 4(3)

**Other: Pharmacists, Nutritionists, Allergy Paediatric Nurses, Physicians, General Practitioners*
***Other: Ministry of Health & Welfare, Research*
****Other: Research, Continued Professional Development (CPD) & Continuing Medical Education (CME) resources, allergy training, completed allergy modules*

4.4.2 Settings (hospital/home) of BMC and ML in children with CMA based on HCPs reports

Descriptive statistics was used to describe what type of milk challenges (BMC/ML) used healthcare professionals in IgE and non-IgE CMA and where these challenges were performed (hospital or home)/.

IgE mediated CMA

The number of healthcare professionals' responses regarding the use of BMC and ML in hospital or home in IgE mediated CMA was graphically presented in Figure 1.

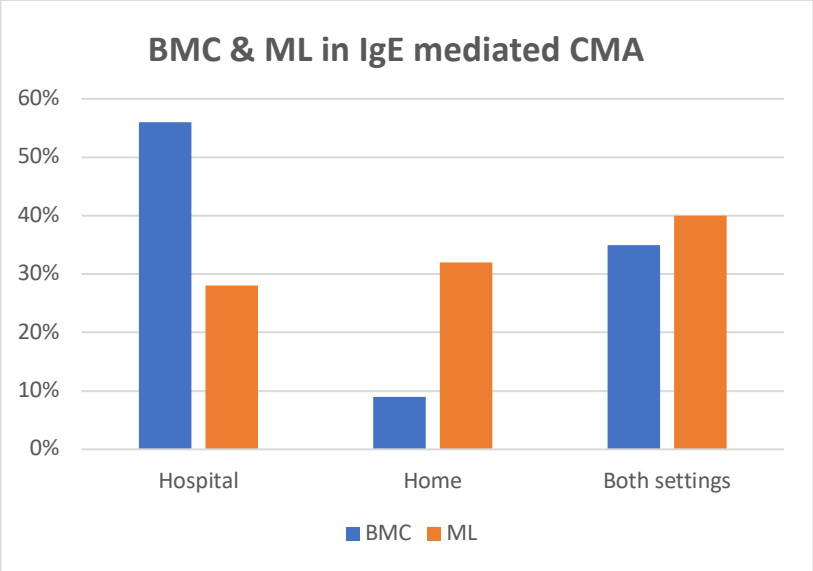


Figure 4.1: Settings (hospital/home) of BMC and ML in children with IgE-mediated CMA

Ninety-three (82%) HCPs indicated that they used BMC to identify patients with IgE-mediated CMA able to tolerate BM products before tolerating uncooked milk. Fifty-two (56%) respondents stated that they conducted these challenges in a clinical setting, 8 (9%) in a home-based setting, and 33 (35%) reported using both settings (Figure 4.1). For ML, 68 (60%) HCPs stated that they used this approach to determine the development of tolerance to BM in different forms. Nineteen (28%) respondents reported that they used the ML approach in a clinical setting, 22 (32%) in a home setting, and 27 (40%) in both settings (Figure 4.1).

Non-IgE-mediated CMA

The number of healthcare professionals' responses regarding the use of BMC and ML in hospital or home in non-IgE mediated CMA was graphically presented in Figure 4.2.

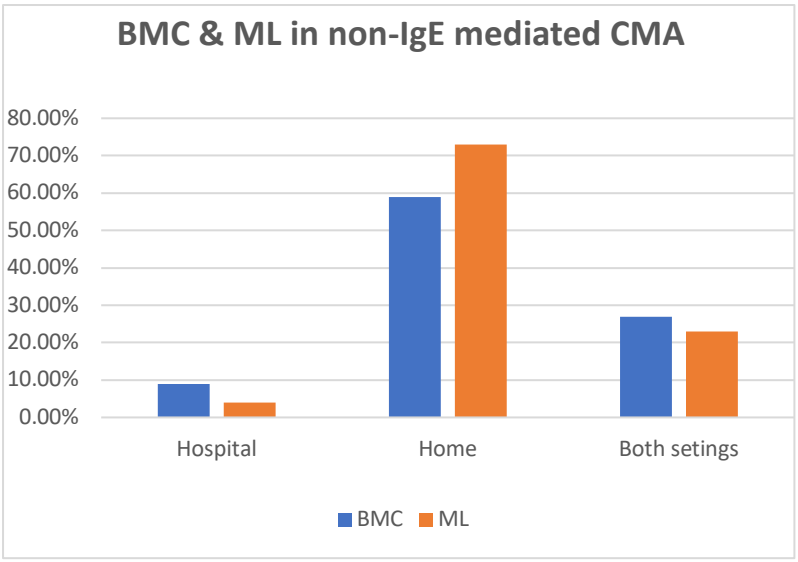


Figure 4.2: Setting (hospital/home) of BMC and ML in children with non-IgE-mediated CMA

Eighty-six (75%) of the respondents stated that they used BMC to determine the development of tolerance to BM in patients with non-IgE-mediated CMA. Eight (9%) HCPs reported that they challenged their patients in a clinical setting, 51 (59%) used home-based challenges, and 27 (31%) reported using both settings (Figure 4.2). In terms of using the ladder approach (ML), 77 (68%) HCPs reported that they used the ML to identify children able to tolerate a range of BM-containing foods. Three (4%) HCPs reported that they used ML in a clinical setting, 56 (73%) at home, and 18 (23%) reported using both settings (Figure 4.2).

Statistical analysis was performed using Pearson's Chi Square test to indicate whether there was an association among the type of CMA (IgE or non-IgE mediated CMA) and the choice of setting where a BMC or ML was performed by HCPs (Table 4.2). Statistical tests and Chi-Square values are referred to the appendix 10.

Table 4.2: Percentages of HCPs responses regarding the choice of BMC setting in IgE and non-IgE CMA

BMC settings in IgE and non IgE-CMA reported by HCPs			
BMC Setting Total Responses (N=179)	**IgE mediated CMA**	**Non-IgE mediated CMA**	**p value (Pearson chi-square test)**
			<0.001
Hospital	52 (56%)	8 (9%)	
Home	8 (9%)	51 (59%)	
Both	33 (35%)	27 (31%)	

A greater number (52 (56%)) of hospital-based BMC responses were indicated in IgE-mediated CMA, with a larger number (51 (59%)) of home-based BMC being used in non-IgE-mediated CMA (Table 4.2). Pearson's Chi -Square test (appendix 10) indicated that the choice of BMC setting (clinic/home) was statistically significantly ($p < 0.001$) associated with the type of CMA (IgE-/non-IgE-mediated).

Table 4.3:Percentage of HCPs responses regarding the choice of ML setting in IgE and non-IgECMA

ML settings in IgE and non-IgE mediated CMA reported by HCPs			
ML Setting Total Responses (N=145)	**IgE mediated CMA**	**Non-IgE mediated CMA**	**p value (Pearson chi-square test)**
			<0.001
Hospital	19 (28%)	3 (4%)	
Home	22 (32%)	56 (73%)	
Both	27 (40%)	18 (23%)	

A considerable number of respondents used ML challenges/introductions at home in both IgE- {22 (32%)} and non-IgE-mediated CMA {56 (73%)}. In IgE-mediated CMA a reduced number {19 (28%)} of healthcare professionals suggested a ML challenge/introduction in hospital, while in non-IgE mediated CMA a very small number {3(4%) of healthcare

professionals suggested a ML challenge or introduction in hospital. Pearson's Chi -Square test (appendix 10) indicated that the decision about where to perform a ML challenge/introduction (hospital/home) was also statistically significantly ($p < 0.001$) associated with the types of CMA (IgE/non-IgE-mediated).

4.4.3 Safety of BMC and ML at home/outside the medical settings in IgE and non-IgE mediated CMA

Descriptive statistics was performed to describe graphically which place (hospital or home) was safe for a BMC or ML introduction according to healthcare professionals' opinions (Figure 4.3).

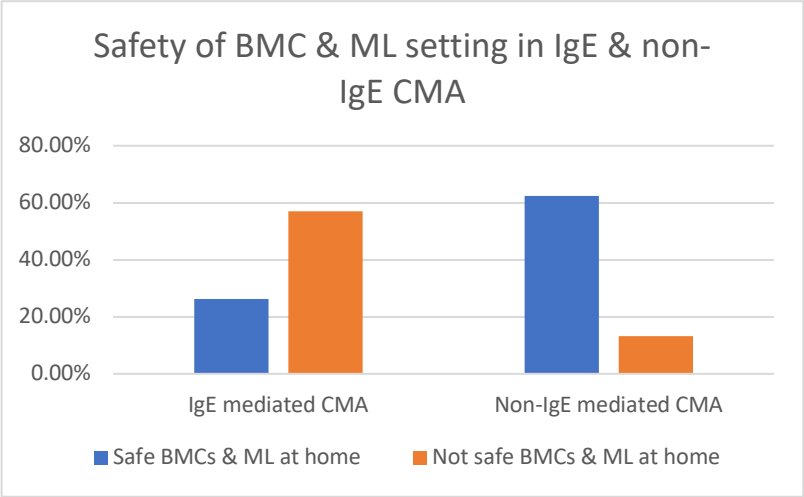


Figure 4.3: Participants' views regarding the setting where BMC & ML are conducted in patients with IgE and non-IgE mediated CMA

In terms of IgE-mediated CMA, 30 (26%) respondents stated that the home was a safe place to conduct BMC or ML, whereas 65 (57%) HCPs (Figure 4.3) considered the home/outside the clinical setting as a non-safe place to conduct both BMC and ML, due to the potential for severe symptoms (Table 4.5).

In n terms of non-IgE-mediated CMA the majority of HCPs (71 (62%)) considered the home/outside the clinical setting as a safe place to conduct both BMC and ML. A small

proportion of respondents {15(13.2%)} reported that the home was not a safe place for either BMC or MLs (Figure 4.3).

Statistical analysis was performed to identify whether the type of CMA (IgE or Non IgE-mediated CMA) has influenced HCPs' recommendation regarding the appropriate setting for the performance of BMC or ML. Pearson's Chi Square test (appendix 10) indicated that HCPs responses regarding the safety of milk challenges was statistically significantly ($p < 0.001$) associated with the types of CMA (IgE/non-IgE-mediated)(Table 4.4).

Table 4.4: Percentage of HCPs responses to home safety of milk challenge in IgE and non-IgE mediated CMA

HCPs responses regarding home safety of milk challenges in IgE and non-IgE mediated CMA			
Milk Challenges (BMC & ML) Safety Total Responses (N=181)	**IgE mediated CMA**	**Non-IgE mediated CMA**	**p value (Pearson chi-square test)**
			<0.001
Safe at home	30 (26%)	71 (62%)	
Non safe at home	65 (57%)	15 (13%)	

Table 4.5: Summary of the most frequently reported symptoms by HCPs for BMC & ML

Clinical Symptoms	IgE mediated CMA				
	Clinical setting *N (%)		Home *N (%)		P value (Pearson chi-Square test)
	BMC	ML	BMC	ML	(p=0.3)
Urticaria	68 (60)	34(30)	32(28)	25(22)	
Vomiting	55 (48)	24(21)	33(29)	22(19)	
Angioedema	49 (43)	23(20)	10(9)	9(8)	
Runny nose & eyes	49 (43)	19(17)	13(9)	12(11)	
Nausea	48 (42)	20(18)	16(14)	22(19)	

Wheezing	39 (34)	15(13)	3(2)	4(3)	
Diarrhoea	34 (30)	17(15)	42(37)	35(31)	
Anaphylaxis	32 (28)	9(8.0)	-	-	
Symptoms	**Non- IgE mediated CMA**				
	Clinical setting *N (%)		**Home *N (%)**		**P value (Pearson square test**
	BMC	**ML**	**BMC**	**ML**	**p=0.8**
Atopic eczema	20(18)	8(7)	43(38)	37(32)	
Abdominal pain	18(16)	4(3)	41(36)	32(28)	
Diarrhoea	15(13)	6(5)	37(32)	35(31)	
Gastro-oesoph.	12(11)	4(3)	32(28)	30(26)	
reflux	7(6)	2(1)	22(19)	17(15)	
Colic	6(5)	4(3)	17(15)	12(10)	
Food aversion	5(4)	4(3)	43(38)	29(25)	
Constipation					

In IgE mediated CMA, the respondents reported that urticaria and vomiting were common symptoms during hospital and home BMC and ML. Angioedema usually occurred in hospital-BMC/ML and diarrhoea at home-BMC/ML. Thirty-two (8%) respondents reported anaphylaxis in hospital-BMC and 9 (5.2%) participants in hospital-ML (Table 4.5).

In non-IgE mediated CMA, the participants stated that symptoms like atopic eczema, abdominal pain and diarrhoea often occurred in hospital-BMC/ML while constipation, atopic eczema, abdominal pain, diarrhoea and gastroesophageal reflux commonly occurred at home-BMC/ML (Table 4.5).

Differences between symptoms and setting of a BMC or ML in IgE and non-IgE CMA

Statistical analysis was performed to evaluate whether there were differences between a BMC or ML setting (hospital and home) and symptoms in IgE mediated CMA. Pearson's Chi Square test (appendix 10) indicated that symptoms in children with IgE mediated CMA were statistically significantly (hospital versus home BMC :$p < 0.001$ and hospital versus home ML) associated with the place that a BMC and ML were conducted (table 4.6). According to HCPs' responses a larger number (table 4.5) of symptoms was observed in

hospital BMC and ML compared to the symptoms at home BMC and ML in IgE-mediated CMA. These findings indicated that the majority of HCPs performed BMC and ML in hospital in children with IgE-mediated CMA due to complicated and serious allergic reactions. However, the results showed that a significant number of symptoms were observed at home BMC and ML in children with IgE -mediated CMA, according to HCPs' reports.

In mild to moderate non-IgE mediated CMA, Pearson's Chi Square test (appendix 10) did not indicate a statistically significant (hospital versus home BMC: P value=0.3 and hospital versus home ML: P value=0.8) association between symptoms and a BMC or ML setting (table 4.6). According to HCPs' responses a larger number (table 4.5) of symptoms was observed at home BMC and ML compared to the symptoms in hospital BMC and ML in non- IgE mediated CMA. These findings indicated that the majority of HCPs indicated BMC and ML at home in children with mild to moderate non- IgE mediated CMA due to less complicated and serious allergic reactions.

Table 4.6: Associations between IgE and non-IgE mediated CMA symptoms and setting (hospital/home) of BMC and ML

Symptoms	Settings: Hospital versus home	P value (Pearson Chi Square test)
IgE-mediated CMA	**Baked Milk Challenges**	**<0.001**
	Milk Ladder	**<0.001**
Non-IgE mediated CMA	**Baked Milk Challenges**	**0.3**
	Milk Ladder	**0.8**

4.4.4 Parental anxiety associated with BMC and ML as reported by HCP

Descriptive statistics was conducted to show graphically parental anxiety according to HCPS' responses during a baked milk challenge or milk ladder introduction. Among the 114 HCPs, 18 (15.8%) HCPs reported that parents were anxious with hospital-BMC; 24 (21.1%) with home-BMC; 46 (40.3%) with BMC either in hospital or at home (both settings); 16 (14.0%) with hospital-ML; 26 (22.8%) with home-ML and 41 (36.0%) with ML in both settings. The majority of HCPs reported that parents are anxious during the procedure of BMC/ML either in hospital or at home (Figure 4.4).

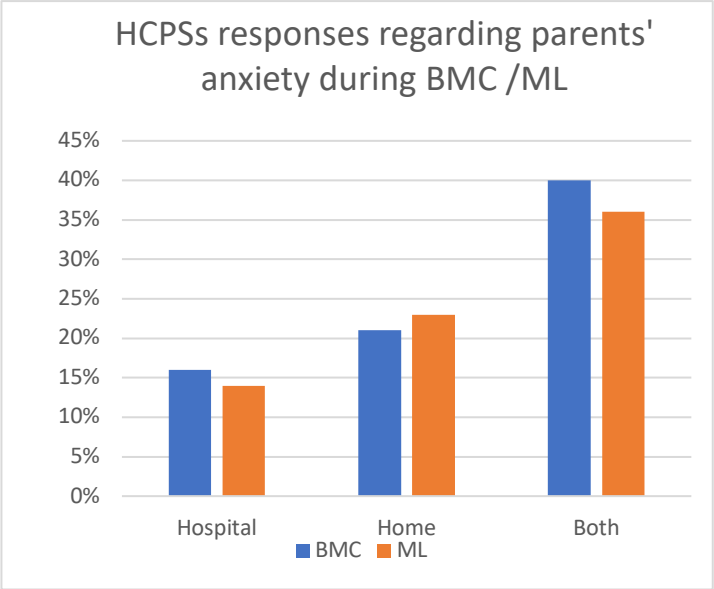


Figure 4.4: HCPs' views regarding parental anxiety and milk challenge setting (home/hospital)

Statistical analysis was conducted to assess whether the type of milk challenge (BMC or ML) has influenced parental anxiety in the different settings (hospital/home). Pearson's Chi Square test (appendix 10) indicated that HCPs responses regarding parental anxiety during a milk challenge was not statistically significantly ($p < 0.86$) associated with the type of milk challenges (BMC/ML) (Table 4.7.)

Table 4.7: Percentages of HCPs responses related to parental anxiety in hospital or home BMC and ML

HCPs responses regarding parental anxiety in hospital and home settings for BMC and ML			
Milk Challenge Setting Parental Anxiety Total Responses (N=171)	**BMC**	**ML**	**P value (Pearson chi-square test)**
			0.863
Hospital	18 (16%)	16 (14%)	
Home	24 (21%)	26 (23%)	
Both	46 (40%)	41 (36%)	

4.4.5 Differences across countries regarding the setting of BMC and ML in IgE and non-IgE mediated CMA

Statistical analysis was conducted to assess if there is an association among milk challenge setting preferences and HCPs' country of residence in IgE and non-IgE mediated CMA. In the UK and USA, a considerable number of HCPs reported that they preferred home BMC in IgE – mediated CMA and non-IgE mediated CMA. However, Pearson's Chi Square test (appendix 10) indicated that there was not a statistically significant (association among different countries and BMC/ML settings in IgE and non-IgE CMA. HCPs responses regarding parental anxiety during a milk challenge was not statistically significantly ($p < 0.86$) associated with the type of milk challenges (BMC/ML) (Table 4.6).

From the rest countries, in IgE mediated CMA the majority of HCPs reported that preferred to perform a BMC in the hospital (table 4.8). However, there was not found any significant statistical association among the countries and setting of preference of BMC and ML performance. Statistics tests are presented in appendix 10.

Table 4.8: Percentages of BMC setting preferences across HCPs' country of residence in IgE and non-IgE mediated CMA

	BMC setting preference					
	IgE –mediated CMA			**Non-IgE-mediated CMA**		
Country	Hospital	Home	Both	Hospital	Home	Both
USA	**19%**	**25%**	**18%**	**13%**	**19%**	**18%**
UK	**33%**	**63%**	**53%**	**25%**	**49%**	**57%**
REST	**48%**	**13%**	**29%**	**63%**	**32%**	**25%**

In the UK and the USA, a considerable number of HCPs reported that they preferred home-BMC in IgE and non-IgE CMA. From the rest countries, the majority of HCPs reported that preferred to conduct a BMC in the hospital (table 4.8)

Table 4.9: Percentages of ML setting preferences across HCPs' country of residence in IgE and non-IgE mediated CMA

	ML setting preference					
	IgE -mediated CMA			Non-IgE mediated CMA		
Country	Hospital	Home	Both	Hospital	Home	Both
USA	**26%**	**32%**	**7%**	**33%**	**19%**	**16%**
UK	**32%**	**32%**	**64%**	**33%**	**43%**	**68%**
REST	**42%**	**36%**	**29%**	**33%**	**68%**	**16%**

Table 4.10: Association among milk challenge setting preferences and HCPs' country of residence in IgE and non-IgE mediated CMA.

Countries (USA,UK,Rest)	Settings (hospital versus home	P value (Pearson chi-square test)
IgE-mediated CMA	**Baked Milk Challenges**	**0.17**
	Milk Ladder	**0.87**
Non-IgE mediated CMA	**Baked Milk Challenges**	**0.38**
	Milk Ladder	**0.32**

4.4.5 Guidelines used in BM introduction

The majority of HCPs reported that a combination of medical history (MH), skin prick test (SPT)/milk specific IgE levels, and severity of symptoms were considered in order to decide whether and where to carry out BMC/ML, due to the inconsistencies in guidelines. The minority of HCPs reported following international guidelines or a hospital policy. The valuable number of participants reported that they follow national/regional guidelines and they referred to the MAP or BSACI guidelines that had been published at the time of the survey (table 4.11) (Luyt et al., 2014; Venter, Brown, Shah, Walsh, & Fox, 2013)

Table 4.11: Indicators and clinical guidelines used in conducting a Baked Milk Challenge and Milk Ladder

Clinical Guidelines	Hospital * (N (%))		Home *(N (%))	
	BMC N=85(97)	ML N=63(72)	BMC N=81(92)	ML N=59(67)
Medical History/SPT/sIgE/ Severity of symptoms	40(47)	23(45)	34(45)	22(37)
National/Regional/Local	23(27)	19(37)	25(33.3)	29(49)
International	12(14)	6(12)	8(11)	4(7)
Hospital policy	9(11)	3(6)	5(7)	4(7)

N=Total number of responses

4.5 Discussion

This is the first study that has investigated how and where HCPs introduce BM-containing foods for the management of IgE and mild to moderate non-IgE mediated CMA. According to the findings of this study the majority of HCPs use BM-containing foods in the form of a BMC/ML not only to determine tolerance to baked milk but also to help children introduce increasing amounts of these foods into their diet to develop milk tolerance and outgrow their milk allergy. However, HCPs reported that some children may experience an immediate or delayed IgE or non-IgE-mediated allergic reaction during the introduction of BM- containing foods.

This study provides a statistically significant ($P<0.001$) data regarding the IgE mediated CMA symptoms and settings of BMC and ML. According to this data, a larger number of symptoms are observed in the hospital compared to symptoms that take place at home during a BMC or ML. This means that the majority of HCPs 'prefer to perform a BMC or ML in hospital in children with IgE-mediated CMA due to complicated and severe allergic reactions. One important result from this study is the finding that in IgE mediated CMA 32 (28%) HCPs reported anaphylaxis in clinic-based BMC and 9 (8%) in clinic-based ML challenges, but there were no reports of anaphylaxis in BMCs/MLs conducted at home. This

finding is consistent with previous studies, which have reported the development of anaphylaxis in some IgE-mediated CM allergic children after ingestion of BM-containing foods in hospital (Kim et al., 2011; Mehr et al., 2014). Mehr et al (2014) identified clinical predictors of reactions to baked cow's milk. These predictors included children with asthma requiring preventer therapy, IgE-mediated clinical reactions to more than three foods, a prior history of anaphylaxis to cow's milk, and highly atopic children. The study involved BM challenges in which increasing amounts of BM were introduced over a number of BM were introduced over a number of hours over the same day; 27% of children did not pass these oral food challenges. This shows that baked milk challenges carry a risk in those with IgE-mediated CMA, and in a number of children with severe forms of non-IgE-mediated CMA. Hence, these findings suggest that, as argued by Mehr et al (2014), it is not safe for children with these clinical indicators to undergo BMC or ML at home; such procedures should always be carried out in a hospital setting where such indicators are present. The findings from this survey highlight that in IgE-mediated CMA, there were no cases of reported anaphylaxis at home during baked milk challenges. This could be due to successful individual risk assessment and HCPs having chosen an appropriate setting accordingly. This is supported by the fact that there were more IgE-mediated reactions associated with baked milk challenges in the clinical setting compared with the home.

Our survey did not identify a significant (P value=0.8) association among milk challenge setting preferences and HCPs' country of residence in IgE and non-IgE mediated CMA. This may be due to the fact that in the USA and UK the use of baked milk is more well-known in the management of CMA compared to the rest countries of the world. However, further investigation is required to explore HCPs' experiences related to the use of BMC and ML in the rest countries of the world. Healthcare systems differ between countries and many European countries may not be able to provide food challenge facilities for all food allergic patients as considerable hospital resources are required (Muraro et al., 2014). Such challenges are time-consuming with long waiting lists, a major problem in many allergy clinics. For practical reasons, allergy services attempt to address this issue by suggesting initial introduction of BM-containing foods at home based on a clinical assessment. The findings from this survey indicate that the decision regarding the location of challenges in the majority of cases is based on an individualized clinical assessment looking for such specific parameters as: sIgE levels, skin prick tests, severity of previous symptoms, severe

forms of non-IgE-mediated CMA such as Food Protein-Induced Enterocolitis Syndrome or mixed IgE- and non-IgE-mediated CMA.

In terms of guidelines that were considered by HCPs for the use of BM products in clinical practice, this research highlights that there is inconsistency of guidelines on the optimal management/treatment of patients with CMA for the introduction of BM-containing foods. The results of this study indicate that the place (hospital/home) chosen by HCPs for the introduction of BM-containing foods depends upon the type of CMA (IgE or non-IgE-mediated) and severity of symptoms (mild/moderate/severe). The majority of respondents reported that home is not a safe place for the introduction of BM-containing foods in IgE-mediated CMA, whereas this procedure could be safely carried out at home in cases of mild to moderate non-IgE-mediated CMA. These findings are consistent with the National Institute for Health and Care Excellence (NICE,2011) UK Food Allergy Guidelines that do not recommend food challenges to be conducted at home in IgE mediated CMA, but that do say that in mild to moderate non- IgE-mediated CMA the challenge food can be safely introduced at home (Mendonça, Cocco, Oselka, Sarni & Solé, 2011). In contrast, the BSACI guidelines recommend that, for selected IgE mediated milk allergic children (those without poorly controlled asthma, multiple/complex allergy, or severe reactions with trace of milk allergen exposure), a BM-containing food introduction protocol can be implemented at home (Luyt et al., 2014).The debate regarding the introduction of BM-containing foods for the management of IgE-mediated CMA will be discussed further in Chapter 7, Section 7.1. Looking beyond the UK guidelines, the findings of this survey have also clearly highlighted the inconsistency of international guidance on food challenge and/or the gradual introduction of baked cow's milk in a matrix or milk ladder. The WAO and EAACI recommend that milk challenges should be conducted in a safe, well-equipped environment that is supervised by a medical team, and have published guidelines for milk oral food challenges. However, these guidelines do not provide any specific guidance on conducting baked milk challenges or implementing milk ladders (Fiocchi et al., 2010; Muraro et al., 2014; Sampson et al., 2012).

Another Important finding of this study is that there is a significant association (P value<0.001) between the type of CMA (IgE and non-IgE) and BMC or ML settings (home/hospital). This means that HCPs' decision about the appropriate and safe place of a BMC or ML performance is influenced by the children's type of CMA (IgE or mon IgE-

mediated CMA). However, choosing the safest challenge setting remains a difficult decision that concerns not only HCPs, but also carers.

Parental anxiety is another factor that was considered by HCPs reports in this study. Our findings indicated that parental anxiety is not statistically significantly (P value=0.8) associated with the setting of a BMC or ML. A considerable number of HCPs reported that the families were anxious when BMC (46 (40%)) or ML (41 (36%)) were conducted, whether at home or in a clinical setting. This data is consistent with the findings of previous studies, which have shown enhanced parental anxiety for the procedure and the risk of reaction on the day of a food challenge (Knibb et al., 2012; Soller, Hourihane, & DunnGalvin, 2014). Parental anxiety is usually related to the severity of symptoms and the possibility of fatal anaphylaxis in IgE-mediated CMA (Lau et al., 2014). Additionally, a study found a positive post challenge effect in patients' and their families' food allergy health-related quality of life, although this effect declined over time in parents/patients thus emphasising the need for regular clinical contact with carers and patients after food challenge (Soller et al., 2014). However, there is no published data on the experience and psychological impact of BMC/ML protocols (standardised process and doses of challenged foods) in parents and CM allergic children. Due to the fact that the BM-containing food introduction can be a long process (some children follow the milk ladder for more than 2 years), a better understanding of parents'/children's perceptions regarding the BM-introduction would be helpful for HCPs to provide optimal care to children and support parents throughout this approach.

4.5.1 Strengths and limitations

This is the first study that has systematically collected data about how BMCs and MLs are conducted in practice and this data provides a baseline which could be used to examine any changes that occur in this practice over time. All data collection, analysis and interpretation were conducted by the same researcher to minimise the effect of researcher bias. Another strength of this survey is that the majority of the responders are specialised/have practical experience in paediatric food allergy and their patients are mainly infants and children with IgE-and non-IgE mediated CMA. Most participants work in secondary and tertiary care and more than 50% of their weekly workload consisted of patients with food allergy (Table 3.1). The sample reflects the range of different clinical roles who may have responsibility for the care of CMA patients.

There are several limitations of this study. Although this is a global study, our sample size is relatively small and respondents were mainly from the UK and USA; there were more limited number of responses from HCPs working in other European countries and in other countries around the world. Thus, the data may be most representative of these populations. Additionally, although the questionnaire included all the appropriate definitions (classification of IgE and non-IgEmediated- CMA, BMC, ML), a limitation may be derived from the different criteria that HCPs used regarding the recognition, diagnosis and management of the complex clinical expression and presentation of CMA across the world. If the survey were repeated, the data from UK respondents may now be different because after the completion of this study, the MAP Milk Allergy Guidance (2017) and the BSACI Guidelines (2014) were published (Luyt et al., 2014; Venter et al., 2017) and may have resulted in changes to practice. Further studies with an enhanced participation of healthcare professionals worldwide could provide internationally comparable data on the use of baked milk challenges and graded reintroduction of foods in a larger population.

4.5.2 Conclusion

Due to the limited information on the reintroduction of BM-containing foods within and outside of the clinical setting, a global survey of HCPs was conducted to provide data regarding their perspectives and clinical practice in this area; the first of its kind. The key finding of this study was that there is the potential for severe/anaphylactic reactions in BMC and ML, and, in recognition of this, HCPs seemed to make decisions about the appropriate venue for a BMC/ML taking this into account. In addition, the results indicate a huge discrepancy in the decision making of HCPs regarding “where” and “when” BMC/ML should be carried out. There is still a debate regarding the appropriate place (home/hospital) in IgE-mediated CMA and clinical trials are required to provide evidence on the safety of this procedure at home.

Furthermore, HCPs reported increased parental anxiety in both approaches, either during BMC or ML. According to these findings clear guidelines are required for “where” (hospital/home) and “when” (appropriate time for BM-reintroduction) to challenge BM-containing foods based on risk factors such as asthma and IgE-testing (SPT, milk sIgE). Moreover, “standardised “and “validated” BMC and ML protocols are needed to guide HCPs and enhance the safety of this approach. The development of safe home-based, baked milk

introduction plans based on individualised risk assessment could help many countries who may not be able to provide food challenge facilities due to limited hospital resources. Further research is required to explore country-specific advice and compare different settings and clinical practices regarding the use of BM-containing foods. A larger sample size with a sampling frame inclusive of more countries and clinicians from tertiary, secondary, and primary care should be conducted. In summary, the findings of this novel study demonstrate that there is a clear need for universal guidance, considering country-specific needs, on the safe introduction of baked milk products.

Chapter 5. Investigating parents' experiences regarding re-introducing baked milk foods in children with Cow's Milk Allergy in the United Kingdom

5.1 Overview

In chapter 4, HCPs study showed that in many cases a baked milk introduction takes place at home in IgE and mild to moderate non-IgE CMA according to HCPs reports. However, there are not studies that have investigated how and when parents or caregivers conduct these challenges at home, what type of support and guidance have been given by HCPs, what are their opinions about a milk ladder plan, and what suggestions they could provide for improving a milk ladder plan.

This chapter describes a qualitative study that explored parents' experiences, understanding, and their level of satisfaction in using milk in baked goods and the impact or outcomes of these products in the management of their children's milk allergy. Semi-structured individualised phone interviews were conducted with mothers in the UK who have children with IgE-and Non-IgE-mediated Cow's Milk Allergy and who were introducing baked milk foods into their child's diet. Participants were recruited via social networking sites and their data has been analysed using thematic analysis.

5.2 Background

5.2.1 Rationale for the study

Two things led me to decide to conduct this study. The first was the HCPs opinions regarding the parental anxiety during a BMC process. The majority of HCPs participated in my survey responded that parents were anxious during a BMC (chapter 3, section 4.4.5). The second thing was a post in the UK website of Mumsnet chat room. A mother with a CM allergic child asked 'Has anyone else heard of the Milk Ladder? My son is two and his paediatrician wants me to start the milk challenge from home. Any ideas, have you heard any research on the use of baked milk foods? I realized that listening to and understanding mothers' perceptions on the impact of BM-reintroduction in their children's diet could provide useful data to

examine the usefulness, effectiveness, appropriateness, acceptability and safety of BMCs and milk ladders.

In the UK, paediatric allergy clinics suggest home reintroduction of baked milk products either with or without a formal oral food challenge in hospital. Different versions of milk ladders, based on clinician preferences, are given to parents of children with CMA to help them to start gradually re-introducing baked milk in the diet of their children at home (Luyt et al., 2014). However, as the survey of HCPs described in Chapter 3, home baked milk re-introduction is not appropriate for all children and for several cases it is still unclear which patients are optimal candidates for undertaking a baked milk challenge in hospital or following a milk ladder process at home. In addition, there is not universal agreement regarding the process, optimal recipes and doses to use for these challenges (Venter et al., 2013).

Several important issues prevent clinicians from ensuring that optimal outcomes could be achieved in the use of baked milk for CMA children. In particular, there are no clinical trials that have evaluated the efficacy and safety of a ML process and "when" and "where" (clinical setting vs home) these challenges should be conducted. The appropriate place (clinical setting versus home) for the introduction of baked milk foods and the association of these foods with acceleration of milk tolerance development are the subject of much controversy (Dang, Peters, & Allen, 2016; Leonard, 2016; Leonard, Caubet, Kim, Groetch, & Nowak-Węgrzyn, 2015). In IgE mediated CMA, several studies have reported anaphylactic reactions and treatment with epinephrine during introduction of baked milk foods (Bartnikas et al., 2012; Mehr et al., 2014; Turner & Boyle, 2014; Turner et al., 2013).

Food anaphylaxis is associated with maternal anxiety and mothers of food-allergic children are more anxious compared with mothers of children with no chronic diseases (Lau et al., 2014; Mandell, Curtis, Gold, & Hardie, 2005). A substantial number of studies have reported that paediatric food allergy has an impact on health related-quality of life, daily family activities, social events and emotional well-being of parents/carers (Allen & Martin, 2010; Knibb, Barnes, & Stalker, 2015; Knibb et al., 2012; Soller et al., 2014; Williams & Hankey, 2015); (Indinnimeo et al., 2013). Uncertainty by experts also contributes to increased worry and concern for parents and lower self-efficacy around CMA management. In particular, although dietitians that are experts in food allergy can provide advice and sufficient

information in the management of CMA, they may not be trained to manage the emotional needs of parents of CMA children. Health care professionals need to undertake a training on emotional support or a psychologist may be required to provide this kind of support in an allergy clinic. (MacKenzie, Grundy, Glasbey, Dean, & Venter, 2015; McHenry & Watson, 2014)." Findings from a recent quantitative survey (based on a postal questionnaire) demonstrated that the majority of parents whose children have been diagnosed with IgE mediated egg and milk allergy reported that having baked milk and egg challenges conducted in tertiary care (rather than at home) alleviated their concerns about the dietary management of their child's allergy (Lee, Mehr, Turner, Joshi, & Campbell, 2015).

In summary, for IgE mediated CMA the introduction of baked milk products in the home versus a medically supervised setting remains a challenging question that concerns not only healthcare professionals but also parents. The range and doses of BM-containing foods given in the ML are not standardised and validated in IgE and mild to moderate non-IgE-mediated CMA. In the UK, different versions of milk ladders, based on hospital's own individual protocols, are given to parents of children with IgE and non-IgE- mediated CMA to help them to start gradual re-introduction of baked milk at home. While there are some quantitative studies on this issue, there are no qualitative studies to enhance our understanding of how parents experience and manage the reintroduction of milk at home.

This study aimed to address this gap utilising a qualitative research approach since this is well-suited to explore parents' perceptions and experiences regarding compliance, acceptability, practicability and safety related to the use of baked milk containing foods at home. Understanding parental perceptions is of utmost importance in terms of assuring the validity of any future guidelines and will contribute to an effective CMA dietary management and to better targeting of dietary advice to parents/carers, whose children will be candidates for a BMC or a ML plan in the future. Additionally, the evidence from this study is expected to help allergy services and healthcare professionals to provide optimal care to children during reintroduction of baked milk foods and contribute to the eventual standardisation of tools used for this purpose.

5.2.2 Aims and objectives

The aims and objectives of this study were as follows:

Aim: To explore parents' perspectives regarding the use of baked milk containing foods into their child's diet.

Objective: To conduct interviews with a selected sample of parents of children diagnosed with IgE and non-IgE-mediated CMA in order to qualitatively explore and analyse parents' experiences, perceptions, and concerns on the use of baked milk containing foods (a milk ladder plan), in the UK.

In order to meet the objective of the study the following questions have been addressed using a qualitative approach:

I. How do parents experience the impact or outcome of the home-reintroduction of baked milk foods (milk ladder) into their children's diet at the time that they are still allergic to "raw" milk?
II. What are parents' perceptions about the usefulness of a milk ladder plan as a dietary management of their children's CMA?
III. What are parents' concerns associated with the home-reintroduction of baked milk products (milk ladder)?

5.3 Methods

5.3.1 Rationale for a qualitative approach

This study has followed a qualitative methodology using a thematic analysis approach to meet the objective above. The focus of this research is to elicit participants' experiences and their perceptions, perspectives and interpretations of a particular situation. Qualitative methods have been used to investigate and describe the participants' perceptions, beliefs, opinions and experiences in their own terms (Snape & Spencer, 2003). Qualitative research seeks new information and knowledge in order to improve therapies or services and it can be conducted with a range of methods and traditions (Tong, Sainsbury, & Craig, 2007). There are many approaches to qualitative research (e.g. grounded theory) and many methods of data collection (e.g. interviews). The researcher's self-reflection and pre-understanding are

essential parts in planning and analysing qualitative research in order to minimise bias issues of his/her own influence (Watts et al., 2017).

This study conducted in-depth interviews with individuals. In-depth interviews are an excellent way of generating new insights rather than imposing existing knowledge or preconceptions and qualitative methods provide the best way to understand patients' or caregivers' needs and concerns (Wann-Hansson, Hallberg, Klevsgård, & Andersson, 2005). The in-depth and extended discussion was guided by the interviewer using a semi-structured interview style with predefined questions and specific topics of discussion that allowed open-ended responses and clarification of the research context (Kallio, Pietilä, Johnson, & Kangasniemi, 2016). The information gathered using this approach could facilitate our understanding of the effects of home -baked milk reintroduction and its impact in children with IgE and non-IgE mediated CMA from the perspective of parents.

5.3.2 Study design

Semi-structured one-to-one interviews were carried out to allow the researcher to be flexible in exploring any relevant and interesting materials that might be mentioned by the parents. An interview schedule (section 4.3.5, Table 4.1) was designed for the purpose of this study. Using open-ended questions, the researcher allowed parents to respond in their own words and provide meaningful, rich and naturally explanatory responses. The participants were given a choice of Skype, Messenger call, video conferencing and/or telephone call, depending on their availability. All interviews were anonymised and digitally-recorded.

5.3.3 Identification and eligibility of participants

Potential participants were identified and recruited through social networking sites, such as parental groups and forums. Recruitment mainly took place through online platforms, such as personal blogs, Facebook and Instagram. A significant benefit of on-line recruitment from social networking sites is the ability to target people who share a strong interest in a particular area. This study recruited parents who have experienced BMCs or a ML process

for the management of their children CMA. A selected sample of parents who are primary caregivers was used. Participants were included in the study if they met the following criteria:

Inclusion Criteria

Participants satisfied eligibility criteria if they were parent(s)/guardian(s), who:

- have introduced baked milk products/or a milk ladder plan with their child/children who have been clinically diagnosed with CMA
- have children diagnosed with concomitant allergies
- are willing to be interviewed via telephone, messenger, skype or video-conferencing
- are able to communicate verbally
- are UK residents

Exclusion criteria

Exclusion criteria were as follows:

- parents who have not introduced baked milk containing foods/a milk ladder plan in their children's diet
- parents who were not able to give informed consent
- parents who were unable to speak English

5.3.4 Recruitment and informed consent

Administrators of social media groups were provided with information regarding the study and were asked whether they would be willing to post an advertisement for participants on their group page. An advertisement poster (appendix 11) was designed specifically for this study, which contained a brief description of the study, a clear statement of the participants' inclusion criteria and the researcher's contact details to request further information and/or express interest in participating in this study. Interested participants were briefly screened for eligibility. If they met all study criteria, a participant information sheet (appendix 12) that gave a detailed explanation of the study and invited parents to participate in the audio-taped interview, the interview schedule (Table 5.1), the demographic questionnaire (appendix 13), and a consent form with a reply slip **(**appendix 14) were sent via email. The interview date,

time and the means by which the interview would be conducted (e.g. by telephone), were scheduled according to participants' preferences, convenience and availability. All documents provided adequate information and made sure that potential participants were fully informed before signing the consent form and participating in the study. Parents had two weeks to consider the study information and decide if they wish to participate. Participants were asked to sign and return the written consent from with the reply slip and the demographic questionnaire to me by email. Those interested in participating were sent an email, thanking them for their willingness to participate and confirming the interview arrangements.

The interviews were carried out between February and July 2017. Participants' involvement was no more than the length of an interview. Each interview was anticipated to last approximately 30 minutes, depending on how much the parents wished to share, and took place over phone/messenger call. Participants who attended the interview received a voucher of £10 as a gesture of thanks.

5.3.5 Sampling Strategy

Several studies suggest that saturation is essential to qualitative sampling (Ando, Cousins, & Young, 2014; Guest, Bunce, & Johnson, 2006; Latham, 2013). According to the theory of saturation, the collection of new data from additional participants does not provide any further insights on the issue under investigation, and thus data collection can cease (Mason, 2010). Saturation is the gold standard to ensure that adequate and quality data are collected. In qualitative analysis, the number of participants may vary from one to a hundred or more and represent a suitable and sufficient sample size, since this decision is based on the achievement of a rich and convincing analytical narrative rather than on statistical logic (Crouch & McKenzie, 2006). Indeed, many qualitative studies have achieved data saturation with fewer than twenty interviews therefore in thematic analysis a relatively small sample is likely to be adequate to understand common perceptions and experiences among a group of relatively homogeneous individuals (Mason, 2010); Guest et al., 2006). Hence, there are no clear guidelines as to the exact number of participants required to reach data saturation. Instead, a number of factors such as heterogeneity of the population, the number of selection criteria, the scope of the study, the nature of topic, type of data collection methods

use and the budget and resources available, might influence the sample size and therefore saturation of a qualitative study (Baker & Edwards, 2012).

The concept of saturation was used to justify the sample size in this study. It was assumed that data saturation could reasonably be expected in this study because the participants were recruited according to some common criteria such as homogeneous group of participants (parents of children diagnosed with CMA, UK residents, and all participants have used baked milk challenges/a milk ladder approach for the management of their children's CMA) and also all participants were asked a similar set of questions. At the beginning of the recruitment it was not known how many participants were required to reach data saturation for this study. The recruiting process was open to new information being presented. When the research questions were answered the recruiting process closed.

5.3.6 Data collection

Prior to starting the interviews, basic demographic data was collected for parents and their children. Individual interviews were conducted verbally by telephone or Facebook messenger call and lasted between 20 and 30 minutes. The use of semi-structured interviews helped to explore some relevant and interesting matters mentioned by parents. The interview schedule **(**table 5.1) was developed based on the existing literature with consideration of the research question, and was refined with the help of the academic supervisors and an external academic advisor with experience in qualitative research. All interviews were digitally recorded, transcribed verbatim, and anonymized. The interview schedule (table 5.1) was developed based on the existing literature (Braun and Clark, 2006) with consideration of the research questions. The interview schedule consisted of four topics that were driven by the research questions. Each topic included narrow questions that were part of the broader overarching research questions. These questions were designed to inform analysis and provide answers to the overall research question regarding the parental experience of baked milk introduction. The semi-structured design of the interview aimed to use the schedule to ensure that the broad topics of interest were covered with all interviewees, however, the individual questions were selected according to each participant case. Hence, not all interviewees were asked the same questions; the approach was tailored for each interview according to the flow of the guided conversation. The semi-structured interview therefore provided a guide for the interviews and facilitated a conversation

between the researcher and interviewee, and allowed a degree of freedom and adaptability in gathering data about the experience of the interviewee.

The questions were prepared ahead of time of the interview to help the interviewer: to be prepared, well-organised, and competent during the telephone interview; to achieve optimum use of interview time; to explore the topic with interviewees comprehensively; and to keep the interview focused on the desired topic. The interview schedule was divided into two parts. The first part included a set of open-ended questions and aimed to explore the mothers' experience with baked milk challenge(s) conducted in hospital. The second part included five topics and each topic had a set of 4 to 5 prompts related to different aspects of mothers' experience with the use of a milk ladder during the management of their children's CMA. Some mothers were asked questions from part one, and others from part two (depending on the method of baked milk introduction). The interview schedule thus provided a long list of questions from which the interviewer selected those appropriate to the discussion. The main topics that was attempted to cover the interview are outlined below:

- Mothers' experiences during the implementation of the milk ladder
- What kind of support have mothers received during the baked milk products introduction?
- What impact has the introduction of baked milk products into the diet of their children had?
- How does the implementation of the milk ladder influence the mothers' daily life?
- What are mothers' concerns during the process of milk ladder implementation?
- What ideas have mothers to improve this process?
- What are mothers' preferences regarding the place and time of baked milk products introduction?

The interview schedule was piloted to assess how long it would take. The semi-structured guide was designed to cover the duration of 20-30 minutes; a comfortable length for a telephone interview. The interview schedule was refined with the help of the academic supervisors and an external academic advisor with experience in qualitative research. All interviews were digitally recorded, transcribed verbatim, and anonymised.

Table 5.1: Interview Schedule

Topics

A. Use of Baked Milk Challenge (BMC)

I am going to ask you some questions about your experience and your child's experience of the baked milk challenge.

1. Why did your child have the baked milk challenge?
2. How long ago was the challenge?
3. What was the venue of challenge?
4. Which food(s) was/were used in the challenge(s)?
5. Were you/your child a bit anxious before the challenge?
6. What happened on the day of challenge?
   - Was the baked milk challenge process well explained to you?
   - Were you/your child nervous/anxious during/after the challenge? If yes so did the nurse/doctor put you/your child at ease?
7. Did your child have any problems with eating the challenge foods due to taste?
   On a scale of 1-10, how nice did it taste to your child?
8. Has your child experienced an allergic reaction during the challenge(s) or later? If yes so could you tell me about that?
   - What impact has it had on the child?
   - Has your child received any medication or adrenalin at the end of the challenge?
   - Any other problems for your child since the challenge?

B. Use of a Milk ladder process

Appropriateness of the milk ladder process and the place that it is carried out

I am going to ask you some questions about your experience and your child's experience of the milk ladder.

1. How did you decide to take up the milk ladder challenge? Who recommended you the milk ladder that your child undergoes/underwent?
2. Was the milk ladder process well explained to you? Were you nervous / anxious about doing it? If yes so why?
3. How did you explain what would happen to your child? How did your child react?
4. Did it become easier to do? Why? How long did that take? Why?
5. Which place (home/hospital) do you think that would be more suitable for this process to be carried out and why?

Compliance

1. What kind of baked milk containing food did you use at the beginning of the milk ladder process?
2. What foods of the milk ladder did your child tolerate?
3. In which order have you used the baked milk containing foods throughout the milk ladder process?
4. How long have you used/been using the milk ladder and how easy or difficult did you find it?
5. Is there anything that could have made your experience better?

Acceptability

1. Did your child have any problems with eating the challenge foods due to taste?
   On a scale of 1-10, how nice did it taste to your child?
2. According to your opinion what are the advantages/disadvantages in the use of the baked milk products?

For example:

- What about the cost of baked milk products?

- What about the palatability of these products?
- Did you observe any changes in your child's eating behaviour and health after use of the baked milk products?
- Was your family quality of life influenced by any of these changes?

Practicability

1. Which kind of baked milk products would you prefer to give your child and why?
2. Was it easier for you to use commercial or homemade baked milk products? Did you face any difficulties to identify/prepare these foods?
2. Did you find helpful (or not) the recipes and doses of the baked milk products and why?
3. What was your knowledge about the amount of milk protein in the baked milk products that you have used? (Before and after introducing the baked milk products)
4. What type of support did you have during the use of baked milk containing foods?
- Was the information provided helpful to you? What was most helpful/what was not?

Safety

1. How was the overall experience for you and your child? Has the milk ladder experience changed how you and your child feel about risk? If yes so in what way? Are you and your child feeling less anxious overall? Can/did you manage better your child condition?
2. Has your child experienced any allergic symptoms during the milk ladder process? If yes so has your child received any medication following the allergic reaction?
3. Have you got any concerns about the milk ladder process?
4. Do you think that you could improve the milk ladder that you have used and if yes so how?
5. How would you advise other mothers about introducing baked milk products in their children's diet?

5.3.7 Data analysis

Data was analysed using thematic analysis as described by Braun and Clarke (2006). Thematic analysis is the most common form of a qualitative analytic method that are conducted in six phases as it has been described in Figure 5.1 (Braun & Clarke 2006).

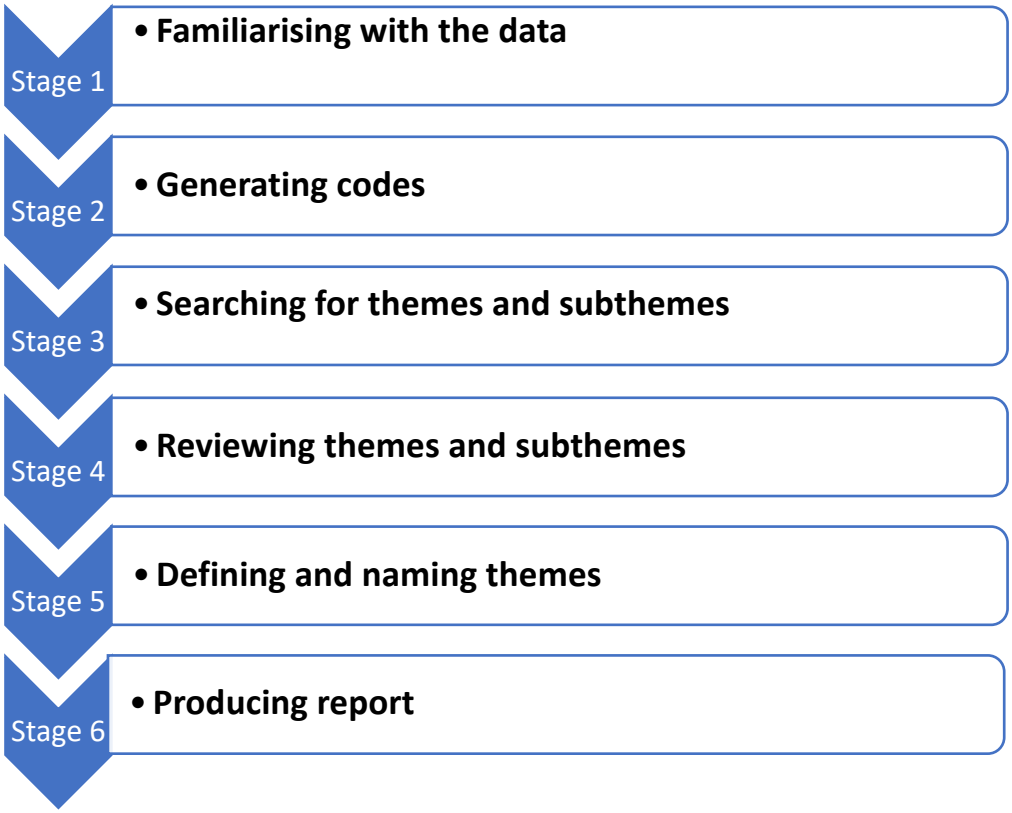


Figure 5.1: Thematic analysis according to Braun & Clarke's (2006) approach

Thematic analysis was used to answer the research questions related to parents' experiences, perceptions and views about the usefulness of a milk ladder plan as a dietary management of their children's CMA. In this study, an inductive approach has been used to identify, analyse, and report themes that come from parents' data (King 2004). A second researcher checked the accuracy of transcriptions going through the recording while reading the transcriptions. After checking the accuracy, the transcripts were read twice prior to their analysis. Qualitative data analysis software NVivo 11 (QSR International Pty Ltd; Doncaster, Victoria, Australia) was used for the coding of data. NVivo is a computer-assisted qualitative data analysis software package that has been used to facilitate the management and analysis of data.

In the first stage, the interviews were transcribed immediately after each interview and transcripts were read to familiarise myself with mothers' data. In the second stage, initial codes were generated for each individual transcript in the nodes of NVivo. The initial codes were broad and detailed. All texts that were relevant to research questions were coded. A list of codes was produced and similar or identical codes were grouped. In the third stage

conceptualisation between codes and different levels of themes was initiated and the major themes were described. In the fourth stage, themes were reviewed, rephrased, and reorganised and also subthemes were created. Themes were compared with coded text passages to check if they represented the actual content of the transcript. Themes provided an explanation of the important experiential items that were found during the process of analysis. In the next stage, themes were defined and refined by writing a short summary for each individual theme and subtheme. At this stage, the development of themes and subthemes were discussed with my academic supervisors to check for clarity, and agree on final themes. In the final stage, a report was written that presented in the section of the results and illustrated themes and subthemes and participants' experiences with their own words and my interpretative commentary.

5.3.8 Ethics considerations

The Science Faculty Ethics Committee (SFEC) of the University of Portsmouth approved this study, on 25th May 2016 (appendix 15). In qualitative research, informed consent, confidentiality and trust are the main considerations. All participants received written information about the study and signed a consent form. In the participant information sheet, it was clearly stated that the participant was free to withdraw from the study at any time for any reason, and with no obligation to give the reason for withdrawal. In the consent form it was clearly stated that parents would get the opportunity to discuss with the Investigator before making a decision about their participation in the study. This research study followed the policy for Research Data Management as described in the Research Data Management Policy published in April 2015 at the University of Portsmouth.

Participants were aware that the data will be anonymous and confidential and stored according to Data Protection Act. Each participant was given a study number for data analysis and dissemination of outcomes. All collected data, including digital recorded telephone interviews, and hard copies of the transcripts, were secured in a locked cabinet and any electronic records were stored in password protected files. Only the researcher and authorised academic supervisors have access to the encryption key.

5.4 Results

5. 4. 1 Characteristics of participants

The study recruited mothers that followed a BM-reintroduction approach, from different parts of the UK, through social networking sites. In total twenty-two semi-structured individualised phone or Messenger call interviews were conducted. Fifteen mothers that participated in this study had children suffering from mild to moderate non-IgE-mediated CMA and seven mothers had children with IgE-mediated CMA. The mothers were well educated with over a third having a graduate or postgraduate qualification and the majority of them worked part time. The mothers' ages ranged from 21 to 44 years (median = 34.5years) and the age of children ranges from 15 months to 8 years (median = 2.5 years). Over a third of children were diagnosed by a combination of the clinical symptoms history and SPT, IgE, and/or oral milk challenge, and more than a half of the children had a concomitant allergy either food allergy such as egg, peanut, sesame, shellfish, multiple food allergy or eczema/asthma. The majority (20 (91%)) of mothers reported that they didn't have an initial BMC in hospital. From the total of seven IgE-mediated CMA patients, only two children had baked milk challenge in hospital before they commenced reintroduction of baked milk foods at home. Children had completed different steps of the milk ladder. One child had reacted in step 1 (malted biscuit), 6 children passed steps 2-4 (digestive biscuits/cupcakes) and reacted to Scotch pancakes/muffins, 7 children passed steps 5-7 step (shepherd pies/lasagne/pizza) and reacted to chocolate, 3 children passed 9-11 (yogurt/cheese/sterilised milk) and reacted to pasteurized milk, and 5 children completed the milk ladder. The time spent during the process of the milk ladder ranged from 0 - 36months (median = 12 months). The majority of children experienced skin reactions during BM-reintroduction. Characteristics of mothers and their children are detailed in **Table 5.2.**

Table 5.2: Characteristics of participants

Characteristics	Total Number of Participants (N)=22
Education:	
Postgraduate	9
Undergraduate	8
College	5

Characteristics	Total Number of Participants (N)=22
Employment:	
Part time	15
Stay home	3
Full time	4
Child's gender;	
Male	13
Female	9
Diagnosis:	
Non-IgE-mediated Cow's Milk Allergy	15
IgE-mediated Cow's Milk Allergy	7
Method of diagnosis:	
History of symptoms	9
History of symptoms & SPT/IgE/Oral milk challenge	13
Child's other food allergy:	
Not any	9
Soya	4
Multiple food allergies	2
Egg	2
Sesame	1
Peanut	1
Wheat	1
Egg & selfish	1
Egg & peanut	1
Other health conditions:	
Eczema	11
Not any	6
Asthma	3
Asthma & Eczema	2
Hospital Baked Milk Challenges:	
No	20
Yes	2
Different steps of the milk ladder had completed by children:	
0 (reacted to the 1st step)	1
2-4 steps	6
5-7 steps	7
11 steps	3
Completed	5
Symptoms experienced children during BM-reintroduction:	18
Skin reactions	4
Itching	5
Loose stools	5
Abdominal pain	5
Behaviour changes-hyperactivity	2
Vomiting	4
Sleeping disturbances	1
No symptoms	

The coding process of this study was documented using the approach outlined by Ando et al., (2014). In the first stage, codes that provided information relevant to the research questions were collated and a codebook was generated to ensure saturation of data. During the coding process, some codes were repeated and thus, they were clustered under the same codes. An initial coding was carried out to reduce any loss of data related to questions of research. The codebook comprised 36 codes in 5 themes. **Table 5.3** shows the codebook with the initial coding, contributory codes and themes.

Table 5.3: The Codebook with the initial coding, contributory codes and themes

Initial Coding from individuals n=total number of participants	Contributory codes	Themes & Sub-themes
Different types of milk ladders (n=7) **Confusion with different milk ladders (n=7)** **Lack of healthcare support (n=17)** **Advice from other parents (n=10)** **Delayed diagnosis of CMA (n=7)** **Lack of follow up (n=10)** **Limited time of HCP's appointment (n=4)** **No clear explanation about the baked milk introduction (n=7)** **Use of social media to gather information and get answers to milk ladder queries (n=17)**	Delayed CMA diagnosis Confusion with different types of milk ladders for reintroduction of milk in baked forms Lack of communications between parents and healthcare professionals Gathering information from media and CMA supporting groups	Theme: Understanding the milk ladder implementation – a lack of clarity and support Subtheme: Which ladder, and when to stop and when to climb? Subtheme: It's one appointment, then you're on your own
Increased anxiety (n=14) **Anxiety for next steps (n=7)**		Theme: Introducing baked milk at home: Inexpertise and anxiety

Nervous due to painful symptoms (n=9) **A list with a description of potential reactions and guidelines how to cope with these (n=5)** **Lack of directions regarding the delayed reactions (n=2)** **Confused with the causes of symptoms (n= 10)** **Changes of behavour or awake at nights (n=3)** **Confused with the dose of food trial and any association with side effects (n=5)** **Difficult to communicate with the toddler and recognize side effects due to the trial food (n=5)** **Place of food re-introduction (n=15)** **Confidence with hospital food introduction (n=10)** **Convenient with home food introduction (n=10)** **Dealing with the balance between milk ladder step up and step back based on side effects of foods (n=3)** **Time spent in each step of the milk ladder (n=8)**	A list with a description of potential reactions and guidelines how to cope with these Confused with the causes of symptoms Increased anxiety with allergic reactions associated with baked milk Nervous due to painful symptoms Reluctant to milk ladder due to the lack of communication in young children Confidence with hospital food introduction – (all mothers with IgE mediated CMA children & some mothers who had experienced severe reactions Convenient with home food introduction – all mothers with non-i9IgE mediated CMA children Uncertainty regarding the time spent on	Subtheme: Discriminating between a reaction and an illness Subtheme: Making them poorly to make them better Subtheme: Preferences on the milk ladder setting (hospital/home)

	each stage of the ML Confusion with time spent in each step of the milk ladder	
Food choices (n:22) **Negative mothers' experience with food choice in the milk ladder (n=22)** **Foods in the milk ladder do not always cater to concomitant food allergies (n= 10)** **Frustrated with the reading of the food package labels (n=7)** **Frustrated with the unhealthy food options (n=14)** **Lack of a list with recipes and further details regarding the quantity of milk, cooking time and temperature for the foods in each stage (n=12)** **Lack of alternative or equivalent food options (n=15)** **Lack of knowledge regarding the amount of milk protein in the products (n= 16)** **Time consuming to organise the food introduction (n=3)** **Positive mothers' experience with food choices in the milk ladder (n=20)** **Children happy with the taste of most foods (n=8)** **Less concerns eating out or nursery or as a family (n=8)**	Foods in the milk ladder do not always cater to concomitant food allergies Lack of alternative/equivalent/healthy food options Lack of knowledge regarding the amount of milk protein/temperature & time of baking/quantity of milk for each step/list of recipes Frustrated with the reading of the food package labels	Theme: Living with the milk ladder: making it work Subtheme: Balancing the rigidity of the milk ladder with the demands of real life Subtheme: Returning to normality – removing the restrictions of a dairy free diet

Low cost of foods (n=20)

In the stage of generating the initial codes I coded each segment of data that was relevant to or addressed the research questions. I did not have pre-set codes and did not code every piece in the text. The initial codes were developed as I worked through the coding process. All the potential codes were identified and highlighted, and all relevant data was systematically coded. A long list of the different codes was produced and provided a preliminary idea about data. Next, all the quotes related to each individual code were compared and, as result, some codes were modified, and some new codes were generated. The next stage was focused on the broader level of themes and involved sorting the different codes into potential themes. All codes identified were grouped under potential themes and subthemes. As Braun & Clarke (2006) explained, a theme is characterised by a pattern that captures something significant or interesting about the data or research questions. The codes were evaluated and those codes that appeared to fit together were grouped together to make an initial theme. For instance, several codes related to mothers' concerns regarding the limited information provided by healthcare professionals, and insufficient time for milk ladder consultation.

' I collated these codes in an initial theme called "Understanding the millk ladder implementation – a lack of clarity and support'. Other codes were around mothers' concerns related to allergic reactions during baked milk introduction and the safety of this process. I collated these codes in an initial theme called 'Introducing baked milk at home: Inexpertise and anxiety'. Other codes were related to mothers' experiences regarding the impact of milk ladder foods on their children' diet. I collated these codes in an initial theme named 'Living with the milk ladder: making it work'. Some codes formed main themes and other codes formed subthemes. At this point a collection of a list of themes and subthemes was produced. All codes identified were grouped under potential themes and subthemes. The themes and subthemes were then reviewed to ensure that the quotes underpinning them and experiences they described were coherent. At this stage, one of the initial subthemes was remove. In particular the subtheme related to '"delayed CMA diagnosis" was subsequently removed since, on further reflection, it described a part of mothers' general experience of having a child with CMA but not specifically to baked milk introduction. The exact grouping of subthemes and the titles of these and the themes were revisited to ensure that they accurately reflected the full story of the mothers' experiences.

5.4.3 Description & interpretation of results

According to the qualitative findings, the milk ladder appears to be simple to implement in theory but mothers experienced difficulties to implement it in real life. Not only that but the support they got from HCPs was minimal both in helping them to understand the theory but also in supporting them to apply it in practice. This made it an anxious and rather lonely journey for the mothers.

Based on the thematic analysis three themes and six sub-themes emerged and are presented in this section, with quotations to illustrate them. The themes are presented in a chronological progression through the findings: (1) initial understanding of the milk ladder implementation (2) starting the milk ladder and (3) managing the milk ladder in day-to-day life.

The symbol (q) indicates the interview from which the quotation originates and if it is derived from a mother of children with IgE or non IgE-mediated CMA.

Theme 1: Understanding the millk ladder implementation – a lack of clarity and support

The majority [77%] of mothers expressed that there was limited healthcare support during the implementation of the milk ladder, and they needed further clarification for the introduction of baked milk containing foods into the diet of their children with IgE and non-IgE mediated CMA. This theme is divided into 3 subthemes:

Subtheme 1: It's one appointment, then you're on your own

This subtheme describes mothers' experiences of starting out with the milk ladder, the information they received from HCPs during the process, and the support (or lack of) that they received to implement the milk ladder. At the start of the milk ladder journey mothers were not familiar with it, and, understandably, looked to HCPs for information about the milk ladder and how to implement it. However, mothers commented that during the initial consultation they were not given sufficient time to become familiar with the new information provided for the baked milk reintroduction or to ask questions regarding this process. A sheet with the milk ladder and written guidelines on how to complete this process were given, but mothers felt they needed more time to digest all the information, to understand how it can be used and how they could go through the milk ladder step by step. Mothers described their experience as follows:

"*I was given the paper of the milk ladder when we had got the consultant and dietitian appointment. We had limited time for a quite lot of information to be shared, so I left not knowing how long to spend in each level of the milk ladder and I did not have access to anyone to get an answer to that." (q3, non-IgE CMA)*

'Our dietitian provided us just some basic things ….she actually gave us a sheet with the twelve step milk ladder and advised us to go through very slowly with this. She said all the information is written on the paper and if I had a problem to contact them by phone' (q21-IgE mediated CMA)

Some participants expressed their preference for a follow up plan that could give them the opportunity to be educated by healthcare professionals and get as much information as they could regarding the development of their children's milk tolerance via the gradual reintroduction of baked milk foods. Some of them mentioned that there was a lack of dietetic support for allergy management in their hospital or community setting, and there were long waiting lists for allergist/paediatrician appointments in the clinic. They complained that the milk ladder is a long process and they required further health care services support either via phone or email to feel confident with this process. Mothers expressed their disappointment as follows:

"We had an annual paediatric consultant appointment. So, for two years we managed to see the consultant. Unfortunately, our dietetic services, there should be five dietitians but there is not anyone in post."(q3, non-IgE)

"The support is still there with the dietitian b#
#ut is more driven by me ... so if I contacted them saying okay we just tried the milk ladder and had a reaction they are like okay fine stay and try again for another six months but they are not very proactive… anymore ... in terms of contacting us... review things... which makes me feel quite isolated."(q14, non-IgE)

"I think that they actually could plan for us to go back again but nobody contacted us about it." (q10, IgE CMA)

Hence, the limited time during the initial consultation seems to be a barrier between the communication of mothers and healthcare professionals about the milk ladder. Although mothers were provided with written guidelines, they realised at home that additional practical information was required. For the majority of mothers, the baked milk introduction was a difficult and long process and they felt that further healthcare support, either in the form of follow-ups or phone/email contact would be incredibly helpful. In essence, the mothers expressed an experience of loneliness and lack of support for managing the milk ladder process. They felt inexpert at managing this process, but could not gain regular access to dietetic support for working with the milk ladder.

Subtheme 2: Which ladder, and when to stop and when to climb?

Following on from the lack of ongoing support for implementing the milk ladder, mothers also expressed their experience of disconnect between the theoretical implementation of the milk ladder and the reality. While they initially found the information that they have given by healthcare professionals clear, when they started the milk ladder, they found it difficult to implement in practice. For example, it wasn't always clear to them how and when to move between the stages of the milk ladder.

"I thought it was clear ... but then when we intended to do it ... then actually I realised that it was not particularly clear in terms of how long you leave between you know moving to each stage... and how
much you give for how long… so it has been quite trial and error … specifically to my little boy and how he reacts to make sure going slowly enough for him … does that make sense?" (q14, non-IgE CMA)

"Our dietitian provided us with some basic things. She gave us a sheet with the twelve-step milk ladder and advised us to go through very slowly with this. She said: the information is written on the paper and if I had a problem to contact them by phone or email….." (q21, IgE CMA)

Due to the lack of communications with healthcare professionals highlighted in subtheme 1, some participants joined support groups in social media to find answers to their queries. Some mothers mentioned that the use of social media was an excellent way to gather

information for the milk ladder and food reintroduction. They had the opportunity to discuss their concerns or queries with other parents who had similar experiences and obtain practical solutions for any issues that arose during the introduction of baked milk products. Interviewees believed that mothers who had used a milk ladder plan had better experience and could provide better help during this long process than could healthcare professionals. They felt that someone needs to have first-hand experience of implementing a milk ladder to really understand what it involves:

" Not at all ... What I had was from the CMPA charity that I joined... that's the only support ... And that's fantastic support because all moms that have been through it or are going through it and they know more they seem to know more than doctors and other professionals … I think you have to experience it yourself to totally understand it" (q2, non-IgE CMA)

Mothers commented that they were aware of the different types of milk ladders, and several participants attempted to complete different milk ladders to identify which ladder could benefit their children. They reported that HCPs recommended alternative versions of a milk ladder. For instance, some mothers reported that their healthcare professional provided them with the 12-step milk ladder that worked better for their children's desensitisation compared with the 4 or 5 stages milk ladder. In particular, they expressed this as follows:

"We received a different version of the milk ladder initially from the dietitian, which was only 5 steps. We didn't get the MAP version and that [the version given] failed very fast in terms of jumps between the stages. So, I decided to use the MAP version because my son is quite allergic. He reacts to very small amounts of milk..." (q14, non-IgE CMA)

"There are differences between milk ladders, and this is very confusing. It should be one standard milk ladder that all hospitals within the country should follow because this is so confusing. (q6, non-IgE CMA)

Mothers with IgE-mediated CMA children said that they used a milk ladder to introduce baked milk foods into the diet of their child and they were confused with the different version of milk ladders and they did not know which milk ladder was more suitable for their child. However, according to the iMAP guidelines the milk ladder can only be followed by infants and children with a mild to moderate non-IgE-mediated CMA (Venter et al., 2017).

There is still no evidence that supports the use of the milk ladder in IgE-mediated CMA that may result in severe or immediate type allergic reactions. HCPs usually rely on BSACI guidelines that suggest the use of a milk ladder with caution at home (Luyt et al., 2014).

"The difficulty is that I found that it has been varied in the types of milk ladders. There is not just a generic milk ladder, there is one that has got 12 steps, and there is one has got 4 or 5 steps. And then the difficulties that I got is that for now she is on stage 2 in a milk ladder or stage 3 according to different type of milk ladder… I suppose my main concern is that it doesn't seem like a standard milk ladder, everyone uses their own milk ladder and it doesn't feel that there is enough information to go with it." (q7, IgE-CMA)

Hence, the experiences of these mothers are that following a milk ladder can be a confusing experience complicated by the existence of multiple different milk ladders. Moreover, the mothers were often not sure how to know when to climb the milk ladder by moving onto the next stage. The lack of sufficient information regarding the BM-reintroduction led mothers to seek advice from sources such as social media or other parents with CM allergic children and even to switch to a different milk ladder than that given to them by their HCP. The majority of mothers found support in social media such as Facebook/Instagram instead of healthcare services because they needed additional information to increase their knowledge, for instance regarding the amount of milk protein in the BM product, time spent in each step, alternative/equivalent food options in milk ladder and recipes. This perspective emphasises the need to improve healthcare support during this long period of baked milk introduction and ensure not only the efficacy of the milk ladder approach but also the safety of this procedure. Another important issue indicated by these results is that mothers felt confused with the different versions of milk ladders and expressed that they would have preferred a standardised approach for the baked milk foods reintroduction with standard guidelines that could optimise the milk ladder process in terms of practicability, acceptability and safety for their children.

Theme 2: Introducing baked milk at home: Inexpertise and anxiety

Mothers described great apprehension about introducing baked milk at home. Generally speaking, they described the concerns they had about their ability to recognise potential allergic symptoms during reintroduction of baked milk foods into their child's diet. They were also concerned about how they could cope and manage an allergic reaction at home.

Subtheme 1: Discriminating between a reaction and an illness

Mothers described their lack of knowledge or criteria to help them to distinguish between an allergic reaction and an illness. They also highlighted that several allergic symptoms are similar with common conditions in infancy or early childhood and that this could be confusing. For example, mothers said it was very hard to distinguish the difference between an allergic reaction and a cold or teething because some symptoms seemed so similar and their children were too young to provide a detailed description of their condition. They felt that the management of allergic reactions during food trials should be based on recognition of symptoms and not on assumptions of them. They discussed that a list with a description of potential symptoms during reintroduction of baked milk food would help them in identifying symptoms and providing better care for their children. Some mothers expressed this as follows:

"Sometimes we think like you know gastro- symptoms could be caused by different things. We don't really know…just guessing a lot….."(q5, IgE-CMA)

"It would be very helpful if we could have a full description of the potential reactions when we are doing the milk ladder."(q18, non-IgE CMA)

"I felt that we would be unable to identify if he was having a reaction to the milk ladder trial or to an unidentified allergen…Between the ages of 12-24 months there wasn't really a time where he was settled and not suffering either with a food related reaction, teething or cold/viral illness so during this time I didn't attempt the milk ladder."(q22, non-IgE CMA)

Other parents had to deal with the balance between milk ladder step up and step back based on side effects of milk containing foods.

"…the symptoms that we look for in order to know whether or not to proceed…Sometimes, they are not specific, and they are difficult to separate from another…The actual impact for a child with an IgE CMA is looking for symptoms in order to know that it could conflict with the

next step up is difficult because the symptom is so difficult to be clear….so, it is quite difficult for me as a parent." (q5 IgE CMA)

Hence, the recognition of symptoms during an allergic reaction to a baked milk containing food was not clear from mothers' perspective. They need education how to recognise and manage symptoms through the milk ladder process.

Subtheme 2: Making them poorly to make them better

Mothers described the fears and anxiety they experienced during their children's milk reintroduction, due to the possibility for allergic reactions. They felt responsible for any pain caused to their child and found it incredibly difficult to observe their child in pain. Mothers described feeling nervous and anxious because they observed their child suffering symptoms after baked milk food consumption; a situation in which they felt helpless to provide appropriate care.

"Yes, it makes me very anxious, it makes me nervous. I feel sick because I know I am potentially giving a substance that is going to make him very poorly and then if he reacts and he did react we have to watch him in pain…"(q6, non-IgE)

Some mothers assumed that their children avoided eating the trial food because they associated discomfort or pain with this food. The young age of children was another important barrier between the communication of mothers and children. Following an unsuccessful baked milk food trial, some parents with young children were reluctant to engage with the milk ladder and avoided re-attempting food reintroduction. They reported that it is very hard for them to expose their children to pain:

"He is too young to communicate so he could be crying because he is in pain or he could be crying because he is teething or because for any other reason…it is very difficult for me. At present the milk ladder should be left until he is an age that he can talk and he can tell that there is pain or feels uncomfortable. I don't want to put him back in pain until he is able to communicate with me. As mum I don't want to put him through a pain again."(q6, non IgE CMA)

Mothers further commented that they felt distressed because they had already experienced their child's allergic reaction prior to diagnosis. Some mothers were nervous in the first steps of the milk ladder and when they became more familiar with the process they felt relaxed with the good progress of their children.

"I felt guilty for feeding these foods and causing his suffering. The more we failed the more I became stressed with labels on food and anxious about reintroducing…I figured out what I was doing but psychologically it was hard. I had to tailor the ladder attempts to my son. Instead of trying for three days then moving on, which is what I was told to do, I noticed my son would react anywhere from six hours with hives or tummy pain to seven days with eczema. By day 5 the reaction was appearing even when I stopped then it was still causing reactions and would take weeks to manage his skin. I learned to try one meal wait a week. Then try another meal and wait a week, try a meal again and every other day if ok after that third week consider a pass. It took longer but in the long run this worked better for his skin as I could manage it quickly when we failed a step" (q20, non-IgE CMA)

"I was also anxious to feed my child something that I know has previously caused him a great deal of pain, discomfort and suffering…I worry that if I continue to attempt the milk ladder and it continues to cause pain then my son would develop a food aversion." (q22, non-IgE CMA)

Hence, mothers were reluctant to engage with the milk ladder and believed that it would be better to attempt this process later when their children will be able to communicate and express properly their discomfort. Interestingly, some mothers reported that their children associated pain with the challenge food and avoided eating this food. These mothers reported being satisfied with the milk- free dietary plan, and they did not want to expose their children to a painful process and potential risk of allergic reaction by introducing BM-products. Most of these children had experienced severe reactions and their mothers did not want to see them to suffer from reactions once more.

Subtheme 3: Preferences on the milk ladder setting (hospital/home)

Mothers with non-IgE mediated-cow's milk allergic children were aware that their children were not at risk of severe reaction and found home to be a more convenient place for the baked milk introduction.

"I think he would panic... I think trying at home is better... unless they have a severe reaction. I think if you can do it at home it is better for the child, that's unless yeah there is really severe reaction. I would prefer my boy to be here." (q16, non-IgE-CMA)

"…[I] feel comfortable that we did it at home but if I had had any concerns i.e. anaphylaxis I would have requested a hospital trial" (q18, non-IgE CMA)

However, mothers of children with IgE mediated- cow's milk allergy appeared to experience increased anxiety with home-food reintroduction compared to those mothers that had the opportunity to challenge their children in hospital. Those whose children were challenged in hospital preferred the safety provided by a hospital environment because they did not feel confident to manage any potential severe reactions at home.

"I think if I hadn't done it at the hospital, I would be really anxious about whole thing. And the fact we only do it in hospital gives me confidence and I know they are doing it. I know people in other authorities they need to do it at home." (q12, IgE mediated CMA)

"Actually, I would prefer a challenge for my child in the clinic and I cannot understand why they didn't offer us a challenge in the hospital. During his first food trial at home I was watching his progress with a vigilant eye and thinking how to cope with any potential reaction…I felt really scared and so nervous." (q19, IgE mediated CMA)

Hence, different opinions were stated by mothers regarding the appropriate place for the milk ladder to be conducted. Mothers with non-IgE mediated milk allergic children had a different opinion regarding the setting (hospital or home) for the initial food trial of each step of the milk ladder compared to mothers who had children with non-IgE mediated CMA. Mothers of children with non-IgE mediated CMA were confident with the milk ladder process at home, due to their child having less severe reactions to baked milk. Mothers of children with IgE-mediated CMA were aware that there was a risk of a potential severe reaction to baked milk and wondered why the allergy healthcare services did not offer an initial baked milk challenge (each food of the milk ladder to be challenged firstly at hospital before it is introduced at home) in hospital.

Theme 3: Living with the milk ladder: making it work

This theme describes mothers' opinions regarding the practicability and acceptability of foods included in the milk ladder. The lack of flexibility in the foods to be used at each stage of the milk ladder made it very difficult for the mothers to implement the milk ladder in the face of the demands of real life with a young child. They described many difficulties in adapting the milk ladder to their child's and family's needs due to the very limited range of foods described in the milk ladder. However, they also described benefits to their child and their family once they started to progress through the milk ladder, and the restrictions of a diet free from cow's milk were gradually removed.

Subtheme 1: Balancing the rigidity of the milk ladder with the demands of real life

There were a range of challenges that the mothers described when they came to implement the milk ladder. In particular, they described challenges that appear to relate to a mismatch between the rigidity of the foods given at each stage of the milk ladder, and the demands of real life with a young child. For example, most mothers expressed concerns about the amount and quality of baked milk foods such as biscuits, cakes, muffins, pancakes, pizza, chocolate buttons etc. used in the milk ladders. They were concerned that these foods are high in sugar, fat, and salt (according to paediatric healthy food guidelines, these foods should not be given in large amounts or with great frequency). Some had worked hard to cultivate healthy eating habits in their child, only to be faced with offering them what they felt were large amounts of unhealthy foods in the milk ladder. Mothers expressed their concerns as follows:

"The most frustrating was the choice to start with unhealthy…we had such a healthy diet for her...I remember on day 8 she looked to me and said "Mummy, this is too many biscuits… though you know we hadn't biscuits. I understand that 2 or 3 biscuits are high fat and high sugar diet and she hadn't had anything like that before… I would like to see 3 or 4 suggestions per step and including some healthier options of food based on lower sugar." (q3, non-IgE CMA)

"If a child eats even a chocolate muffin for every day for three weeks it is not good for them. And I find a lot of food in the milk ladder as well, at the bottom anyway, they are all very sweet. I suppose now to be in the stage that we have to give her all these cakes and biscuits and stuff.

Even that have milk she really does not feed very well, but I don't think we have any other option."(q7, IgE CMA)

Mothers whose children also had allergies to other foods commented that foods in the milk ladder do not always cater for concomitant food allergies and that this could make it particularly challenging for them to integrate into their child's diet:

"For the milk ladder even we had not completed the stage 1…that I found it very complicated at all, just because you know he's got so many allergies and I am not just looking for the dairy, I am looking for soya, I am looking for lecithin which is a stabilizer for many products anyway…"(q6, Non-IgE CMA)

"So basically, she can eat mini muffins now but the problem that I got is that there are a lot of milk ladders that contain egg, so I really struggle and the stage for now to give her a wide variety of things that comes in that specific level because they got a lot of egg in. They need to give options to the people who have problems with dairy and eggs, give options to people who perhaps, I don't know, have problem with soya. Because soya is a massive problem, we haven't an issue with soya - but some people do."(q7, IgE CMA)

Some mothers also described the challenges that they had experienced in getting the milk ladder to work when their child was not keen to taste the foods prescribed in the milk ladder. When this happened mothers described that they had struggled to find alternative food options.

'My son is not happy with sweet taste of foods, he refused biscuits, cakes, and snacks like that….I didn't have other food options in the first steps of the milk ladder. For example, he loves savoury snacking'….. q21, IgE-CMA

Mothers also found it difficult to identify equivalent foods that they could use at the appropriate stages of the milk ladder adding time and complexity to their food shopping. They noticed that the process of identification of equivalent foods in the shops was time consuming and, furthermore, that often it was impossible to find equivalent or alternative foods. In addition, mothers reported that the information provided in the food labels of the products were not comprehensive and sufficient to help them to make proper food choices.

For instance, terms such as milk powder/semi-skimmed milk/whole milk/casein/whey confused mothers because they didn't know which of them was suitable for their child. Furthermore, they highlighted that many food labels don't clarify the amount of milk protein present in a product.

"*I get really quite irritated about being in the supermarket and looking labels all the time. My daughter spends the majority of her life in the supermarket and I am looking at labels." (q7, IgE CMA)*

"Take a packet of biscuits. Reading and reading the packages takes long. This takes too much time and I am frustrated, and I need a biscuit that contains milk, no wheat powder and is right for the milk ladder step." (q3 non-IgE CMA)

'*When I started to go and buy digestive biscuits, stand in the shop and pick up all the packets some didn't have any milk, some got milk powder, some soya milk, some of them had whey powder, and I would stand up in the shop for a long time and they didn't give you that information. That was what I found out so I have to be back and think what I have to buy. If you add brands on the milk ladder which again it couldn't be probably allowed to but at least everybody knows what they use….'. (q12, IgE-CMA)*

Some mothers therefore suggested that it would be useful for some food brands to be added in each step of the milk ladder to help the select correct foods for the appropriate step, and to save time when food shopping.

When preparing their own foods for the milk ladder, mothers were also frustrated with not having been provided with meal recipe ideas that clearly detailed the appropriate temperature and time of heating according to each step of the milk ladder.

"It would be really helpful if in the information that they gave you it said "when you make these things make sure that heating them to e.g. I don't know 250 C. That's all they need to do, they need just to put cooking information in a leaflet and if would make it simple. It really would be helpful to have alternative products in a list. Even I use a Facebook group support occasionally for a few suggestions but I need a comprehensive list."(q3 non-IgE CMA)

"…when we get the stage 3 they need to be specific about the temperatures, it needs to be clearly labelled on the leaflet. So, I wasn't given any recipes by the dietitians, the only recipes that I come across had been through on my research on the internet and by joining different groups on Facebook where people share information. If I hadn't done that then I would not have any idea how to progress. Actually, a lot that I learnt about the milk ladder comes through my research and through my group."(q7, IgE CMA)

Hence, mothers felt disappointed with several food options in the milk ladder and they found it very difficult not only to feed their children, but to convince them to consume these products. An improved milk ladder should consider a concurrent food allergy such as wheat or egg allergy and provide alternative and healthy food choices during baked milk containing foods introduction. Lack of clear instructions and additional information related to food labelling, baked milk food recipes, and brand names of suitable baked milk products in terms of milk protein content and degree of baking caused difficulties for the mothers to help their child to follow their milk ladder plan.

Subtheme 2: Returning to normality – removing the restrictions of a dairy free diet

Mothers perceived there to be benefits of the introduction of BM-containing foods into their children's diet. They felt that even though the milk tolerance is slowly built up in their child's digestive system and this process takes longer, it was worth following a milk ladder plan.

The majority [73%] of participants mentioned that the introduction of baked milk foods into their children's diet had a positive impact in the budget and social life of the family. They said that these products are cheaper compared with milk substitute foods and can liberate their children's diet enabling them to join family during meal time and eating out. Some mothers expressed this as follows:

"It's cheaper to buy milk products than imitation milk products. Yes imitation cheese/milk/yogurt that we buy they are all more expensive per litre pot. The dairy free yoghurt/chocolate are really very expensive so the cost on the left seems very reasonable…"(q3, non-IgE CMA)

"Yes, we can go out to eat more without any barrier on which restaurant he can eat in. When we went out we didn't have to worry about taking lot of special allergy foods. It generally makes our daily life easier." (q9, non-IgE CMA)

"As a family we don't feel any more concerns about food when we go out…Just in these steps we are all able to join in and eat as a family." (q11, non-IgE CMA)

The majority of mothers discussed that they would encourage other parents to undertake to implement a milk ladder plan. They explained that while this process may in some cases take a long time to complete the beneficial effects outweigh the negative effects such as a reaction or the slow development of tolerance to milk. The mother quoted below had almost completed the milk ladder with her child, and she felt happy about having gone through the process and was motivated to help other mothers. She felt that it was important that a mother should be confident during the milk ladder process, control her anxiety and has less concerns for any potential reactions (non-IgE-mediated CMA) that could affect her effort and her child during the milk ladder implementation. She expressed as follows:

'I do advise quite a lot because we have almost completed it and I stay on the page to help...I always say go into it confident...don't worry and stress...because you are gonna affect your child…don't look for reactions because you are going to convince yourself…it doesn't matter how long it takes...a lot of people go a lot slower than we did...just do what you can...just ask a lot of questions...and just watch for support around you...and to see where you are getting…there is nothing else you can do really…' (q1-Non-IgE mediated CMA)

Hence, although in the subtheme above mothers described the difficulties they had experienced in implementing the milk ladder, there were benefits once their child began to progress through the milk ladder. In particular, being able to use baked milk products (previously off limits to their child) helped their child's diet to become less restricted and more interesting and enjoyable. It also gave both the child and their family greater scope to engage in social activities related to foods such as enjoying meals out as a family. In addition, these findings show that mothers were able to worry less about their child's diet and safety as their child climbed the milk ladder. Despite the practical difficulties that mothers coped with the milk ladder implementation, they were satisfied with the outcomes of the milk ladder and they would be happy to recommend this intervention to other parents starting a milk ladder and share their experience.

5.5. Discussion

This is the first study that has explored the impact of baked milk introduction in children with IgE and non-IgE CMA from mothers' perspective. Overall, this study emphasises the importance of the milk ladder's improvement, as a tool of BM-introduction, and mothers' awareness and support for them during the introduction of baked milk foods into the diet of their children with IgE and non-IgE-mediated CMA. Taking an overview of the findings resulting from the thematic analysis, three themes and seven subthemes emerged. These are related to the key aspects of the process of milk reintroduction from the mothers' perspective (figure 5.2)

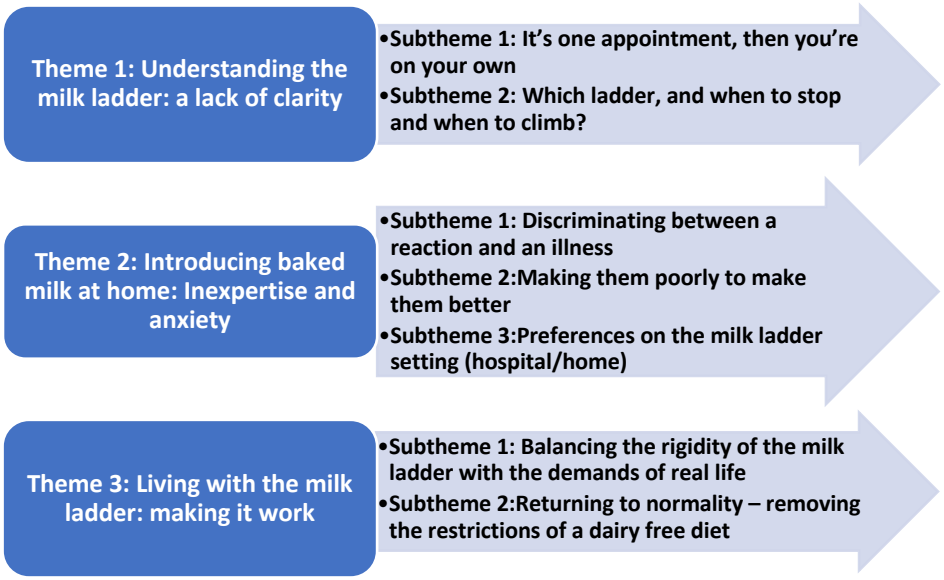


Figure 5.2: Lack of support for implementing the milk ladder: understanding theory and managing reality

In this section, each theme will be discussed in relation to the implications of the findings in the context of the wider literature.

5.5.1 Understanding the milk ladder – a lack of clarity

The main findings that will be discussed in this section is the lack of clarity of the obtained information and the limited time during HCPs' consultation and the lack of follow ups during the milk ladder implementation. One important finding from the mothers' study related to the lack of clarity of the information that mothers received before they started to introduce baked milk products into the diet of their children via a milk ladder. The findings of this study not only confirm that a variety of milk ladders are used in baked milk re-introduction, but also highlights that mothers are confused with the different versions of milk ladders. As has been discussed in Chapter 2 (literature review, section 2.3.2), many allergy clinics in the UK recommend a milk ladder protocol based on their own hospital policy. There is still no national agreement on the use of a standardised milk ladder based on scientific evidence. The main issue is that the milk ladder is not suitable for all children with CMA. Certain groups of CMA children should undertake a baked milk challenge in hospital under medical supervision because they may react to any cow's milk protein and experience a severe reaction. The safety of a milk ladder intervention is discussed below in section 5.5.3. Other practical issues were related to the quantity and frequency of the trial food during the milk ladder implementation. In particular, what are the appropriate doses of the administered trial food (how much and how should they be giving a baked milk containing foods such as muffin, per day/week/month and for how long), and when is the appropriate time to move from one step of the milk ladder to the next one.

Some mothers argued that their child had benefitted from exposure to low doses of milk protein over a longer period, but this argument has not yet been investigated in any systematic way through research. There is still a paucity of data regarding the doses used in BM-food trials and the time spent in each step before children move to the next step. NHS allergy clinics usually recommend to move on the next step on the milk ladder once the child is able to fully tolerate the BM-containing food in the step they are on. This means that the child has been able to eat the food more than once, in the quantities suggested on the milk ladder, without suffering any allergic reactions as a result. Sometimes, it can take

up to 2 or 3 days for allergy symptoms to appear after eating a food. Therefore, it is advisable to allow at least 3 days on a step before considering whether to continue the next step of the milk ladder. However, the recommendations above are based in a theory regarding the milk ladder and the experience of healthcare professionals. A recent study suggests that even when a child has successfully passed a BMC this cannot guarantee that the child will continue to be tolerant to the same food or will not react in the future to the same doses of this food at which they passed the BMC (Dunlop, Keet, Mudd, & Wood, 2018). Hence, the number of steps in the milk ladder seems to be crucial for milk allergic children during BM-reintroduction because it determines the doses of a variety of BM-containing foods consumed by children and the time spent in each step and on the total process of BM-reintroduction. However, clinical trials need to assess and determine the optimal doses and the time spent in each step of the milk ladder before the CMA child moves on the next step.

Mothers also experienced insecurity regarding the preparation of homemade BM-foods because they were not clear how long to cook the foods and at what temperature. Furthermore, mothers were disappointed that they did not receive any information about appropriate commercial BM-products that could be used at each stage of the milk ladder (e.g that were equivalent to the homemade BM-foods in terms of the amount of milk protein they contained). They also found reading food labels very complicated and time consuming These findings indicate that further clarification is required during consultation related to the practical issues of the milk ladder implementation. There is also a need for parents to have access to additional clear written instructions related to the quantity and frequency of administered food trial for each step. Furthermore, information should be provided that can familiarise parents with specific terms such as milk protein and to help them understand how much milk protein is required for each step of the milk ladder. In particular, specific guidance should be given to parents on how to ensure the quantity of milk protein contained in homemade foods, and how to recognise the content and amount of the milk protein ingredients in the food labels of commercial baked milk products.

This research highlights that for some mothers' social media is the main source of information about the introduction of baked milk products due to the limited support by healthcare services. Group online support seems to be easily accessible and appears to provide not only materials such as recipes, ideas for food alternatives, help in recognition

and management of allergy symptoms in baked milk food reaction, but also emotional support between mothers who have common concerns related to their children's food allergy management. However, mothers emphasize that in reality they would prefer better support from healthcare services to ensure the efficacy and safety of implementing the milk ladder. Current research suggests that the internet and social media have become a source of important information in Allergy and Immunology (Dimov & Eidelman, 2015). A systematic review of studies has shown that social media improves education and provides information for disorders such as food allergy, especially platforms such as Facebook, You Tube and Twitter (Patel, Chang, Greysen, & Chopra, 2015). However, the quality and reliability of information provided by social media regarding the management and treatment of food allergy are still questionable and further investigation is required to provide robust evidence (Dimov, Gonzalez-Estrada, & Eidelman, 2016).

A combination of online support run by healthcare professionals may benefit parents and patients in the management of CMA. There are online educational programs led by HCPs that may improve parents'/caregivers' knowledge and support them during long-term treatments such as the milk ladder. Online healthcare support with friendly technology (educational videos, online forums, and live video chats) can provide direct information and allow HCPs/food allergy trained educators to reach and support parents, caregivers and patients from distance (Ruiz-Baqués et al., 2018). In food allergy, education is an essential factor for improving patients' and their family's quality of life. If HCPs, parents/caregivers and patients work together, they can enable greater trust and enhance the effectiveness of the treatment (Ruiz-Baqués et al., 2018).

Another finding from the study is that there is a barrier between the communication of mothers and HCPs due to the limited time during their initial appointment in the allergy clinic. The majority of mothers discussed that they need more time during their visit in clinic to obtain practical advice and discuss any aspects related to the process of baked milk introduction. This finding is consistent with a number of studies in primary healthcare services that have found the length of consultation varies among the countries and is usually determined by healthcare professionals' and patients' characteristics (H. Britt, Valenti, & Miller, 2002; H. C. Britt, Valenti, & Miller, 2005; Cape, 2002; Hutton & Gunn, 2007; Martin, Banwell, Broom, & Nisa, 1999). The majority of these studies showed that the average consultation time in a primary care setting range between 10 to 15 minutes

while the patients reported that they preferred a longer consultation. Interestingly, these studies also found that healthcare professionals that provided a longer consultation prescribed less and offered more advice on health-promoting activities and a healthy lifestyle. However, the length of consultation should be individualised and adapted according to patients and caregivers needs. Appropriate time spent during the clinic appointments and especially in the first appointment could help to facilitate better recognition and management of health, social and/or psychological problems and provide enough information and advice to caregivers. There is currently very little literature examining patient and parental/caregiver satisfaction with the clinic services provided to allergy patients. These findings highlight the need for effective communication between HCPs and parents during the period of milk ladder treatment. Improved communication could be achieved if parents were given the opportunity to contact HCPs through phone or email in case that they had an emergent issue such as an unexpected allergic reaction or a practical query regarding the implementation of the milk ladder.

In addition, mothers expressed their preference for there to be a range of follow-ups from HCPs during baked milk introduction that would help them to implement the milk ladder. The mothers' experience of a lack of follow-ups with HCPs is concerning because it suggests that, in these cases, there has been no assessment of patients' recovery (e.g. stage of milk tolerance), no examination of parents' concerns during the treatment, and no monitoring of whether healthcare professionals' advice was addressed in the period of therapy. The completion of a milk ladder can involve a long process of desensitisation. Indeed, in this study so the mothers had been following a milk ladder for their child for more than two years. Where this is the case, regular follow-ups are required to monitor the growth and nutritional status of milk allergic children, assess the long-term effects of baked milk introduction, and examine milk tolerance Campbell, Park, Kueber, Lee and Hagan (2015) examined how a follow-up visit affects outcomes in patients who received emergency treatment for potentially life-threatening reactions. They found that improving follow-up rates can ensure that the anaphylaxis trigger has been accurately identified and patients had understood which allergy trigger to avoid so to reduce the risk of re-exposure to this allergen or prevent unnecessary food avoidance. This evidence indicates that reassessment and follow-ups can lead to more effective management of patients' food allergy. This is because they can benefit from additional information, education and advice, and they receive further food allergy action plans and documentation including practical guidance

for social activities such as eating out, school, travelling (Campbell et al., 2015)). Thus, while health care follow-ups are a key part of good management of cow's milk allergy and the milk ladder process, it is clear from this study that they are not always available for families. This is potentially dangerous, and leaves a great deal of responsibility for managing the milk ladder on mothers' shoulders; something that they may not feel qualified for. Finally, as highlighted above, it also removes the opportunity for mothers to discuss their queries and concerns with healthcare professionals and get more concrete advice (in response to their real-life experience of trying to implement the milk ladder) about how to work with the milk ladder. This study found that, right at the start of the process, mothers were disappointed with the length of consultation and limited information provided about baked milk introduction by healthcare services.

Hence, one of the major recommendations emerging from the findings above is that there is a need for improvement in quality of healthcare support as follows:

- Provision of detailed written and verbal information and guidance to ensure the safety of baked milk introduction
- Standardisation of the milk ladder
- Appropriate time given during clinic visits to provide effective communication between healthcare professional and parents about the principles and practice of the milk ladder
- Adequate monitoring of milk tolerance progress, compliance with the milk ladder treatment, parents' comprehensiveness during the implementation of milk ladder is required
- Development of an explicit nationally-recommended milk ladder protocol that all healthcare professionals can adhere to, and provide standardised advice to parents/caregivers during the gradual milk introduction process at home

The specific issues that should be addressed by HCPs and clarified with parents during a milk ladder intervention are as follows: the time spent in each step, options for alternative and healthier food choices, recipes for homemade food options, and education on the reading of food package labels and how to recognise allergic symptoms caused by BM-reintroduction. Interventional research (clinical trial) is required to investigate the optimal doses of the food trial and the time spent in each step before the patient moves on the next step. Additional research is required to evaluate how healthcare services can help milk allergic patients and caregivers to have appropriate information and a plan to recognise allergic symptoms and management of allergic reactions.

5.5.2 Introducing baked milk at home: 'Inexpertise' and anxiety

The main findings that will be discussed in this section is the difficulties experienced by mothers with regard to how to recognise and manage allergic reactions experienced by their child during the introduction of baked milk foods at home. This is also linked to the findings around mothers' reluctance to introduce these foods in young children who are not able to communicate with their parents and express their discomfort or the reason for crying. Furthermore, this relates to the preferences expressed by the mothers in this study related to the setting (home/hospital) for baked milk introduction according to the type of CMA (IgE or non-IgE mediated).

An important finding of this study was that mothers felt nervous about giving their child baked milk and responsible for the symptoms caused by the challenge food. This finding is in line with other research in the field of food allergy that provides evidence that mothers may feel responsible for controlling triggers and for vigilance around their child's diet, and they believe that they may have to deal with unexpected reactions in their child's food allergy (Rouf, White, & Evans, 2012). The main symptoms that mothers in the current study observed during their child's BM-reintroduction were eczema, loose/frequent stools, abdominal pain, vomiting, and sleep disturbances. Previous studies have shown nocturnal awakening of children affected by atopic diseases such as mild to moderate non-IgE mediated gastrointestinal allergies and eczema (Camfferman, Kennedy, Gold, Martin, & Lushington, 2010; Foong et al., 2017). There is a lack of studies investigating the association between food allergies and changes to children's behaviour. An association of food allergy and brain activity, leading to changes in emotion and behaviour, has also been identified (Costa et al, 2012). Pain or discomfort caused by food allergic reaction appears to increase anxiety and stress in children, activating brain areas associated with emotional and affective behaviour responses (Costa et al., 2012). However, there is still a paucity of data in this field and clinical trial interventions are required to determine whether there is truly an association between food allergies and changes in children's behaviour.

The findings of this study indicate an association between mothers' anxiety and potential reactions during BM-reintroduction. Mothers experienced distress related to the prolonged milk ladder process and associated symptoms during baked milk food introduction at home.

These findings are in line with current research evidence that indicates parents/caregivers with food allergic children experience increased anxiety and stress due to the unpredictable risk of allergic reactions and also the risks of undertaking food challenges (Lau et al., 2014; Shaker, Schwartz, & Ferguson, 2017).

As has been reported in section 5.5.1, that mothers in this study were disappointed with the limited information that had received from healthcare services in terms of food labels on the commercial baked milk products, clear instruction regarding homemade recipes and alternative foods choices to meet eating habits of their children or any potential recurrent food allergy). In previous studies parents/caregivers have also expressed dissatisfaction with the labelling of allergic food products.

It has been found that inappropriate labelling or misunderstanding of labels' information is associated with parental anxiety and a risk of accidental exposures and unexpected allergic reactions (Ju, Park, Kwak, & Kim, 2015; Sheth et al., 2010). Parents experience increased anxiety in handing responsibility over to their child such as reading food labels of the products, doing cooking, and taking control of the food allergy themselves (Knibb & Semper, 2013). Another qualitative study identified that participants tended to stick to familiar brands/food to reduce the risk of allergic reactions. They also emphasised the importance of individualised advice, which takes into account psychological and environmental factors that may influence the food choices of parents/carers/patients in food allergy (Sommer, Mackenzie, Venter, & Dean, 2012)**.** The findings of this study are in line with the literature that indicates that allergy healthcare services appear to provide limited help in the reduction of parents' stress and in some cases, parents experience prolonged and increased levels of anxiety, especially when there is risk of a life-threatening anaphylactic reaction (Akeson, Worth, & Sheikh, 2007).

Hence, parents who suffer from anxiety during a milk ladder intervention should be supported by healthcare services for example by providing clear information about how to select appropriate foods. This might also help to alleviate some of the anxiety and stress mothers experience when following a milk ladder for their child. Furthermore, adequate education of parents/caregivers regarding the proper choice or preparation of BM-containing foods may reduce their chances of making a mistake when choosing foods for the milk ladder, and reduce the risk of any potential allergic reaction. Based on the experience of mothers in this study, clear education regarding how to recognise and

distinguish allergy symptoms from other symptoms commonly experienced during infancy and early childhood, such as teething or colds, and how to identify the amount of milk protein in commercial milk-containing products may reduce their anxiety and stress. Indeed, there is evidence that educational intervention providing accurate information regarding how to manage the risk of allergic reactions can reduce anxiety and stress in mothers of children with food allergy (Boyle et al., 2017).

According to the iMAP guidelines the milk ladder can only be followed by infants and children with a mild to moderate non-IgE-mediated CMA. It is not suitable for children with an IgE-mediated CMA that results in severe or immediate type allergic reactions (Venter et al., 2017). Although there is no clear evidence for the use of the milk ladder in IgE-mediated CMA, this process is recommended in children that have only cutaneous symptoms on a mouthful of milk allergen exposure, reducing milk sIgE and SPT and no reaction to milk in the past 6 months (Luyt et al., 2014). Although not a quantitative study, one of the major findings to emerge from this study is that from the total of seven children with IgE-mediated CMA only two children had an initial formal BMC in hospital and thereafter they continued the milk ladder at home. The rest of the children (n=5) did not have any BMC in hospital. Their mothers had been advised to attempt the milk ladder at home. However, mothers with IgE-CMA children expressed their preference for a hospital-BMC because they were distressed about any potential severe reaction occurring at home. Most mothers argued that hospital is the appropriate place for BMC because medical care could be immediately provided if there is a risk of severe reactions. There is still a debate over whether BM-reintroduction can take place at home or if BMC should be performed initially in the hospital and, if it is successful, to then continue with the milk ladder at home. Based on the literature review in chapter 2, the baked milk challenge studies have reported mild to moderate symptoms during BMC (Bartnikas et al., 2012), while other studies have described severe reaction and anaphylaxis in introducing a BM-containing food (Nowak-Wegrzyn et al., 2008). Most researchers in IgE-mediated CMA suggest that (due to the potential risk of severe reactions or anaphylaxis) for these patients a supervised-BMC should be conducted in hospital before adding BM-containing food into the children's diet at home (Dunlop et al., 2018; Leonard, 2016; Mehr et al.,2014). In IgE-mediated CMA there are still inconsistencies between guidelines and controversies in the literature. On one hand, according to HCPs survey, healthcare professionals relied on a clinical assessment combined with a detailed medical history and immune biomarkers evaluation before they decided the

setting of the milk ladder. On the other hand, according to the findings of this study, mothers prefer an initial baked milk challenge for each step of the milk ladder to be conducted in hospital, due to the association of baked milk introduction with severe reactions or anaphylaxis.

Turning to the location of baked milk introduction for mild to moderate non-IgE-mediated CMA, as previously highlighted, the updated international iMAP guidelines recommend home-baked milk introduction and the use of the milk ladder at home for these patients (Venter et al., 2017). In this study, mothers with mild to moderate non-IgE mediated cow's milk allergic children expressed their preference for home-baked milk introduction. This is both because their child is not at risk of severe reaction and because the home environment is more convenient and friendly than hospital. Thus, in this case, the recommended practice and parental preference are in clear agreement.

5.4.3 Living with the milk ladder: making it work

The main findings that will be discussed in this section is related to practicability and acceptability of the milk ladder in real life: (1) The lack of food variety and alternative/equivalent food choices to meet needs of milk allergic children such as palatability and cater concurrent food allergies; (2) Difficulties in identifying suitable baked milk products in the food shops and recognising the appropriate ingredients such as the content of milk protein in the food labels; (3) benefits of the milk ladder based on mothers' perspectives.

The mothers described many ways in which the lack of flexibility in the milk ladder became challenging for them in the face of the day-to-day realities of life with young child. One such challenge the mothers described, was feeding their child unhealthy foods on the milk ladder which conflicted with their desire to raise their children to follow healthy eating behaviours. Mothers expressed their dissatisfaction with the quality of BM-containing foods. They felt some of these products to be unhealthy due to their high sugar and fat content. Some mothers reported that they follow a healthy eating plan for their children, and they did not

include sugary foods such as biscuits, muffins and cakes into the diet of their children. They would have preferred to have been able to offer healthier food options that would contribute to a healthy balanced diet improve their children's nutritional status, and promote their normal growth.

Currently all milk ladders distributed by the hospitals are designed only to induce tolerance in CMA children and they do not prioritise healthy options and the promotion of healthy eating habits during the short period in which it is anticipated the milk ladder is used. In line with this priority, the milk ladders focus only on the amount of milk protein, and cooking time and temperature in order to improve children's tolerance to milk containing foods. However, some mothers described that they had been following the milk ladder for more than 2 years (i.e. in these cases the milk ladder could not be considered a short-term intervention). Children's eating patterns, food preferences, and eating behaviours are developed during the first years of life. Thus, in early childhood, children learn "what", "when" and "how" to eat. Furthermore, the establishment and formulation of food preferences in early life influences nutritional habits in adolescent and adult life (Anzman-Frasca, Ventura, Ehrenberg, & Myers, 2017). According to the review of current literature, however, no research has assessed the growth and development of children who have completed a milk ladder plan. Further research is therefore required to investigate if there is an association between refined sugary and fatty foods of the milk ladder and obesity in children who completed a milk ladder. However, there is certainly the potential that the regular consumption of unhealthy foods as part of the milk ladder may set up unhealthy eating behaviours in the longer-term, which could have an effect into adulthood. Hence, this finding indicates that the milk ladder should be designed in a way that promotes healthy eating habits and includes a variety of healthy and alternative food options especially when it is used long-term so to ensure the normal growth and development of children.

It is interesting that despite all the challenges mothers described with understanding and implementing the milk ladder, and the concerns and anxieties they experienced, those that had persevered with the milk ladder felt that the process was overall worthwhile. The findings of the study highlight that the re-introduction of BM-containing foods had a positive impact in the diet of their children. This was because it increased the variety of their diet, improved their social life by enabling them to eat out without fears, and reduced

the expense incurred buying dairy free products. Mothers highlighted that the incorporation of baked milk foods seems to be safe and well tolerated in general**.**

Hence, according to mothers' perspectives, the milk ladder intervention has a negative impact on the quality of the diet of their child related to the introduction of foods with high sugar and fat content, palatability and appropriateness, and suitability of these foods for concurrent food allergies. These findings suggest that the milk ladder should be improved by offering healthier food options, equivalent or alternative foods in case of palatability issues or concomitant food allergies. A list should be provided including brand names of suitable baked milk products and homemade recipes with all appropriate information regarding the content of milk protein in the baked milk containing food, time and temperature of baking, and alternative/equivalent recipes for homemade baked milk containing foods so that to meet any needs for concurrent food allergies. Despite the practical difficulties, limited healthcare support, and stressful process, the majority of mothers were willing to encourage and support other parents that attempted to implement a milk ladder plan. For them, the positive impact of the milk ladder intervention was associated with a less restricted milk diet and the possibility for children to enjoy foods in birthday parties, eating out, and join family meals.

5.5.4 Implications of the findings for practice

Understanding parental perceptions is of the utmost importance to ensure the validity of any future guidelines regarding the safety of BM-reintroduction in IgE-mediated CMA and improvement of the existing milk ladder for an effective dietary management of mild to moderate-non-IgE mediated CMA. It will also enable more appropriate dietary advice to be given to parents/carers of children who will be candidates for baked milk challenge or a milk ladder plan in the future. Additionally, the information from this study is expected to help allergy services and healthcare professionals to provide optimal care to children during baked milk challenges or gradual reintroduction, and may contribute to standardisation of these procedures/protocols.

5.5.5 Strengths and limitations

The study has a number of strengths. Firstly, this is the first study that provide prospectively collected data regarding the impact of BM-reintroduction at home. The choice of a qualitative research approach to collect and analyse data for this important topic provided valuable information and an in-depth understanding about the needs of mothers that introduce BM-products into their child's diet at home. During the semi-structured interviews mothers felt relaxed to tell the story regarding their BM-reintroduction journey. This allowed mothers to discuss what difficulties they had, what they found useful, what improvements could be applied to help their children to achieve a milk ladder completion, what symptoms their children experienced, and what additional information and support they require. Participants not only answered all the questions but also participated in a productive discussion providing a valuable data. Participants were aware that this was a PhD study and the student researcher is based only in the University of Portsmouth and she does not work as an allergy dietitian in a clinic. It was mentioned to avoid any obliged positive responses and it was also emphasised that their valued honest responses would be appreciated and contribute significantly in this research.

The sample size was representative and homogenous and involved the two types, IgE-mediated and non-IgE-mediated of CMA. It also included participants that had completed or found in different steps of the milk ladder obtaining information for mothers' experience in different stages of the milk ladder. According to this methodological approach, information has been obtained for mothers' experience at the first steps, in the middle and completion of the milk ladder.

There are some limitations of this study that have been considered. Firstly, although both parents were invited via the post advertisement, only mothers consented to participate in the study. However, this was understandable; during our discussion in the interviews it was clear that mothers had taken the responsibility for managing the BM-reintroduction and the dietary care of their milk allergic child. They spent time to prepare/or identify suitable BM-containing foods for the trials, searched and gathered information via social media, and visited the allergy clinic to discuss with HCPs their child condition.

Secondly, this qualitative study had limited diversity, as most qualitative studies. The findings may not be generalizable to other populations because the majority of participants had similar social-economic, educational, and ethnic background. However, interviews have been conducted from across England, representing mothers with access to a variety of levels and types of allergy support. Finally, mothers had a busy schedule (part time/full time work, responsibilities for housekeeping, care of their children/or their siblings) and some interviews were conducted during their lunch break at work, at home late at night when their child was sleeping or in the morning with their child/and siblings, so some unexpected interruptions took place that might influence not only the way of interviewer's questioning but also the interviewee's responding. Hence, some interviews were rescheduled to another time or day suitable for mothers to deal with this issue. Mothers also were asked to contact me via email or phone if they wanted to add any further information.

5.5.6 Conclusion

This study has, for the first time, examined the experience of mothers of children with CMA who had introduced BM-containing foods into their child's diet; any healthcare support that mothers had received during their attempting to introduce these foods; any difficulties that they had during this approach; what symptoms their children experience in the re-introduction of BM-containing foods and the additional support that they would like to have during the BM-reintroduction.

Mothers experienced concerns regarding: the lack of awareness on practical guidelines based on evidence for the BM-reintroduction and training in the recognition of symptoms and management of their child's milk allergy; the confusion caused by the use of different version of milk ladders; the quality of care provided by healthcare services during the BM-reintroduction; the BM-reintroduction at home for children with a milk allergy that results in severe or immediate type allergic reactions known as IgE-mediated CMA; the quality of food options to ensure a healthy balanced diet during the long process of the milk ladder; and the lack of alternative/equivalent food options to cater concomitant food allergies or other preferences of children.

This could be rectified by providing further follow ups in allergy clinic or contact with a HCP by phone or email and a list with a description of common symptoms during BM-

reintroduction and of alternative healthier food options including branded foods to help parents to choose appropriate BM-products. Gradual milk reintroduction is usually a long process and the findings from this qualitative study suggest that mothers need an improved milk ladder based on national recommendations, and local healthcare support, to ensure the efficacy and safety of this process. Mothers need training and education to recognise and distinguish allergy symptoms from other common paediatric conditions such as teething, colic, or colds. Practical guidance on how to read food package labels and identify the suitable products for the gradual milk reintroduction and also a list of recipes with homemade BM-containing foods would ensure mothers follow and complete this process in a proper and safe manner. Finally, further research and clinical trials are required to investigate the appropriate doses of challenge foods in the milk ladder, the time spent for each step during BM-reintroduction for the optimal milk tolerance achievement and the appropriate place for the BM-food trials in IgE-mediated CMA to reduce uncertainty and anxiety of mothers and enhance the safety of this approach.

Chapter 6. A quantitative research study: Can immune markers predict milk challenge outcomes in children with IgE-mediated CMA?

6.1 Overview

Understanding of HCPs' and parents' attitudes could help to improve the efficacy and safety of a baked milk introduction. However, these studies did not provide data regarding the appropriate time of a baked milk introduction. Especially, parental study indicated an emergent need of the appropriate time of baked milk re introduction not only to identify children able to tolerate baked milk foods but also to avoid an unexpected baked milk allergic reaction. According to the systematic review in chapter 2, the evaluation of immune markers may provide information on the appropriate time of a baked milk introduction.

The overall aim of this chapter is to assess if immune markers such as SPT and milk sIgE can predict milk challenge outcomes and if it is possible to establish a cut-off for sIgEs and SPTs to CM, that could predict whether a child with IgE-mediated CMA would react to an oral milk challenge. The literature review in Chapter 2 highlighted a variability of the suggested cut-off values for SPT and milk sIgE to predict milk tolerance, due to different statistical methods, age of participants, different type/form of allergen. Therefore, no clear proposed cut-off for SPT and milk sIgEs for predicting milk challenge outcomes have so far been determined (Caubet et al., 2013; L. Ford et al., 2013). This chapter examines the sensitivity and specificity of the immune markers versus milk challenge outcomes by analysing retrospective data from a prospectively collected cohort of paediatric patients seen in Cincinnati Children's Hospital Medical Centre (CCHMC). Statistical analysis used Receiver Operating Characteristic curves to examine the ability of SPT to fresh milk and milk extract and also IgE to milk allergen, total IgE, IgE casein, and IgE β-lactoglobulin to predict baked or unheated milk challenge outcomes.

6.2 Background

6.2.1 Rationale of the study

Diagnosis of milk allergy is difficult in children. In clinical practice HCPs use several methods that include medical histories, clinical examinations, skin prick and serum-specific immunoglobulin E (IgE) tests, food challenge, and supervised elimination diet in order to properly diagnose CMA (du Toit et al., 2010). The clinical history of symptoms is the cornerstone of the initial diagnosis of CMA and it can be supported with the results of SPT and milk sIgE testing. These allergy tests and oral food challenge are used to describe food sensitization patterns, cross-reactions, and to aid in the diagnosis of a food allergy. These tests may also help to indicate and determine if the milk allergy has been resolved (Luyt et al., 2014). The milk sIgE antibody is produced as an immune response to the allergen, in this case milk proteins, and it can be measured using enzyme-linked immunoassay (ImmunoCAP, Phadia, Upsala, Sweden) which is approved by the Food and Drug Administration (FDA)(Ewan & Coote, 1990).

Current research indicates that both allergy tests, SPT and sIgE, show a good sensitivity (the probability that a test will indicate 'disease' among those with the disease) but a low specificity (the fraction of those without disease who will have a negative test result) (Kianifar, Pourreza, Jabbari Azad, Yousefzadeh, & Masomi, 2016). In other words, a positive SPT/increased milk sIgE levels does not necessarily prove that the test results are clinically relevant. For instance, if the CMA diagnosis is based only on the SPT or sIgE test, children without milk allergy may undergo an unnecessary milk elimination diet. Although an oral milk challenge remains the “gold standard” test for the confirmation of the CMA diagnosis, researchers are currently attempting to identify valuable biomarkers that could confirm the initial diagnosis or predict tolerance to milk. This is due to the fact that an oral milk challenge is an expensive and time-consuming process, and there is a potential risk for severe allergic reactions (Bartnikas et al., 2012).

The review of the literature presented in Chapter 2, (section 2.3) identified a few studies which highlighted that clinical tolerance to baked milk has been associated with negative SPT measurement to baked milk and low levels of serum milk specific IgE levels in IgE-mediated-CMA (Bartnikas et al., 2012; Ford et al., 2013; Nowak-Wegrzyn et al., 2008). They found that

the larger the wheal size of the SPT and the higher the serum milk sIgE levels the greater the possibility that children would react during the milk challenge. More recent studies have drawn conflicting conclusions on the value of skin prick tests, serum IgE, and age, as predictors of oral milk challenge outcome. Rolinck-Werninghaus et al. (2012) found that serum food IgE level, young age, and history of eczema, were predictors of a positive food challenge to milk (Rolinck-Werninghaus, Niggemann, Grabenhenrich, Wahn, & Beyer, 2012). However, there are not yet established SPT/milk sIgE diagnostic cut-off values able to predict milk tolerance without the need to perform a milk challenge.

Published data indicates that serum food sIgE equal or less than 0.35 kUA/L achieves optimal negative predictive value for food allergy and a wheal average diameter of 3 mm is generally considered as a positive SPT cut-off value in clinical practice (García-Ara et al., 2001;Van der Valk et al., 2015). However, no standardised cut-offs values and clear laboratory criteria exist to predict which (and at what age) children are more likely to pass a repeat (re-introduction) milk challenge. According to the reviewed studies in chapter 2, SPT and sIgE cut off values may be influenced by the age, the degree of cooking of the food (raw or baked) and the type of allergen used to perform a SPT (commercial extract vs. raw milk) (Bartnikas et al., 2012; Ford et al., 2013; Turner et al., 2013). Consequently, diagnostic/predictive tools such as SPT and sIgE testing cannot yet replace oral milk challenges due to conflicting published data and this important field needs further research. This study aimed to analyse an existing dataset, which was prospectively collected, and assess if SPT and milk sIgE can predict milk challenge outcomes and if it is possible to establish a cut-off for sIgEs and SPTs to CM, that could predict by itself whether a child would react to an oral milk challenge. Monitoring these allergy tests may provide valuable information for the appropriate time of a gradual milk reintroduction or milk ladder and the timing of milk tolerance development that would be extremely useful in daily clinical practice and might help to avoid an unnecessary milk elimination diet for children.

6.2.2 Aims and objectives

This study had the following aim and objectives:

Aim: To assess immune markers (SPT, milk sIgE) prior to baked or unheated milk challenge and evaluate if there is an association between these immune markers and milk challenge outcomes in children with IgE-mediated CMA.

Objectives: As a part of a larger quantitative study, data was extracted and analysed regarding the immune markers and milk challenge outcomes of children diagnosed with IgE-mediated- CMA

6.3 Method

6.3.1 Study design

A subset of retrospective data that was collected prospectively was analysed, from a larger research project referred to as a "chart review" being coordinated by the CCHMC. This research project has been given approval by CCHMC's Institutional Review Board (appendix 16) and there was a data transfer agreement (appendix 17) between the CCHMC and the University of Portsmouth. The Science Faculty Ethics Committee (SFEC) of the University of Portsmouth recommended that there was no need to undertake a secondary ethical review at University of Portsmouth. The decision letter of the SFEC is attached in the appendix 18.

6.3.2 Data collection

Retrospective chart review of patients undergoing oral food challenges (OFCs) prior to December 1, 2015 was performed by the allergy team in CCHMC. Information including clinical history, dietary history, exam findings, demographics, patient's eczema and other atopic disease status, OFC procedures, OFC results, skin prick testing results, and allergen-specific IgE levels prior to OFCs were collected through database analysis and ongoing review of the electronic medical record. Selective data related to milk challenge outcomes and measurements of immune markers such as Skin Prick Testing (SPT) and milk sIgE CMA (i.e.,

electronic patients' records) were transferred into an encrypted flowsheet database and sent to the University of Portsmouth for further analysis. Names of participants were removed and coded, and anonymous data was used.

6.3.3 Data Safety and Monitoring

To safeguard confidentiality and protected health information (PHI), all research records identified subjects only by study ID number. Paper copies of research records were maintained in a locked file cabinet at the Principal Investigator's office in CCHMC. Logs linking identifying information to research records were kept in a separate file locked in the Principal Investigator's office. Access to the hard copy documents were monitored by the Principal Investigator. The computerized database identified subjects only by study identification number. Computer files were password protected on a secure hospital drive, and only the study personnel had access to the database. Investigators and research staff were instructed not to discuss study subjects except as necessary to carry out data collection. Published materials did not reveal the identities of any patient participating in the study. The database will be maintained until all pertinent research has been concluded and published. All paper records were shredded, and all computer files were deleted. No data obtained from this research study was provided to a pharmaceutical, medical device, or biotech company.

6.4 Data Analysis

All statistical analysis was conducted by using IBM SPSS Statistics for Windows version 22.0. Descriptive statistics and frequencies were used to summarise clinical data regarding:

- the number of children who passed or failed (negative=passed, positive=failed) the milk challenges
- the number of children who had a positive or negative result (positive=milk reactive, negative=milk tolerant) to allergy tests. Negative SPT wheal size was defined as equal or lower than 3mm and positive SPT higher than 3mm. Negative milk sIgE measurements were defined as equal or lower than 0.35KUA/L and positive higher than 0.35 KUA/L. As described in the review of literature in Chapter 2 section 2.5: the cut-off of milk sIgE measurements and SPT wheal size above have been proposed by Nowak-Wegrzyn et al (2008).

All reported values, positive and negatives were included in the analysis and reported statistics. Pearson's chi-square test was used for testing associations between immune markers (SPT and milk sIgE measurements) and milk challenge outcomes. A p value less than 0.05 was considered statistically significant.

The receiver operating characteristic (ROC) curve was used to evaluate the performance of immune tests (SPT to fresh milk, SPT to milk extract, total IgE, IgE casein, IgE β-lactoglobulin) versus milk challenge outcomes. In the Receiver Operating Characteristic (ROC) curve the true positive rate (Sensitivity) is plotted in function of the false positive rate (100-Specificity) for different cut-off points. Each point on the ROC curve represents a sensitivity/specificity pair corresponding to a particular decision threshold.

6.5 Results

6.5.1 Clinical characteristics of biomarkers (OFC, SPTs, milk sIgE)

A total of 277 food challenges conducted over 2 years at CCHMC were reviewed and of these, 191 were performed with milk (n=33) and baked milk (n=158). Out of the 191 challenges, 91 (48%) were positive (failed the OFC), and 100 (52%) were negative (passed the OFC). Challenges were performed with different forms of milk. One hundred and forty-seven (77%) milk challenges were conducted with baked milk, of which 71 (48%) were positive and 76 (52%) negative; 33 (17%) challenges were performed with fresh milk, of which 16 (48%) were positive and 17 (52%) were negative; 11 (6%) challenges were carried out with baked milk containing foods (cake, biscuit, muffin, crab, chocolate, wafer, yogurt), of which 4 (36%) challenges were positive and 7 (64%) were negative. Table 6.1 presents the mean that refers to the average of the participants' immune biomarkers measurements (SPT fresh milk and SPT milk extract wheal sizes, and milk allergen, total IgE, casein-IgE and β-lactoglobulin levels) and summarises the results of immune biomarkers and milk challenge outcomes that were classified as positive (patients react to milk/baked milk) and negative (patients tolerate milk/baked milk. The statistical analysis is presented in appendix 19.

Table 6.1: Clinical characteristics of immune markers and oral milk/baked challenges

Immune Markers N=199	Positive N (%)	Negative N (%)	Mean
Oral Milk challenges	91 (48)	100 (52)	-
SPT -Fresh milk (mm)	53 (28)	138 (72)	2.62[1]
SPT-Milk extract (mm)	85 (45)	106 (55)	4.07[1]
Milk Allergen (KU/l)	109 (52)	82 (43)	8.35[2]
Total milk IgE (KU/l)	93 (49)	98 (51)	89.97[2]
IgE-milk casein (KU/1)	54 (28)	137 (72)	3.36[2]
IgE-β lactoglobulin (KU/l)	45 (25)	146 (76)	2.59[2]

[1] SPT wheal size (mm)
[2]IgE levels (kU/L)

6.5.2 Sensitivity, specificity and ROC curves for immune biomarkers (SPTs, Milk Allergen, milk sIgE) in predicting milk tolerance in CMA children

The validity of immune biomarkers was evaluated by calculating their sensitivity and specificity. **Sensitivity** is the ability of a test to identify patients with the disease and measures how often a test correctly generates a positive result for people who have the condition that is tested (true positive rates). A test with 100% sensitivity correctly identifies all patients with the disease. A test with 80% sensitivity detects 80% of patients with the disease (true positives) but 20% with the disease go undetected (false negatives) (Trevethan, 2017). **Specificity** is the ability of a test to identify patients without the disease and measures how often a test correctly generates negative results for people who do not have the condition that is being tested (true negative rates). A test with 100% specificity correctly identifies all patients without the disease. A test with 80% specificity correctly reports 80% of patients without the disease as test negative (true negatives) but 20% patients without the disease are incorrectly identified as test positive (false positives) (Trevethan, 2017).

AUC- ROC Curve is a performance measurement for classification problem at various thresholds settings. Received operating curve is a curve of probability and illustrates the diagnostic ability of a binary classification system (a pass or fail a test method) as discrimination threshold is varied.

The ROC curve shows the cut-offs between sensitivity and specificity. Classifiers that give curves closer to the top-left corner indicate a better performance. As a baseline, a random classifier is expected to give points lying along the diagonal. The closer the curve comes to the 45-degree diagonal of the ROC curve space, the less accurate the test Trevethan, 2017. AUC is calculated to summarise the performance of each classifier into a single measure and it is a general measure of predictive accuracy of a test Trevethan, 2017. With regard to the interpretation of sensitivity and specificity, and ROC curves, it is important to note:

- There is a trade-off between sensitivity and specificity (any increase of sensitivity is accompanied by a decrease in specificity)
- The closer the curve follows the left-hand border and the top border of the ROC space, the more accurate the diagnostic test.
- The area under the curve measures the test accuracy. An area of 1 represents a perfect test and an area of 0.5 represent a worthless test (i.e. no better than chance).
- The tests are classified based on the areas under the ROC curve as follows (Florkowski et al, 2008):

Areas Under the ROC curve (AUROC)	**Category**
0.9-1.0	**Very good**
0.8-0.9	**Good**
0.7-0.8	**Fair**
0.5-0.7	**Poor**
Less than 0.5	**Fail (i.e no better than chance)**

The majority of milk challenge studies used cut-offs to determine positive tests as being more than 0.35 KU/l milk sIgE tests and 3mm for SPT (Bartnikas et al., 2012; Caubet et al., 2013). This study used the above values to categorise the SPT and immune markers as positive/negative to assess milk challenge outcomes. The statistics analysis was related to the established cut-offs above that are used in practice.

A total of 191 SPTs, and laboratory tests (Milk Allergen, total IgE, casein-IgE and β-lactoglobulin) were analysed against milk challenge outcomes by using the ROC curve. ROC curves were created in SPSS by plotting the true positive rate (sensitivity on the y-axis) of the

immune biomarkers against their false positive (1−specificity on the x-axis) for all possible cut -offs. The graphs of ROC curves for the immune biomarkers are presented in figure 6.1 and 6.2.

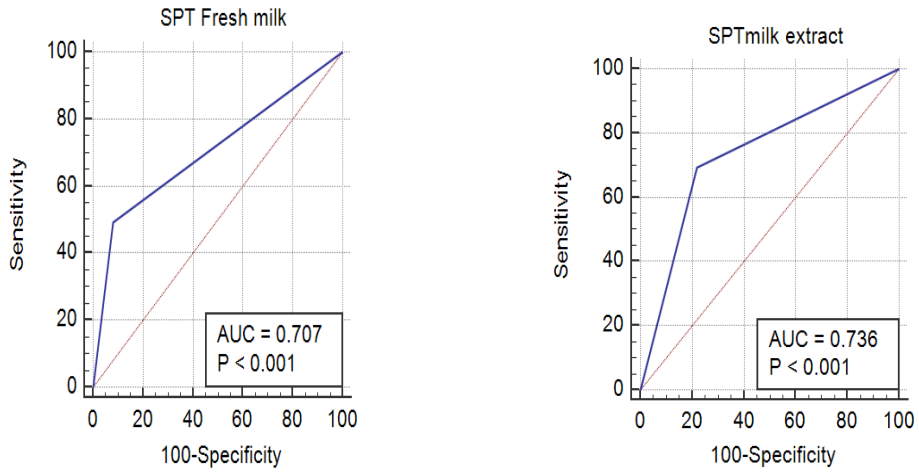


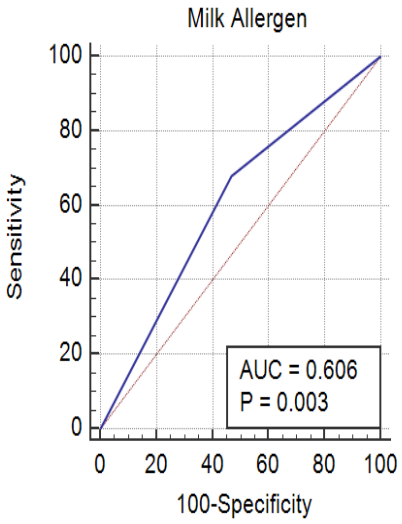
Figure 6.1: Sensitivity, specificity and ROC curves for fresh milk and milk extract SPTs in predicting milk challenge outcomes in children with IgE-mediated CMA (N=191).

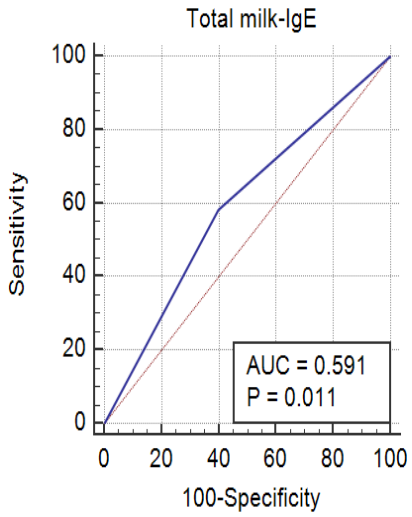
Description of ROC curve plotting related to sensitivity & specificity of SPTs

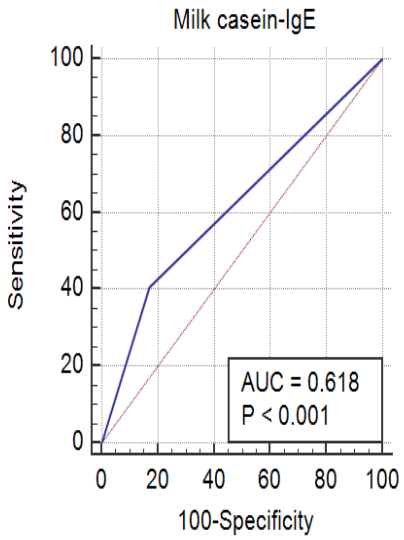
SPT Fresh milk had a low sensitivity with a low percentage of true positives (50%) and a high specificity with a high percentage of true negatives (92%). This means that the SPT to fresh milk correctly detected 50% of those who failed a BMC (i.e. reacted to baked milk); 50% therefore, were undetected. SPT to fresh milk correctly identified 92% of children who passed a food challenge to baked/unheated milk (true negatives) but 8% of children that passed the BMC were incorrectly identified as test positive (false positives**)** (table 6.2). In the ROC curve analysis, the AUC was 0.71 (95% CI=0.63-0.77) and according to the classification of the AUC results mentioned above, this value is categorised as fair indicating that the SPT fresh milk was not a reliable test predictor of BMC outcome (table 6.2).

Similar results were demonstrated by the ROC plotting of SPT milk extract. It had a low sensitivity with a relatively low percentage of true positives (69%) and a high specificity resulting in a moderate percentage of true negatives (78%). This means that the SPT to milk extract correctly identified 69% of those who failed the BMC (i.e. reacted to baked milk) but

31% of those who failed the BMC (i.e. reacted to baked milk) were not detected. The SPT to milk extract correctly detected 78% of children who passed the BMC (i.e. were tolerant to baked/unheated milk; true negatives) but 22% of children that passed the BMC were incorrectly identified as test positive (baked/unheated milk allergic- false positives) (table 6.2). The AUC was 0.73 (95%CI=0.66-0.79) and according to the classification of the AUC results mentioned above, this value was 'fair' and thus indicated that the SPT milk extract was not a reliable test (table 6.2). Statistical analysis of both SPTs is referred to appendix 19.







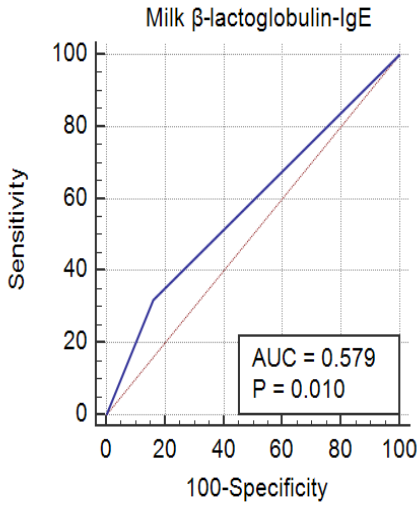


Figure 6.2: Sensitivity, specificity and ROC curves for Milk Allergen, Total-IgE, Milk casein-IgE-, and β-lactoglobulin-IgE in predicting milk challenge outcomes in children with IgE-mediated CMA (n=191)

Description of ROC curve plotting related to sensitivity & specificity of milk allergen, total IgE, casein-IgE, and β-lactoglobulin-IgE

Milk Allergen laboratory test had a high sensitivity with a relatively high percentage of true positives (68%) and a low specificity resulting in low percentage of true negatives (53%). This means that the milk allergen test correctly detected 68% of children who failed the BMC (i.e. reacted to baked/unheated milk; true positives) but 32% children who failed the BMC were undetected by the milk allergen test (false negatives) (table 6.2). Additionally, the milk allergen test correctly detected 53% of children who passed the BMC (i.e. tolerated baked milk; true negatives) but 47% of children who passed the BMC were undetected (false positive). The AUC was 0.61 (95% CI=0.53-0.67) and according to the classification of the AUC results mentioned above, this value is 'poor' and thus indicates that the Milk Allergen laboratory test was not a reliable test due to poor value of AUC (table 6.2).

Total IgE laboratory test had a sensitivity 58% and specificity 60%. This means that the test correctly identified 58% of children that failed the BMC (i.e. reacted to baked milk – true positives) and 42% of children were undetected (false negatives). The total IgE laboratory test correctly detected 60% of children who passed the BMC (i.e. tolerated BM; true negatives) but did not detect 40% of the children who passed (i.e. who did not react to BM; false positives) (table 6.2). The AUC was 0.59 (95% CI=0.51-0.66) and according to the classification of the AUC results mentioned above, this value is 'poor' thus indicating that the total IgE was not a reliable test (table 6.2).

Casein-IgE laboratory test had a sensitivity of 41% and a specificity of 83%. The casein-IgE laboratory test thus correctly detected 41% of children who failed the BMC (i.e. reacted to baked milk; true positives) but 59% of children who failed the BMC were not therefore correctly detected (false negatives). The casein-IgE test thus correctly detected 83% of children who passed the BMC (i.e. tolerated baked/unheated milk; true negatives) but 17% children who passed the BMC were incorrectly identified as test positive (false positives) (table 6.2). The AUC was 0.62 (95% CI=0.55-0.69) and according to the classification of the

AUC results mentioned above, this value indicated that the total IgE was not a reliable test due to poor value of AUC (table 6.2).

Similar results were demonstrated by the ROC plotting of the β-lactoglobulin-IgE laboratory test. It had sensitivity of 31% and a of 84%. The β-lactoglobulin-IgE laboratory test thus correctly detected 31% of children who failed the BMC (i.e. reacted to BM; true positives) and therefore 69% of those children who failed the BMC were undetected (false negatives). The β-lactoglobulin-IgE laboratory test correctly detected 84% of children who passed the BMC (i.e. tolerated BM; true negatives) but 16% children were incorrectly identified as test positive (false positives). The AUC was 0.58 (95% CI=0.51-0.66) and according to the classification of the AUC results mentioned above, this value is 'fair' thus indicating that b-lactoglobulin-IgE is not a reliable test (table 6.2). Statistical analysis of the immune biomarkers is referred to appendix 19. Sensitivity, specificity and AUC of immune biomarkers were summarised in table 6.2.

Positive Predictive Values (PPV) and Negative Predictive Values were assessed using the online MedCalc's software diagnostic test evaluation calculator. PPV is the percentage of the positive outcomes that were correctly classified/identified by the diagnostic test as positive. NPV is the percentage of negative outcomes that were incorrectly specified/identified as positive (False positive values) (table 6.2). Tests for which the PPV/NPV is close to 100 are characterised as reliable diagnostic/predictive tools. As can be seen in Table 6.2, the PPsV for the immune biomarkers results ranged from 56.9% to 84.9% and NPVs from 57.5% to 73.6% and indicated that these tests had not very good reliability.

Table 6.2: Sensitivity, Specificity and AUC of immune biomarkers in predicting milk challenge outcomes in children with CMA

Immune Biomarker tests	Sensitivity (%)	95% CI[1] (%)	Specificity (%)	95% CI[1]	AUC[2]	95% CI[1]	PPV(%)	95% CI[1]	NPV(%)	95% CI[1]
SPT Fresh milk	49.4	38.8-60.1	92.0	84.8-96.5	0.71	0.63-0.77	84.9	73.7-91.9	66.7	61.8-71.2
SPT Milk extract	69.2	58.7-78.5	78.0	68.6-85.7	0.74	0.66-0.79	74.1	65.9-80.9	73.6	66.8-79.4
Milk Allergen	68.1	57.5-77.5	53.0	42.8-63.1	0.61	0.53-0.67	56.9	50.7-62.9	64.6	56.2-72.2

Milk total-IgE	58.2	47.4-68.5	60.0	49.7-69.7	0.59	0.51-0.66	57.0	49.6-64.1	61.2	54.1-67.9
Milk Casein-IgE	40.7	30.5-51.5	83.0	74.2-89.8	0.62	0.55-0.69	68.5	56.9-78.2	60.6	55.9-78.2
Milk β-lactoglobulin-IgE	31.2	22.5-42.5	84.0	75.3-90.6	0.58	0.51-0.66	64.4	51.4-75.7	57.5	53.5-61.5

[1]*Confidence interval*

6.6 Discussion

This study describes a quantitative analysis of the immune markers, SPT wheal sizes and milk sIgE measurements in predicting milk challenge outcomes of milk allergic children. These allergy tests are used by allergists and immunologists to evaluate and manage patients with food allergy. A standard diagnostic approach in CMA uses a patients' clinical history in combination with SPT and milk sIgE values (Jarvinen & Sicherer, 2012). These immune markers are not used only in the diagnosis of CMA, but also in the evaluation of the development of milk tolerance before re-introducing milk or suggesting/conducting a milk challenge in children with CMA (Shek, Soderstrom, Ahlstedt, Beyer, & Sampson, 2004). Few studies have evaluated predictive factors for baked milk challenge outcomes. According to the review of the literature in Chapter 2, previous research indicates that the regular consumption of BM-containing foods may reduce SPT wheal size and milk sIgE values, although reliable predictors of a successful baked milk challenge are not yet well established (Bartnikas et al., 2012; Ford et al., 2013). The food challenge test remains the gold standard in diagnosing CMA and confirming food tolerance. Providing valuable and reliable predictor tools that can identify optimal milk allergic patients able to tolerate BM-containing foods is crucially important because, as has

been mentioned in the previous chapters of this PhD thesis, these children may outgrow their milk allergy faster than those excluding milk completely (Nowak-Wegrzyn & Sampson, 2011).

This study evaluated SPT wheal sizes and milk sIgE values in a retrospective review of children who underwent BMC in the paediatric allergy clinic of CCHMC in the USA who all had IgE

mediated CMA and different forms of milk were used for the challenges. It was found that both SPTs (fresh milk, milk extract) and the immune markers casein-IgE and β-lactoglobulin had a high specificity and low sensitivity. These tests thus largely correctly detected children who failed the BMC (i.e. reacted to baked milk; true positives) but a number of children who failed the BMC were not therefore correctly detected (false negatives) (table 6.2). Their PPV and NPV were generally low and indicated that the immune biomarkers results did not have a good reliability. In addition, the AUC accuracy for SPTs (0.71(95%CI=0.63-0.77) and (0.74 (95%CI=0.66-0.79)) for fresh milk and milk extract respectively) were classified as fair and for the laboratory tests (milk allergen 62 (95%CI=53-67), total IgE 59 (95%CI=51-66), casein 0.62 (95% CI=0.55-0.69), β-lactoglobulin IgE 0.58 (95%CI=0.51-0.66)) as poor. Based on these findings this study cannot provide optimal values in predicting milk challenge outcome. These findings are consistent with previous studies that reported neither milk-sIgE or SPT to commercial milk extracts are reliable in identifying milk tolerance in children with CMA (Nowak-Wegrzyn, 2011). Another study showed that while the levels of milk-sIgE, casein-IgE and wheal sizes of SPT were significantly ($p<0.001$) different between patients who were baked milk-tolerant and baked milk-reactive none of these tests had high sensitivity and specificity (Ford et al., 2013). Bartnikas et al (2012) found that milk SPT wheal size was a better immune marker for BMC outcome compared to milk sIgE levels. Similar results were derived from this research; milk fresh and extract SPT wheal sizes were a more sensitive screening test in predicting milk challenge outcome compared to laboratory tests (milk sIgE levels and milk allergen). According to the review of literature in Chapter 2 (section 2.5), due to the limited evidence regarding the reliability of immune markers, the decision of researchers/physicians about the choice of cut off values relies on individual risk assessment in terms of research setting values. A previous study found that BM-milk tolerant children had smaller wheal sizes (less than 3mm) and lower milk sIgE values (less than 0.35KU/L) compared with BM-reactive children (Nowak-Wegrzyn et al., 2008). However, the Bartnikas et al, 2012 study found that a child with undetectable serum milk sIgE levels and two children with negative milk SPT reacted to a BMC. Another study reported no association between BMC and milk SPT wheal reaction and poor negative predictive value of milk SPT wheal reactions smaller than 7mm (Mehr et al., 2014). In contrast, Faraj et al 2012 and Kim et al 2011 reported a high negative predictive value for a negative SPT reaction. In the Caubet et al., (2013) study it was argued that if a child has milk sIgE with a high negative predictive value (98%) but a low specificity (30%) this test cannot replace the food challenge because some children may react to BM-containing foods. On the other hand, if the negative

predictive value is relatively low (78%) and the specificity of the milk specific IgE test is increased (95%), then more children tolerant to BM-containing foods could be identified (Caubet et al., 2013).

Taken together, the studies above suggest that predictive cut off values may be influenced by the age of patients, the cooking degree, the type of allergen and the population that are used to perform SPT. For instance, it has been indicated from the findings of this study that the use of SPTs (AUC<0.71 and <0.74) may be more accurate in identifying milk allergic children who might be able to tolerate baked or unheated milk than the laboratory tests (AUC less than 0.69). This is in line with Faraj et al, (2012) who argued that SPT Fresh milk may be a reliable marker for identifying milk allergic children who could potentially tolerate baked milk since in their study SPT fresh milk provided a very good NPV of 95%. However, the NPV for SPT fresh milk in this study was not so high at 66.7% (95% CI 61.8%-71.2%). Previous studies have demonstrated that commercial food extracts for SPT have not yet been standardised and they may contain differing concentration of relevant proteins that may confound the results of the studies (Tripodi et al., 2009; Verstege et al., 2005). Similar to SPT, milk sIgE predictive values may be different among populations. Proposed cut off values that have been suggested for milk sIgE vary among studies according to many factors such as patients' ages, geographical differences, different criteria on milk challenge outcome interpretation, test characteristics and methodology, including use of different statistical tests. Therefore, cut off milk sIgE levels that are highlighted from different studies cannot be directly comparable.

Some studies have shown that while larger wheal sizes of SPT and higher levels of serum sIgE are associated with food allergies, these tests cannot provide information about the severity of allergic reactions (Lieberman & Sicherer, 2011) . In addition, in many clinical cases allergic reactions have occurred with negative SPT or sIgE levels (false negatives) because either these tests could not detect the specific allergen or the allergy was non IgE mediated CMA (Sicherer & Sampson, 2014)

Due to the limitations of the tests mentioned above (and as a reflection of the results of this study), oral food challenges are still required either to confirm the CMA diagnosis or the

development of tolerance to milk or milk containing foods. The Double-Blind Placebo-Controlled Food Challenge (DBPCFC) is the gold standard for the diagnosis and detection of the baked/unheated milk tolerance but the disadvantages of these challenges are the potential risk of anaphylactic reactions and also it is a time-consuming and costly procedure (Yanagida, Sato, Asaumi, Ogura, & Ebisawa, 2017). For this reason, a number of other diagnostic/predictive tests are currently under investigation. Promising predictive tools that could potentially identify patients that would be able to tolerate baked milk foods appear to be the Basophil Reactivity Tests (BAT) and the Component-Resolved Diagnostics (CRD). According to the literature review in chapter 2, BAT seems to have promising predictive value as clinically useful test in the reintroduction of baked milk. It has been shown that it can distinguish different phenotypes of children that tolerate baked milk foods while they are still reacting to unheated foods from children who react to both heated and unheated milk (Ford et al., 2013). Current studies have demonstrated that BAT could be useful in cases with unclear results of other diagnostic tests such as SPT or milk sIgE, before the clinician decides to conduct a food challenge

(Santos & Lack, 2016). A recent study found that the BAT had a sensitivity and specificity of 100% (CI: 86%-100% and 68%-100%, respectively) in a small sample IgE-sensitized children (41% of the tested children N=36) (Ruinemans-Koerts et al., 2019). However, the BAT is still not widely used in clinical practice because there is a need to define and validate diagnostic cut-offs values and standardise a protocol of methodology in a larger sample size in multicentre research base.

In recent years the use of a molecular test named CRD (Component Resolved Diagnosis) is internationally accepted for the diagnosis of milk allergy and development of milk tolerance. A number of studies have supported that CRD can improve the specificity of allergy testing. In particular, they have reported that CRD used in CMA has shown greater specificity but lesser sensitivity when compared to traditional SPT and serum specific IgE testing (Bartnikas et al., 2013; D'Urbano et al., 2010). CRD is currently used clinically in peanut allergy but requires further evaluation before being ready for implementation in clinical practice for milk allergy.

However, the findings from these studies have varied, due to different study populations and methods, manners of sensitization, environmental exposures, and degree of sensitization to various food components and CRD cannot yet replace the use of food challenges in the

diagnosis and determination of baked/unheated milk tolerance. Their results should be validated by larger studies in different populations and with a greater age range of participants before applying them in clinical practice. The studies consistently recommend that the initial BM-containing food reintroduction should be based on challenge outcomes and not, yet, on the results of immune markers only because cut off values of SPT and milk sIgE are still under investigation and the current evidence presents inconsistent findings.

To summarise, further multi-centre trials are required that include a larger number of participants that come from different geographic locations with a wider range of population groups and can compare the findings among the centres. Standardised clear diagnostic criteria need to be defined and a confirmation of the CMA diagnosis based on milk challenges is required at the beginning of the clinical trial to avoid children who might have developed tolerance to unheated and heated milk from being included in trials resulting in misleading findings. For instance, in previous studies some participants who passed the baked milk challenges may have been tolerant to unheated milk as well as this had not been tested. It is important also for all parties to be blinded. Further evaluation is required to validate promising tests such as CRD and BAT as reliable predictive tools in the determination of milk tolerance before they are ready for implementation in clinical practice. The majority of immune markers studies provided data for a population with median age from 7 to 9 years. Further studies are required to provide data representative for younger children with CMA (who constitute the majority of CMA patients and who may therefore be considered for baked milk introduction). In the majority of studies, the challenge food was prepared by caregivers and therefore there was no control to ensure that there was equal amounts of milk proteins and temperature of baking of the challenge food was standardised across participants. A standardisation of methods such as food challenge and immune test protocols could ensure that the findings of different studies could be meaningfully compared.

6.6.1 Strengths and limitations

The major strength of this study compared to the previous studies is that CMA diagnosis had been confirmed with milk challenges at the beginning of participants' recruitment. This ensured that those children who had outgrown their milk allergy entirely (and could therefore tolerate both baked and unheated milk) were not included in the study. The failure to do this has been a major issue in previous studies. Another important strength is that this

study utilised a larger sample size (N=191) than the other immune biomarkers' studies that had been systematically reviewed in chapter 2 (a range between N=30 to N=132). Furthermore, this sample size was homogenous because the USA study included a cohort with only milk allergic children. Regarding the statistics methods, this study used a higher level of inferential statistics, a ROC curve analysis to identify optimal cut off values of the immune biomarkers.

An additional strength of this study is that the data was collected as part of a larger study and was stored in a format that was both easy and inexpensive to search. It was an important opportunity to investigate the ability of these immune markers to predict milk challenge outcome in American CMA children, within the limited budget and time constraints of a PhD. Making use of existing data collected over a period of time, using established methods, provided a larger sample with which to address the research question than would otherwise have been possible within the scope of a PhD. Given that the dataset was collected as part of a larger study, this is also a highly efficient use of research data.

This study has some limitations that have been considered. This study used existing data that were not originally collected for the purpose of this research which meant that there may have been some inconsistencies in the methods used. As an example, it was impossible to control the challenge food preparation (e.g. degree of heating) and dose administration, therefore the doses of the challenge food might be over or underestimated. In addition, the decision of the food challenge outcomes, whether a child passed or failed the milk challenge, may differ among clinicians and influence the findings of the study. Different observers may have different opinions about symptoms during the food challenge tests even though a symptom score sheet is advised.

Despite being collected from an established allergy centre and being the largest sample ever used in a study of its kind, a larger sample would have permitted for a more robust analysis of the positive predictive values, sensitivity and specificity of immune markers. However, given the relatively low frequency (in research terms) with which an individual allergy clinic will be conducting milk challenges, it would not be feasible to collect data on this scale without conducting a larger, and more expensive, multi-centre trial. This was beyond the scope of what is possible within this PhD.

Due to the retrospective nature of this study, there is no information available regarding whether the children who passed their milk challenges continued to tolerate BM-containing foods at home or “fresh” milk and dairy products, which would have been useful to provide further context to the findings. Further studies are needed to determine if the findings of this research are replicable in a larger sample. Larger multi-centre trials with a longitudinal prospective design are required to validate whether immune markers can predict/identify patients who are able to tolerate BM-containing foods/fresh milk.

6.6.2 Conclusion

This study was conducted to evaluate if SPT and milk sIgE values can predict milk challenge outcomes and development of tolerance to milk. It found that milk extract and fresh milk SPT were slightly better predictors of milk challenge outcomes compared with milk sIgE levels. However, this study found that both SPT and milk sIgE had poor predictive values for milk challenge outcomes and they cannot currently provide reliable information regarding tolerance development.

The findings of this study are consistent with previous studies which have concluded that there are not currently immune markers that can accurately predict the probability of a positive/negative milk challenge outcome for either fresh or baked milk. Although a milk challenge is an expensive and time-consuming process with a high risk of severe reaction/anaphylaxis, it still remains an invaluable tool in the diagnosis of CMA and determining BM / unheated milk tolerance because of the poor practicability of the evaluated immune markers. Additional studies with a larger sample size, in different population and age groups need to evaluate and validate available immune markers that will be able to predict milk challenge outcomes and identify optimal children for BMC/unheated milk challenge or immunotherapeutic intervention so to reduce the risk of severe reactions or anaphylaxis during this process. Serum specific IgE tests or skin prick tests with a high sensitivity and specificity to predict milk challenge outcomes and tolerance development to both fresh and baked milk are urgently required. This will reduce cost to health care systems and improve patients’ quality of life in terms of their nutritional status, daily living and social life (family meals, nursery, school, eating out).

Chapter 7. General discussion of findings of this PhD

7.1 Overview

This chapter collates the overall findings of the three studies of this PhD research, starting with a concise overview of the rationale and aims of the overall programme of research. The main findings of the three studies are summarized in relation to current literature and the strengths and limitations of this programme of research are considered. Bringing together these studies, in conclusion, the implications of the research findings are explained, and future research needs are outlined.

7.2 Rationale and aims of this thesis

CMA is the most common food allergy in childhood with a prevalence ranges from 1.9 to 4.9% and has an adverse nutritional impact on the children and negative psychosocial impact on the child and their families **(**Fiocchi, et al., 2010; Lau, et al., 2014). Current research indicates that ssuccessful re-introduction of BM-containing foods may accelerate milk allergy resolution and assist in establishing a normal diet, by avoiding unnecessary elimination diets which impair proper nutrient intake and affect socialisation (Meyer et al., 2017). However, there is a limited data on the impact of BMC and milk ladders from either a health care professional or parent perspective.

The rationale for this research was built on the need to explore important aspects if the use of baked milk containing foods used in the management of CMA, which has received little research attention to date. In previous years, the cornerstone of the management of CMA was solely based on the strict avoidance of all CM and food containing milk in the diet of patients (Fiocchi et al., 2010). However recent studies suggest that a majority (75%) of milk allergic children are able to tolerate baked milk products and may outgrow their CMA faster than those children who cannot tolerate these foods (Kim et al., 2011; Nowak-Wegrzyn et al., 2008). It is common practice in the UK and internationally that allergy clinics establish their own BMC and gradual milk introduction (milk ladders) protocols to assist cow's milk allergic children. However, the problems with these are that both BMC and ML protocols have not been clinically validated and standardised and there is also a paucity of research investigating BM-reintroduction in children with IgE and mild to moderate non-IgE- mediated

CMA. Due to the lack of clear guidance regarding BM-reintroduction at home, the decision about a BMC and ML process is based on individual clinical assessment and clinical experience. It is still unclear which patients are optimal candidates for BM-reintroduction due to the lack of reliable indicators for identifying these. In IgE-mediated CMA, there is no universal agreement regarding "when" and "where" (clinical setting vs home) initial BM-containing food introduction should be conducted. This PhD research therefore aimed to fill important gaps in our understanding around: (i) the use of BM-products in clinical practice and the guidelines that are followed by HCPs in the management of IgE and mild to moderate non-IgE CMA; (ii) the re-introduction of BM-containing foods at home and how mothers manage baked milk products into the diet of their children and also what guidance and support they have during this process; (iii) the use of immune markers such as SPT and milk sIgE and their ability to predict if a child passes or fails an oral milk challenge.

Three studies were conducted with the aim of providing urgent answers in these important aspects of cow's milk allergy management using a quantitative and qualitative methodology approach:

1. A survey was carried out to evaluate the attitudes and practices of HCPs on the conduct of BMCs and graded re-introduction of BM/milk ladder in IgE and mild to moderate-non-IgE mediated CMA.
2. Semi-structured interviews were conducted as part of a qualitative study to explore mothers' experience of the re-introduction of BM-milk containing foods into the diet of their children who are diagnosed with IgE and non-IgE-mediated CMA.
3. As a part of a larger quantitative study ('chart review"), data was extracted and analysed to assess immune markers (SPT, milk sIgE) prior to BMCs and evaluate if there is an association between these immune markers and milk challenge outcomes in children with IgE-mediated CMA

Understanding HCP and parents' perceptions is of the utmost importance in terms of the validity of any future guidelines, and this could contribute to an effective CMA dietary management and to a better targeting of dietary advice to parents/carers, whose children will be candidates for a BMC or a ML plan in the future. Additionally, the evidence from this study is expected to help allergy services and healthcare professionals to provide optimal care (further support with follow ups/phone calls/via emails, education in person or using

social media, detailed written advice and guidelines) to children during reintroduction of baked milk containing foods, and contribute to the eventual standardisation of tools such as validated milk ladder protocols and available reliable allergy tests used for this purpose. Validated reliable immune markers such as SPT and milk sIgE would help to provide valuable information for the appropriate time of BM-reintroduction and the timing of milk tolerance development that would be extremely useful in daily clinical practice and might help to avoid an unnecessary milk elimination diet for children.

7.3 Summary and implications of findings

Recently the introduction of baked milk containing foods into the diet of milk allergic children in the form of a milk ladder has become well-recognized as a part of CMA management. However, there is paucity of evidence regarding the efficacy and safety of the protocols such as milk ladders that are used in BM-reintroduction. Cow's milk is one of the most common foods responsible for a fatal anaphylactic reaction in children and this emphasises the need to provide a safe procedure in terms of decisions regarding the place (hospital/home setting) and the appropriate time that BMC should be conducted, supervision by HCPs, and standardised protocols. The following sections present a summary of the research findings in the light of literature.

7.3.1 Findings in relation to the use of BMCs and MLs by HCPs in clinical practice

This research has provided the first research into of the use of BM-containing foods in clinical practice for the management of IgE and non-IgE-mediated CMA by HCPs worldwide. The HCPs who participated in the survey reported that they use a BMC and ML to determine the development of tolerance to BM and unheated milk in milk allergic children who were following an elimination diet. This finding is in line with previous studies that suggest BM-reintroduction should be the next step after a milk free diet not only to determine if children tolerate or still react to baked milk but also to help milk allergic children to develop tolerance more quickly (Dupont, 2013). One of the key findings of this study was that BM-reintroduction may cause severe allergic reactions and anaphylaxis in IgE-mediated CMA. This finding is consistent with previous studies that observed severe reactions and anaphylaxis during an initial BMC in hospital (Kim et al., 2011; Mehr et al., 2014). Hence, these findings emphasize the need to perform an initial BMC under medical supervision in

hospital, prior to introducing BM-containing foods at home in IgE mediated CMA rather than at home, in the case of IgE mediated CMA.

In this study, HCPs reported also that the most common symptoms at home were eczema, abdominal pain and diarrhea. Current studies reported similar symptoms such as abdominal pain, eczema, and pruritus at home, after passing a BMC at hospital(Bartnikas et al., 2012); Mehr et al., 2014) . Children experienced these symptoms one week later after eating the same challenge BM-containing food that they had tolerated in hospital. Hence, these findings indicate the need for home guidance on how to introduce BM-containing foods in the diet of milk allergic children in terms of time and degree of cooking, suitable recipes for homemade BM-foods, and list of appropriate commercial BM products available in food stores.

This PhD research has highlighted discrepancies associated with the time ("when" to introduce BM products) and the appropriate place ("where" to introduce BM-products e.g. hospital/home) of BM-reintroduction. There were HCPs that reported home as a safe place for BMC in IgE mediated CMA and they reported that their decision was based on a detailed clinical assessment combined with allergy tests and the policy of the hospital. Since a majority of children can tolerate BM-products, there is still a debate over whether BM-containing foods could be directly introduced at home or an initial BMC should be performed under medical supervision in hospital. Current guidelines regarding the BM-reintroduction led to more controversies than clarity of the appropriate place for a BMC.

BSACI guidelines recommend in IgE-mediated CMA, home-BM containing food gradual introduction (milk ladders). In children who have had only mild symptoms (only skin symptoms) on noteworthy exposure (e.g. mouthful of fresh milk) and no reaction in the past 6 months, and hospital-BM-containing food introduction in milk allergic children that had experienced previous CMA symptoms affecting breathing and gut or circulation, less severe reaction on trace exposure, severe or poorly managed asthma, multiple or complex allergy, no significant reduction in IgE and their parents are unable to comprehend or adhere to protocol (Luyt et al., 2014). According to the finding of Luyt et al, (2016) the majority (80%) of HCPs (116 respondents) advised home-BMC in the UK current clinical practice (Luyt et al., 2016). This finding is similar with the results of this research that found that 65% of HCP

suggest home-BMC in IgE-mediated CMA. BSACI guidelines and the results above indicate a huge discrepancy among current guidelines that suggested by:

- **WAO Guidelines (2010):** … all dietary interventions and avoidance strategies be reevaluated on a yearly basis, ideally after oral food challenges carried out under medical supervision in hospital…. (Fiocchi, Brozek, et al., 2010)
- **ESPGHAN Guidelines (2012):** So as not to prolong unnecessary dietary restrictions, supervised CMP challenges are required... (Koletzko et al., 2012)
- **MAP Guidelines (2013) and (2017):**…no child with IgE-mediated food allergy should have a challenge in primary care or community settings. All those remaining children diagnosed as mild-moderate non-IgE mediated CMA are suitable for home challenge. (C. Venter, Brown, et al., 2017; C. Venter et al., 2013).

Hence, a national and international agreement of BM-reintroduction guidelines based on current evidence is required to enhance feasibility, effectiveness, and safety of a BMC and milk ladder approach.

7.3.2 Findings in relation to mothers' experiences with the BM-reintroduction

To our knowledge this is the first qualitative study to explore mothers' perceptions, experiences, understanding and level of satisfaction in introducing BM-containing foods into the diet of their children. Considering mothers concerns and suggestions on the use of BM-containing foods, valuable data were obtained regarding influences in mothers' and children's quality of life and psychological condition, allergy symptoms that their children experienced, the level of compliance during a completion of a milk ladder, the level of comprehensiveness of this procedure, the level of support from allergy services, difficulties in terms of food choices and taste, and difficulties with food availability and preparation.

Mothers felt there were important benefits to BM-reintroduction, such as liberalisation of their child's diet to include a variety of food rich in milk protein that would not only improve their child's nutritional status but also their quality of life by allowing children to enjoy food without fears in birthday parties, nursery, eating out and enjoying family meals. However, mothers were disappointed with the quality of food options in the milk ladder. Many steps of the milk ladder included baked milk products with high content of sugar, fat and/or salt

such as cakes, biscuits, muffins, waffles, pizza etc. that they felt should be replaced by healthier food options. In addition, some of the participants had children with a concurrent food allergy or multiple food allergies and they had difficulties complying with the foods in the steps of the milk ladder, because they found it very difficult to identify baked milk products free of other food allergens such as egg or wheat. This study showed that gradual BM-reintroduction is a long process and in many cases its completion may take more than 3 years. Healthier food options and alternative foods that meet the needs of those concomitant/multiple food allergies should be considered by allergy services. Healthier food choices in the milk ladder should be considered in all steps because eating patterns and behaviour are developed and established during the first years of life and influence nutritional habits in adolescent and adult life (Anzman-Frasca et al., 2017).

Mothers with IgE-mediated CMA children expressed their concerns regarding the place of BM-reintroduction, and they wondered why allergy services did not offer a BMC in hospital. They preferred to introduce initially a BM-containing food in hospital because medical care could be immediately provided if any potential severe reaction occurred. The issue regarding the appropriate place and time for a BMC and also the guidelines followed by HCPs before they decide to introduce a BM-containing food have been discussed in the section 6.3.1. Generally, mothers were anxious about the side effects of BM-containing foods, especially those mothers that were in the first steps of the milk ladder. Education and a list of written information regarding the common symptom during BM-reintroduction could help mothers to recognise and distinguish allergy symptoms from common ailments in infancy, such as teething or colds. Current studies have shown that providing appropriate information regarding how to manage the risk of allergic reactions can reduce anxiety and stress in mothers of children with food allergy (Boyle et al., 2017).

Increased anxiety of mothers was also associated with practical issues such as the lack of their knowledge regarding the quantity of milk protein and how they could identify this in the labels of food package or how to assess this protein in homemade foods. They felt that any misunderstanding of the reading of food labels or during the preparation of homemade BM-containing foods could be possible and might therefore cause an unexpected allergic reaction in their child. Hence, mothers need to be educated and informed how to read food labels, how the milk protein is indicated in the food package, what is the appropriate quantity of milk protein in each step of the milk ladder and gain any other practical guidance that

could facilitate the gradual introduction of BM-containing foods and ensure the safety of this process. Mothers expressed also concerns about the different version of milk ladders.

Many studies have highlighted the importance of individualised advising and a formulated nutritional intervention, providing appropriate information regarding dietary plans/dietetic protocols in combination with advice and education, considering psychological and environmental factors that may influence the food choices of parents/carers/patients in food allergy (MacKenzie et al., 2015; Sommer et al., 2012). Hence, a very important finding of this study is the limited communication between mothers and HCPs. Mothers reported that there was not enough time during consultation to discuss in detail the milk ladder process and the written information did not sufficiently address their queries. Consequently, they asked advice or discussed their queries with other parents having children with CMA through social media instead of healthcare services. HCPs should consider a standardised milk ladder so mothers do not become confused and can be confident that provide proper care, based on scientific evidence, is being given to their child. Milk ladders should be validated regarding the number and ranges of steps, doses of BM-containing foods and the frequency that these foods should be given so to ascertain the milk ladder's efficacy, feasibility and safety.

7.3.3 Findings in relation to immune markers such as SPT or milk sIgE and their ability to predict milk allergic children that can tolerate immune markers.

This PhD also evaluated and analysed a dataset related to the predictive ability of immune markers in the development of milk tolerance. This study found that both SPT and milk sIgE had poor predictive values for milk challenge outcomes and it was not feasible to identify PPV for failing milk challenges in terms of SPT wheal sizes and milk sIgE levels. It was not possible to identify specific positive cut off levels for SPT or milk sIgE that could predict a child's reaction to milk.

Current studies present inconsistent data regarding specific cut off values for SPT and milk sIgE that can predict milk tolerance or reactivity. Hence, reliable cut off values for these allergy tests are not established yet (Cuomo et al., 2017). The variability of cut off values for the immune markers is due to heterogenicity of milk challenge protocols that are used from different research centres and the different characteristics of participants in each study. Factors such as the age of participants, allergen (milk extract/fresh milk/baked milk), cooking

degree of the challenge food, different quality of methodology regarding the sample size, statistical methods and variations in the chosen level of predictive value (e.g. 90% vs. 95%) may substantially change the proposed cut-offs.

In a clinical setting, establishment of reliable immune markers such as SPT and milk specific IgE could replace food challenge and reduce the risk of severe reaction or anaphylaxis during challenges. Food challenge is usually an inconvenient and expensive process which may cause distress to young children and their parents as it is lengthy and potentially risky. In contrast, SPT is simple to apply, cheap and relatively safe with a very low risk of anaphylaxis. However, it cannot be conducted if patients use antihistamines, have serious atopic eczema or children are less than 4 years because may they feel distress with this process.

Specific IgE can be measured reliably and reproducibly in the serum of children, regardless of their age, use of antihistamines or severe atopic eczema, and there is not any risk of side effects, making these two tests much more attractive for diagnosis of tolerance to milk containing foods than performing food challenges.

7.4 Overall findings and implications for practice from this programme of research

The findings of individual studies, and implications of these, have been discussed in the relevant chapters and previous sections. However, there are overall findings that can be drawn from examining this programme of research as a whole. These have important implications for the implementation of the milk ladder in practice. Specifically, while the individual studies examined questions about the what, where, how and when of baked milk introduction, taking into account the findings of the full programme of research allows us to discuss these questions more comprehensively. Thus, the following sections will examine what lessons the clinical evidence, healthcare professionals' practices, mothers' experiences and immune biomarkers' evaluation derived from this thesis have for (i) whether and when baked milk should be introduced (ii) where baked milk should be introduced and (iii) how the ongoing process of baked milk introduction should be managed.

7.4.1. 'Whether' and 'when' should baked milk be introduced?

Reflecting on the findings of all three studies in this research, there are some important findings related to the decision of whether and when baked milk should be introduced. The survey of HCPs indicated that it is a common clinical practice to challenge patients with baked milk products and include these foods into their diet in increasing quantities, although there is still very little known about the appropriate time of baked milk introduction and who are the optimal patients to start a milk ladder plan. According to the literature review in chapter 3, section 3.5, immune markers such as SPT and milk specific IgE may be useful predictive tools because they could provide information regarding the appropriate time at which to conduct a milk challenge. Healthcare professionals reported in the survey that they currently combine these tests with a clinical assessment to identify optimal patients for baked milk introduction. However, the immune biomarkers study discussed in chapter 6 showed that SPT and milk specific IgE had poor predictive values for the outcome of food challenges and they are not therefore reliable tests to provide information regarding the appropriate time of baked milk introduction and identify children able to tolerate baked milk products.

These findings therefore indicate that it is not currently viable for healthcare professionals to replace milk challenges with the measurement of immune markers to confirm if a child is tolerant to different forms of milk or milk products. Hence, BMCs and a milk ladder plan remain the most appropriate and reliable predictive tools to confirm the level of milk tolerance in children with CMA. However, according to the literature review (section 3.5) and the findings of the USA study, SPT wheal sizes and milk sIgE levels can be considered with other predictive characteristics such as patients' age and presence of other allergic diseases to identify patients who might be able to tolerate different forms of baked milk foods, and thus make good candidates for BMC and ML. However, the age of a child should be considered not only from clinical aspects but also from practical and ethical perspectives. The qualitative study highlighted that mothers' experience an ethical dilemma in cases where baked milk introduction causes pain and discomfort in a young child un able to communicate and describe his/her condition and feelings. According to the qualitative study, mothers worried about the right time of baked milk introduction and were reluctant to start a milk ladder due to the young age of their child and his/her inability to communicate any symptoms they might be experiencing. The lack of both reliable immune markers and a consensus approach among HCPs to deciding when to introduce baked milk indicate the

need for there to be clear guidance that should take into account parental feelings and preferences when deciding whether to start BM introduction.

Nevertheless, despite the difficulties implementing the ML, mothers highlighted clear benefits to their child and family once their child started to develop tolerance. Milk allergic children could include a variety of milk products that provide a liberation of their diet and improved their nutritional status. It also helped to improve the quality of life of the child and their family.

7.4.2. 'Where' should baked milk be introduced?

From the survey of HCPs presented in Chapter 4, it was evident that HCPs introduce baked milk products in the form of BMC and ML both in hospital and at home. The HCPs' decision about the appropriate place for the introduction of these foods mainly depended upon the type of CMA (IgE-mediated or mild to moderate IgE-mediated) in question. In IgE –mediated CMA, HCPs reported selecting the hospital as the appropriate place for BMC due to the potential severity of allergic reactions. The majority of HCPs reported that home is not a suitable place for BMC in IgE-mediated CMA again due to a risk of severe reactions and anaphylaxis that need medical support. However, there is a contradiction between these findings and those of the qualitative study examining mothers' experiences of introducing baked milk. In particular, there were mothers whose children had IgE-mediated CMA but had followed a milk ladder plan at home without having undergone an initial BMC to determine tolerance in hospital. In these cases, mothers expressed that they would have preferred their child to undertake an initial BMC in hospital, but that their allergy clinic did not offer this. As discussed in the literature review (section 2.4.2) and in this chapter (section 7.3.1), the BMC procedure requires medical supervision because severe allergic reactions are associated with baked milk introduction in IgE mediated - CMA. However, as has also been discussed (section 7.3.1.), there is still a debate related to the appropriate setting for baked milk introduction. As demonstrated by the findings of the qualitative study, this lack of consensus seems to affect mothers' quality of life and their psychological condition. Interestingly, the findings of the HCPs indicated that parents may be anxious about baked milk introduction regardless of the location; HCPs reported parental anxiety whether baked milk was introduced at hospital or at home. The qualitative study of mothers' experiences had a similar finding; in particular, mothers of children with a non-IgE-mediated CMA preferred a home-based baked milk

challenge, whilst mothers of children with an IgE-mediated CMA felt more confident and secure with a hospital-based-MC. Therefore, allergy healthcare services not only should assess and individualise each case, but also consider mothers' preferences in terms of BMC setting (hospital/home) to help them to feel confident with this process and reduce their anxiety and stress.

HCPs also reported instances where children experienced symptoms at home during BMC. Such experiences were also highlighted during the qualitative study. Mothers discussed not only the difficulties they experienced in recognising the symptoms of allergic reactions caused by baked milk introduction but also the lack of guidance by HCPs how to recognise and manage this. In the survey of HCPs, they identified common symptoms children that experience during baked milk introduction in hospital or at home in IgE and non-IgE mediated CMA. Allergy healthcare services could provide a list of these symptoms and a guidance how to manage these symptoms at home so that to help parents to provide better care to their children and control their anxiety caused during baked milk introduction at home.

7.4.3. 'How' should the process of baked milk introduction be managed?

Looking across the three studies, the findings of this programme of research highlight two key considerations for how baked milk introduction should be managed in practice. In particular, there are findings related to the information and support provided not only to mothers, but also to HCPs working with cow's milk allergic patients. Additionally, this research identified issues with the (lack of) flexibility of the milk ladder to accommodate the real-world practicalities of baked milk introduction in young children, that should be reflected upon for practice.

Provision of guidance, information and support during the milk ladder process

A combination of discrepancies regarding the management of the baked milk introduction (as indicated by HCPs' survey) and the lack of a milk ladder protocol based on evidence (as indicated by the literature review in chapter 2) may lead to limited guidance being provided to parents of children that follow a plan of baked milk introduction. HCPs require clearer national and international guidelines and a milk ladder protocol based on evidence in order to provide proper support in CMA patients and their parents during baked milk introduction.

This gap in allergy health services was also highlighted by mothers in the qualitative study of this research. Mothers' anxiety was associated with the low quality of allergy health services in terms of limited parents' education regarding not only the recognition of symptoms and management of allergic reactions in baked milk introduction, but also practical issues such as recipes and labelling of baked milk products, trial food doses and time spent in each step of the milk ladder, alternative or equivalent foods, and unhealthy food options in the milk ladder. HCPs and parents' communication should be improved not only during consultation in the hospital visit, but further support is also required either with regular follow up arrangements or direct contact with a dietitian/allergist via phone or email. Mothers were usually confused about the side effects of a food trial and appeared to need direct guidance on questions such as: Is the symptom related to the reaction to food trial? Is it necessary to postpone the milk ladder for later? When is the appropriate time to re-attempt a food trial after a reaction to a step of the milk ladder? What medicine should I use to relieve the symptoms caused by a food trial? According to the findings of the qualitative study, for all the questions mentioned above, mothers sought to find out answers using social media due to the lack of clarity in the information provided by healthcare services and their communication with HCPs. Social media may be a good source of information. However, health care should not be provided by parental groups in social media. Each case is individual and there are many medical, social, and psychological factors that need to be taken into account for each milk allergic patient.

Thus, HCPs' input is essential throughout the implementation of the milk ladder to ensure the safety of the process and reduce parental anxiety. Until a national/international milk ladder protocol is established based on evidence, the milk ladders that are currently recommended to parents should include further healthy options and alternative/equivalent foods to meet the needs of children for a balanced diet and provide choices in case of other concomitant food allergies. The milk ladder information should include recipes with the appropriate information related to temperature and time of heating, brand names of commercial baked milk products with label information regarding the protein milk content and any other related allergy ingredients so parents and caregivers would be able to identify the appropriate product when shopping. This research has shown that, the implementation and completion of a milk ladder may be a long process for some children. Hence appropriate guidance and healthcare support is required to help parents to follow all the steps of the milk

ladder safely and to reduce their anxiety and concerns improving quality of life of both mothers and children.

Flexibility in the baked milk introduction for real-world implementation

The survey of HCPs highlighted that they need to implement best practice with baked milk introduction in the context of limited resources and access for patients to specialists such as allergists/immunologists, allergy dietitians, psychologists and nurses. However, the qualitative study indicates that there is a need for HCPs to spend more time engaging with parents about the milk ladder. This is both spending time during the parents' initial visit in the clinic clarifying information related to milk ladder completion and encouraging parents to directly contact them by phone or email if they have queries or any other issues during milk ladder implementation. Parents' preferences on the appropriate time of baked milk introduction should be considered by HCPs and regular follow ups should be arranged when it is appropriate.

In non-IgE mediated CMA, the guidelines such as MAP and iMAP guidelines are clear and enhance HCPs' clinical practice in the management of baked milk introduction. However, modification of the milk ladders is required in terms of the introduction of healthy food options, alternative foods, and consideration of foods suitable for those with concurrent food allergies. Milk ladders should also be clearer about how much time should be spent in each step of the milk ladder. The ideal option would be to introduce only one milk ladder based on a national or even international agreement in order to avoid any confusion for parents or misunderstanding during the process. A list with potential symptoms and guidance about their management, also in addition to practical guidance regarding the implementation of the milk ladder should be provided by HCPs. Psychologist input may need to be recommended in cases in which mothers cannot control their anxiety during the milk ladder process. According to the literature review in chapter 2, in IgE mediated CMA, due to inconsistencies between the guidelines and lack of consensus from HCPs regarding the appropriate setting (hospital/home), an initial BMC should be undergone in hospital to test tolerability before a milk ladder plan is recommended at home. The immune biomarkers study described in Chapter 7 suggests that HCPs should not rely solely on a SPT or milk sIgE levels before they decide to introduce baked milk products because these immune biomarkers are not reliable predictors. A careful clinical assessment should be used combining immune markers assessment with a consideration of other predictive indicators

such as age, previous_anaphylaxis to milk or milk products and other concurrent allergic diseases. The strong feeling around the location of baked milk introduction as highlighted by mothers in the qualitative study underline the important of considering parents' preferences regarding the implication of the milk ladder before HCPs recommend a milk ladder plan at home.

7.5 Future research needs

The important findings identified by this programme of research into the introduction of baked milk highlights that milk ladder protocols need to be evaluated and validated in terms of the range of foods in the milk ladder, the doses of foods and the time spent in each step of the milk ladder.

Further understanding is also required on the effect of the wheat and fat matrix in the baked milk containing foods and its importance in the allergenicity of milk. Additionally, clinical trials are needed to investigate the risk of recurrence of milk allergy once tolerance is initially achieved, and to evaluate whether the passing of BMC or completing of milk ladders provides a guarantee for prolonged tolerance to baked milk products or unheated milk. Additional research might also explore how best to provide standardised information for parents regarding the gradual milk introduction at home. For example, in the Allergy UK site parents could be asked what changes they believe that are required in the IMAP milk ladder.

There are a few studies that have investigated immune markers that can predict BMC and milk ladder's outcome. There are not yet proposed cut-off values that can be used to predict tolerance to baked or unheated milk. Larger cohort studies and standardised BMC and milk ladder protocols are required to establish reliable immune markers that could predict reactivity to baked milk and unheated milk. Specific cut off values for SPT and milk sIgE values should be validated by clinical trials before they replace the baked or unheated milk challenge. Further clinical trials are required to assess how many children developed milk tolerance after the milk ladder intervention, how many children react, and what symptoms experienced the CMA children during a milk ladder process at home. Growth and nutritional status of children that follow a milk ladder plan for a long period of time need to be assessed. Further qualitative studies are required to evaluate parental anxiety associated with the long process of a milk ladder plan. Parameters or characteristics that predict advancement of

baked milk intake in different forms, including milk challenge/milk ladder characteristics, immune markers such as IgE levels, age of patients, and presence of other allergic diseases need to be investigated

7.5 Strengths and limitations

This programme of PhD research has a number of key strengths. Firstly, this research included a representative population that provided generalisability of the study findings. The HCPs survey involved participants from different regions of the world and the qualitative study with mothers involved participants from different areas of the UK and they had experienced a different level of allergy services. Secondly, the use of a qualitative approach helped to gain an understanding of mothers' perceptions regarding the efficacy and safety of a milk ladder process. During the interviews, mothers were keen to share their thoughts, feelings and experiences about this process. Although mothers had a busy schedule (work and family commitments), they showed a high interest for the topic and were happy to share with me their experiences related to BM-reintroduction into the diet of their milk allergic child. The children of the interviewees had achieved different steps in the milk ladder, and this gave the possibility to collect information regarding mothers' experiences at the beginning, middle and completion of the milk ladder. Finally, prospective recruitment of participants occurred in all studies. HCPs and mothers' data were prospectively collected, thus limiting recall bias. The data was collected, recorded and analysed by the same researcher to minimise researcher bias.

Methodological issues and limitations have already been described in detail within the previous chapters. However, there are also some limitations to the research which are noteworthy when interpreting the study findings. Firstly, the participants of the USA study were patients seen in Cincinnati Children's Hospital Medical Centre (CCHMC) so may not be representative of the larger allergic population and caution should therefore be applied regarding the generalisability of the study findings. However, a single clinic may be insufficient to recruit a viable sample size required to determine cut-off for SPT and milk sIgEs for predicting baked milk challenge outcomes.

Further multicentre trials could provide a better basis for the subsequent generalisation of findings. However, they are very expensive and complex in terms of co-ordination, quality

control and data management. Secondly, although as an allergy dietitian practised in this area I had the experience to interview and collect information of mothers' experiences, my interpretation and conclusions may differ if the same data was collected and analysed by other researchers. In the qualitative studies, different interpretations and conclusions could be derived based on the same data by other researchers because they are based on the personal characteristics and individual skills of each researcher and the data could be more easily influenced by the researcher's biases and idiosyncrasies (Bengtsson, 2016). This type of research is based on the opinions and judgment rather than statistical results and are unique in itself so it is difficult to replicate the findings. In addition, data was collected from residents living in the UK and the findings cannot necessarily be generalised to a larger population in different part of the world. Finally, the HCPs questionnaire was self-reported and the data may be biased by the time in which a HCP fill out the questionnaire. Usually, HCPs have a very busy schedule and many tasks so the understanding of questions and the quality of their responses could be biased by their limited time to complete the questionnaire. Although, HCPs were involved in the management of CMA, their responses may be influenced by their level of expertise and training and also the policy of the hospital/clinic that they were based on.

Even though a major strength of self-report data is that it comes directly from participants and personalises data and facilitate elaboration of responses, a self-response bias may occur. As it was mentioned above, the respondent may be tired or feel time pressure, or had an understanding issue with the context of the question and further explanation was required. However, self-report data for both research methodologies, a survey and a phone semi-structure interviews, are most suitable when attitudes and beliefs of participants are explored because personal opinions cannot be accessed and observed (Duffett et al., 2012)

7.6 Overall conclusion

This research has provided the first in-depth examination of the use of BM-containing foods in clinical practice for the management of IgE and non-IgE-mediated CMA by HCPs world-wide; the first UK exploration of mothers' experience of introducing BM-containing foods into the diet of their children with IgE and non-IgE mediated CMA; and an analysis of USA data derived from a large scale pediatric clinical allergy setting to provide information

regarding immune markers' ability to predict milk allergic children who might be optimal candidates for a gradual milk reintroduction or a milk ladder. There is growing evidence to suggest that introduction of BM-containing foods may be required to develop milk tolerance since regular consumption of BM-containing foods seems to promote the development of tolerance to milk containing products and unheated milk. Milk is an important source of protein in young children and unnecessary milk restricted diets should be avoided. Adding a variety of BM-containing foods rich in milk protein can broaden children's diets, and has the potential to enhance their nutritional status and quality of life. A baked milk challenge is used to introduce a full portion of BM-containing food in a day in hospital and milk ladder to re-introduce milk containing foods gradually at home over a number of days/weeks/months/years. It is a process that re-introduces milk products gradually in stages, starting with foods that contain only a small amount of well-cooked milk and progressing towards un-cooked milk products and fresh milk. However, there is still limited evidence related to the appropriate time to start baked milk re-introduction in IgE and mild to moderate non-IgE mediated CMA and a debate on "where" (hospital or home) to re-introduce BM-products in IgE-mediated CMA.

This PhD research provided data that indicates that immune markers such as milk extract and fresh milk-SPT and milk sIgE have poor predictive values for milk challenge outcomes and cannot currently provide reliable information regarding the appropriate time of a gradual milk reintroduction or a milk ladder and the development of milk tolerance. These findings are in line with previous studies which had concluded that BMC and unheated milk challenges remain the gold standard in determining baked or unheated milk tolerance because of the poor practicability of the evaluated immune markers. Further research is required to provide reliable cut-offs values for SPT and milk-sIgE to provide valuable information regarding the suitable time for a milk ladder in CMA. Providing reliable immune markers not only contribute in determining of milk tolerance but also in reducing the cost to healthcare service system and in improving milk allergic children's health related-quality of life. Clear guidance is required to certain the feasibility and safety of BM-reintroduction and help HCPs in their decision regarding the appropriate place for this process in IgE-mediated CMA. Standardisation and validation of BMC and milk ladder protocols based on scientific evidence are fundamental to guide HCPs and parents/cares enhancing the safety of this approach. Education and practical guidance in mothers on how to complete the milk ladder could contribute to reduce mothers' anxiety and uncertainty about this procedure and

encourage them to attempt and achieve the optimal milk tolerance achievement for their milk allergic children. Healthcare services support is essential during BM-reintroduction not only to educate parents/cares but also to psychologically support parents ensuring the efficacy and safety of the BM-reintroduction ta home.

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Appendix 18: University of Portsmouth SFEC Ethical Review-USA study

Appendix 19: Statistical analysis of immune biomarkers and milk challenges

Appendix 20: UPR16 form – Ethics Review Checklist

Appendix 1: Research letter "Use of baked milk challenges and milk ladders in clinical practice: a worldwide survey of healthcare professionals"

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RESEARCH LETTER

Use of baked milk challenges and milk ladders in clinical practice: a worldwide survey of healthcare professionals

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To the Editor:

In previous years, the cornerstone of the management of Cow's Milk Allergy (CMA) was solely based on the strict avoidance of all cow's milk (CM) and foods containing CM from the patient's diet [1]. More recently, the importance of baked milk (BM) introduction into the diet of children with CMA has become well-recognized as a part of CMA management. Current research suggests that 75% of children become tolerant to baked/heated forms of CM such as muffin and waffles before they become tolerant to pure/uncooked forms of CM [2]. It has been demonstrated that children who tolerated BM were 28 times more likely to become tolerant to CM compared to those children who were not able to tolerate these foods [3]. Further, the ingestion and incorporation of BM-containing foods into the children's diet seemed to accelerate the resolution of CMA without any adverse effects on children's growth, intestinal permeability, or the severity of coexisting diseases such as asthma, atopic dermatitis and allergic rhinitis [4]. Identification of CMA children who are able to tolerate BM in a variety of forms can also contribute to a liberalized diet that improves the quality of life of patients. This strategy may additionally help to avoid an unnecessary restriction of BM-containing foods or to prevent a severe reaction that could be provoked with the uncooked milk; children reactive to BM appear to be at higher risk of systemic reaction than those children that tolerate BM but still remain allergic to uncooked milk [2, 5].

In the United Kingdom, CM is one of the most common foods responsible for a fatal anaphylactic reaction in children less than 16 years of age, and food allergy is the main cause of a fatal anaphylactic reaction outside the hospital setting [6, 7]. It is difficult to estimate how many people die each year from food anaphylaxis and to confirm the trigger that caused these tragedies. We are aware of a fatal anaphylactic reaction in a child following eating a milk product outside the healthcare

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setting in the United Kingdom, two years ago. This further emphasizes that the decision to challenge at home should not be taken lightly and that there is a risk of severe reactions, even anaphylaxis.

At the time of completion of this survey, few guidelines were available on BM introduction. In the United Kingdom, the MAP Milk Allergy guidelines provide information on the initial diagnosis and the management of mild to moderate non-IgE-mediated CMA in primary care using a milk ladder (ML) [8]. The British Society of Allergy and Clinical Immunology (BSACI) guidance for home introduction of BM-containing foods in IgE-mediated CMA was first published at the end of the survey period [9]. However, there are no studies indicating which patients are optimal

candidates for home introduction of BM. Additionally, there is no universal agreement for the criteria used to classify the severity of allergy symptoms as mild, moderate, or severe and no reliable biomarkers that can be used to indicate the safety of home introduction of milk-containing foods. This study was conducted to explore what guidelines and approaches are currently being used by healthcare professionals (HCPs) across the world and what their experiences have been in introducing a full portion of a BM product as a challenge (BMC) over 1 day or as a more gradual introduction over a number of days/weeks before moving on to other baked milk foods, as per a ML approach.

Methods

A web-based global survey was conducted to capture the views of HCPs using a BMC and/or a ML. An electronic questionnaire (see supporting information) was developed consisting of 23 short questions which could be completed within approximately 15 min. The main sections of the questionnaire were:

• Characteristics of HCPs including: professional background and level of allergy training, practice setting (private/hospital-primary/secondary/tertiary care) and amount of time spent consulting patients with food allergies, percentage of respondents from various countries and guidelines that HCPs considered before they made the decision about the setting of BMC/ML.

- Were these challenges used and where were these challenges performed?
- What was the HCPs' opinion on the safety of homeBMC and ML?
- What was the HCPs' opinion on parental anxiety in BMC/ML process?
- What symptoms were observed?

An initial pilot testing of the survey was carried out on a group of HCPs practising in different parts of the world to ensure the clarity of questions. Ethical permission of the study was provided by the University of Portsmouth Science Faculty Ethics Committee. HCPs involved in the diagnosis and management of CMA were invited to complete the online questionnaire. The participants were identified through international professional organizations [Food Allergy and Intolerance Specialist Group of British Dietetic Association (FAISG), British Society for Allergy and Clinical Immunology (BSACI), American Academy of Asthma Allergy and Immunology (AAAAI), American Dietetic Association (ADA), International Network for Diet and Nutrition in Allergy (INDANA), Allergy Society of South Africa (ALLSA), Australasian Society of Clinical Immunology and Allergy (ASCIA), Dietitians Association of Australia (DAA), Word Allergy Organisation (WAO)].

A reminder email was sent 4 weeks later. The survey was carried out between January and April 2014. The Bristol Online Survey was used to analyse and describe the results. Descriptive statistics were used to summarize data using a combination of tabulation and graphical description. Further statistical analysis data were entered and analysed using IBM (IBM Corp., Armonk, NY, USA) SPSS Statistics for Windows version 22.0. Pearson's chi-square test was used: (i) to determine whether or not there was a statistically significant relationship between the use of BMC and ML; (ii) to test whether or not a statistically significant association exists between the settings (clinical/home) regarding where to perform BMC/ML and the types of CMA (IgEand non-IgE-mediated CMA). A P value less than 0.05 was considered statistically significant.

Results

Characteristics of HCPs study participants

A total of 114 HCPs completed the questionnaire and provided data on their clinical practice regarding using either a BMC and/or a ML in both IgE- and non-IgEmediated CMA. The largest groups of respondents were dietitians with an interest in allergy [52 (46%)] followed by paediatric allergists/immunologists [46 (40%)]. The majority of participants [106 (93%)] indicated that they were involved in the management of IgE- and non-IgEmediated CMA in infancy and childhood. Most of the participants were based in the United Kingdom [56 (49%)], followed by the United States [20 (18%)], and were practicing in secondary care/hospital [52 (39%)] followed by tertiary care/specialist centre [42 (37%)]. HCPs reported that they based their decision regarding BM introduction on an individualized clinical assessment (medical history, SPTs, laboratory tests) and national/regional guidelines. Demographic features of all respondents are shown in Table 1.

Settings (hospital/home) of BMC and ML in children with CMA based on HCPs reports

IgE-mediated CMA. Ninety-three (82%) HCPs indicated that they used BMC to identify patients able to tolerate BM products before tolerating uncooked milk. Fifty two (56%) respondents stated that they conducted these challenges in a clinical setting, 8 (9.0%) in a homebased setting, and 33 (35%) reported using both settings. For ML, 68 (60%) HCPs stated that they used this approach to determine the development of tolerance to BM in different forms. Nineteen (28%) respondents reported that they used the ML approach in a clinical setting, 22 (32%) in a home setting, and 27 (40%) in both settings.

Non-IgE-mediated CMA. Eighty-six (75%) of the respondents stated that they used BMC to

determine the development of tolerance to BM. Eight (9%) HCPs reported that they challenged their patients in a clinical setting, 51 (59%) used home-based challenges, and 27 (31%) reported using both settings. In terms of using the ladder approach (ML), 77 (68%) HCPs reported that they used the ML to identify children able to tolerate a range of BM-containing foods. Three (4%) HCPs reported that they used ML in a clinical setting, 56 (73%) at home, and 18 (23%) reported using both settings. Choice of challenge setting (clinic/home) was statistically significant ($P < 0.001$) associated with the type of CMA (IgE-/non-IgE-mediated). A greater number [52 (56%)] of hospital-based BMC responses were indicated in IgE-mediated CMA, with a larger number [51 (59%)] of home-based BMC in non-IgE-mediated CMA. The decision about where to perform milk ladder challenges (hospital/home) was also statistically significantly ($P < 0.001$) associated with the types of CMA. A considerable number of respondents used ML challenges/introductions at home in both IgE- [22 (32%)] and non-IgE-mediated CMA [56 (73%)].

However, choosing the safest challenge setting remains a difficult decision that concerns not only HCPs, but also carers. The majority of HCPs [71 (62%)] considered the home/outside the clinical setting as a safe place to conduct both BMC and ML in non-IgE-

Table 1. Demographic characteristics of respondents

Characteristics	Options	Respondents (n= 114) (%)
Professional background	Dietitians	52 (46)
	Paediatric Allergist/Allergist/ Immunologist	32 (28)
	Paediatrician with Allergy interest	14 (12)
	Other*	16 (14)
Practice settings	Secondary Care/Hospital	52 (39)
	Tertiary Care/Specialist Centre	42 (37)
	Private Practice	19 (14)
	Primary Care/Community	16 (12)
	Other†	4 (3)
Allergy training	Work-based experiential learning	50 (38)
	Speciality in Allergology/ Immunology	36 (27)
	Postgraduate Dip in Allergy	10 (8)
	MSc in Allergy	8 (6)
	PhD in Allergy	8 (6)
	Postgraduate Cert in Allergy	4 (3)
	Other‡	16 (12)
Food allergy weekly workload	>50%	62 (54)
	<50%	52 (46)
CMA patients seen by HCPs	Infants/children	106 (93) 37
	Adults	(32)
Participated countries	United Kingdom	56 (49)
	North & South America	24 (21)
	Oceania, Africa, Asia	20 (18)
	Europe	14 (12)
Guidelines for hospital BMC	Medical history/SPT/IgE	40 (35)
	Regional/National	24 (21)
	International	12 (11)
	Hospital policy	9 (8)
Guidelines for home-BMC	Medical history/SPT/IgE	39 (34)
	Regional/National	26 (23)
	International	6 (5)
	Hospital policy	5 (4)
Guidelines for hospital-ML	Medical history/SPT/IgE	24 (21)
	Regional/National	26 (23)
	International	4 (4)
	Hospital policy	3 (3)
Guidelines for home-ML	Medical history/SPT/IgE	22 (19)
	Regional/National	30 (26)
	International	4 (3)
	Hospital policy	4 (3)

*Other: Pharmacists, Nutritionists, Allergy Paediatric Nurses, Physicians, General Practitioners.

†Other: Ministry of Health & Welfare, Research.

‡Other: Research, Continued Professional Development (CPD) & Continuing Education (CME) allergy training, Medical resources, completed allergy modules.

mediated CMA because there is no risk of severe reactions, with an exception in the case of severe forms of non-IgE-mediated diseases, such as Food Protein

Induced-Enterocolitis Syndrome (FPIES). The most commonly reported symptoms experienced by the patients were reported as atopic eczema and abdominal pain in both hospital and home-based challenges (Table 2). In terms of IgE-mediated CMA, 30 (26%) respondents stated that the home environment was a safe place to conduct either approach whereas 65 (57%) HCPs considered the home/outside the clinical setting as a non-safe place to conduct both BMC and ML, due to potential severe symptoms (Table 2).

Discussion

The results from this survey indicate that 32 (28%) HCPs reported anaphylaxis in clinic-based BMC and

9 (8%) respondents in clinic-based ML challenges, but none at home. This finding is consistent with previous studies, reporting that some children develop anaphylaxis after ingestion of baked milk-containing foods such as a muffin/pizza in hospital [3, 10]. Mehr et al. identified clinical predictors of reacting to baked cow's milk [10]. These included children with: asthma requiring preventer therapy, IgE-mediated clinical reactions to more than three foods, a prior history of anaphylaxis to cow's milk, and highly atopic children. They indicated that such children should undergo BMCs in hospital. This study by Mehr involved challenges with increasing amounts of BM being introduced over a number of hours over the same day and 27% of children did not pass these oral food challenges [10]. This

Table 2. Summary of the most frequently reported symptoms by the HCPs for BMC & ML

	Clinical setting N (%)		Home N (%)	
Clinical symptoms	BMC	ML	BMC	ML
IgE-mediated CMA				
Urticaria	68 (60)	34 (30)	32 (28)	25 (22)
Vomiting	55 (48)	24 (21)	33 (29)	22 (19)
Angioedema	49 (43)	23 (20)	10 (9)	9 (8)
Runny nose & eyes	49 (43)	19 (17)	13 (9)	12 (11)
Nausea	48 (42)	20 (18)	16 (14)	22 (19)
Wheezing	39 (34)	15 (13)	3 (2)	4 (3)
Diarrhoea	34 (30)	17 (15)	42 (37)	35 (31)
Anaphylaxis	32 (28)	9 (8.0)	–	–
Non-IgE-mediated CMA				
Atopic eczema	20 (18)	8 (7)	43 (38)	37 (32)
Abdominal pain	18 (16)	4 (3)	41 (36)	32 (28)
Diarrhoea	15 (13)	6 (5)	37 (32)	35 (31)
Gastro-oesoph. reflux	12 (11)	4 (3)	32 (28)	30 (26)
Colic	7 (6)	2 (1)	22 (19)	17 (15)
Food aversion	6 (5)	4 (3)	17 (15)	12 (10)
Constipation	5 (4)	4 (3)	43 (38)	29 (25)

N, number of responses.

shows that baked milk challenges carry a risk in those with IgE-mediated CMA, and in a number of children with non-IgE-mediated CMA. The findings from this survey highlight that there were no cases of reported anaphylaxis at home during baked milk challenges. This could be due to successful individual risk assessment and choosing an appropriate setting accordingly. This is supported by the fact that there were more IgEmediated reactions associated with baked milk challenges in the clinical setting compared with the home.

Healthcare systems differ between countries and many European countries may not be able to provide food challenge facilities for all food allergic patients as considerable hospital resources are required [11]. Such challenges are time-consuming with long waiting lists, a major problem in many allergy clinics. For practical reasons, allergy services attempt to address this issue by suggesting initial introduction of BM-containing foods at home based on a clinical assessment. The findings from our survey indicate that the decision regarding the location of challenges in the majority of cases is based on an individualized clinical assessment looking for such specific parameters as: sIgE levels, skin prick tests, severity of previous symptoms, severe forms of nonIgE-mediated CMA such as FPIES or mixed IgE- and non-IgE-mediated CMA. Parents' and children's anxiety is another factor that is considered by HCPs. A considerable number of HCPs reported that the families were anxious when BMC [46 (36%)] or ML [41 (36%)] were conducted either at home or in a clinical setting. A better understanding of parents' perceptions regarding the use of BM forms would be helpful for HCPs to provide optimal care to children during introduction of BMcontaining foods.

This survey has clearly highlighted the lack of international guidance on challenge/gradual introduction of baked cow's milk in a matrix (ML). The World Allergy Organization, European Academy of Allergy and Clinical Immunology and PRACTALL consensus report recommends milk challenges in a safe, well-equipped environment that is supervised by a medical team and have published guidelines for milk oral food challenges but these guidelines do not focus on BMC or a ML process [1, 11, 12]. However, as the survey was completed, the BSACI guidelines were published and data from UK respondents may now be different.

In conclusion, our findings suggest that there are a number of inconsistencies between the use of BMC and ML. We suggest that a larger sample size with a sampling frame inclusive of more countries and clinicians from tertiary, secondary, and primary care should be conducted. There is a clear need for universal guidance, taking into account country-specific needs, on the safe introduction of baked milk products.

Acknowledgements

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Conflict of interest
The authors declare no conflict of interest.
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Supporting Information

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Additional Supporting Information may be found online in the supporting information tab for this article:
Data S1. Questionnaire: Use of baked milk challenges & milk ladders in clinical practice.

Appendix 2: Poster presentation ”Use of baked milk challenges in clinical practice: a worldwide survey”

Athanasopoulou et al. Clinical and Translational Allergy 2015, 5(Suppl 3):P148
http://www.ctajournal.com/content/5/S3/P148

Clinical and Translational Allergy

POSTER PRESENTATION Open Access

Use of baked milk challenges in clinical practice: a worldwide survey

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From Food Allergy and Anaphylaxis Meeting 2014
Dublin, Ireland. 9-11 October 2014

Background
Current research suggests that graded introduction of cow's milk, starting with baked milk, may be used as a prognostic indicator for outgrowing Cow's Milk Allergy (CMA).

Aim
This survey aimed to investigate the attitudes and practice of health professionals on the use of baked milk challenges and graded introduction of milk products in IgE and non-IgE mediated CMA in different regions of the world.

Method
The participants were identified by National and International Health Professional Associations and completed the survey online.

Results
Of 113 participants 51(45.1%) were dietitians, 31(27.4%) Paediatric Allergists and Paediatricians, 15(13.3%) Allergists/Clinical Immunologists, 2(1.9%) General Practitioners, and 14(12.4%) other health scientists. 14 (12.7%) of participants reported that they use baked milk challenges to confirm the diagnosis in CMA and 82(73.2%) to determine tolerance to milk. 52(46%) perform baked milk challenges in IgE mediated CMA in hospital, 8(7.1%) suggest home and 33(29.2%) in both places. In non-IgE mediated CMA, 8(7.1%) conduct these challenges in hospital, 51(45.1%) at home and 27 (23.9%) at both places. 17(15%) of responders stated that they use graded introduction of milk containing foods (Milk Ladder) to diagnose CMA and 80(70.8%) to determine tolerance. 19(16.8%) carry out the Milk Ladder in IgE CMA in hospital, 22(19.5%) use it at home and 27(23.9%) in both places. 3(2.7%) perform graded milk introduction in non-IgE mediated CMA in hospital, 56(49.6%) suggest home, and 18(15.9%) in both places.

Conclusion
Data indicates that most of the health care professionals use baked milk challenges and graded introduction of baked milk foods to determine the development of tolerance to cow's milk in clinical practice.

	IgE mediated CMA		Non-IgE mediated CMA	
	°BMC *N(%)	¨GMI *N (%)	°BMC *N(%)	¨GMI *N (%)
Hospital	52 (46.0)	19 (16.8)	8 (7.1)	3 (2.7)
Home	8 (7.1)	22 (19.5)	51 (45.1)	56 (49.6)
Both	33 (29.2)	27 (23.9)	27 (23.9)	18 (15.9)

* N: number of participants, °BMC: Baked Milk Challenges, ¨GMI: Graded Milk Introduction

Figure 1

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Appendix 3: Poster presentation: "Establishing whether specific immune markers can predict tolerance/reactivity during food challenges to different steps of the milk ladder in children with IgE mediated Cow's Milk Allergy"

Establishing whether specific immune markers can predict tolerance/reactivity during food challenges to different steps of the milk ladder in children with IgE mediated Cow's Milk Allergy (CMA)

Postgraduate student, SHSSW
Yiota Athanasopoulou

Supervisory Team
Dr Carina Venter, Prof Tara Dean, Dr Efrem Eren

BACKGROUND

- Cow's Milk Allergy (CMA) is one of the most common food allergies and may be responsible for up to 13% of fatal food-induced anaphylaxis (1)
- Its prevalence varies between 0.25-4.9% in paediatric population (2,3)
- 75% of children with CMA become tolerant to baked forms of cow's milk such as muffins/cupcakes/waffles (4)
- Baked milk products may accelerate the resolution of CMA in children (5)
- There is no clear evidence on "when" (appropriate timing) and "where" (hospital v home) to start graded milk introduction/milk ladder
- There is limited data on prognostic indicators (immune markers) in the identification of milk tolerance

METHODOLOGY

An exploratory research study utilising a quantitative approach that consists of two phases to meet the following objectives:

Phase 1 - Web-based survey was conducted:

- ❑ To provide data on the use of baked milk challenges and graded milk introduction (milk ladder) in clinical practice
- ❑ To evaluate the opinions of healthcare professionals in conducting baked milk challenges & graded milk introduction/milk ladder in CMA

A short questionnaire consisted of 23 questions was completed online by 113 healthcare professionals. Participants' were identified by the UK & International Health & Professional Organizations.

Phase 2- Experimental study will be carried out:

- ❑ To evaluate the milk ladder in terms of its efficacy and patient safety
- ❑ To establish whether immune markers can predict tolerance/reactivity during food challenges based on a milk ladder

Eligible children 1-5 yrs old with IgE mediated CMA will undergo:

- ✓ Four challenges with foods containing milk as listed in four steps of the milk ladder to determine tolerance to milk over a period of 3-4 months at 4-6 weeks intervals
- ✓ Blood tests (milk specific IgE, Milk Components, Basophil Activation Test)
- ✓ Skin Prick Testing

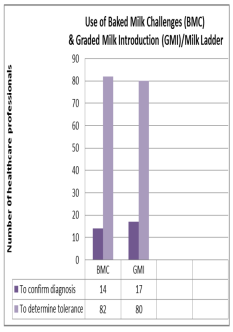
RESULTS - PHASE 1

Table: Use of BMC & GMI in hospital versus home

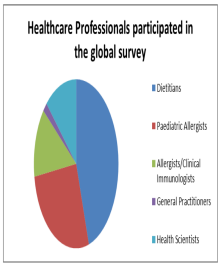
	IgE mediated CMA		Non-IgE mediated CMA	
	*BMC *N(%)	†GMI *N (%)	*BMC *N(%)	†GMI *N (%)
Hospital	52(46.0)	17 (15.9)	8 (7.1)	3 (2.7)
Home	8 (7.1)	21 (19.6)	51(45.1)	56 (49.6)
Both	33 (29.2)	26 (24.3)	27(23.9)	18 (15.9)

*BMC: Baked Milk Challenges, †GMI: Graded Milk Introduction, *N: Number of participants*

Comment: Of 113 health care professionals 21(19.6%) suggest graded introduction of milk /milk ladder at home and 26(24.3%) in both places (home/hospital)



Comment: Histogram graph presents that **14(12.7%)** of participants use baked milk challenges to confirm the diagnosis in CMA and **82(73.2%)** to determine tolerance to milk and also **17(15%)** of responders use graded milk introduction/milk ladder to diagnose CMA and **80(70.8%)** to determine tolerance



RESEARCH PROGRESS TO DATE:

PHASE 1

- Data analysis in progress
- Abstract accepted for European Academy of Allergy & Clinical Immunology (EAACI) Conference – Food Allergy & Anaphylaxis Meeting (FAAM) October 2014
- Preparation of a paper

PHASE 2

- Protocol completed & reviewed
- NHS R&D ethical approval in progress
- Recruitment of participants planned for November 2014

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University of Portsmouth — School of Health Sciences and Social Work

Appendix 4: Poster presentation "Investigating parents' experiences in re-introducing baked milk foods in children with cow's milk allergy"

824 Peanut Allergy Documentation in Electronic Medical Records

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RATIONALE: The electronic medical record (EMR) is an important tool for communication among providers. Use of the EMR for recording peanut allergy (PA) has not been investigated.

METHODS: The EMR of a tertiary care medical center was reviewed for patients with at least one well child primary care visit between January 1 and December 31, 2016. Comparisons were made between children who had PA on their problem list (PL), updated by physicians, vs allergy list (AL) alone, updated by multiple individuals.

RESULTS: Of 884 charts reviewed, 453 charts had PA on PL and 872 had PA on AL. There were no differences in age or gender between children who did and did not have PA on PL. However, children with PA on their PL were more likely to have an allergy referral, evaluation by an allergist, additional food allergies, epinephrine prescriptions, and repeat peanut IgE levels ($p<0.001$ for each). Children with PA on PL were more likely to have peanut allergy confirmed by an allergist than children with PA on AL alone (OR 1.8, $p=.01$). Among children with PA on AL alone, 17% were considered tolerant to peanut, compared to 5% with PA recorded on PL ($p<0.001$). However, PL could not be relied upon to capture PA consistently, as 67% considered definitely PA by the allergist did not have PA on their PL.

CONCLUSIONS: There is inconsistency with how PA is communicated in the EMR, which was associated with significant differences in how PA is confirmed and managed.

825 Barriers preventing Canadian parents of children with food allergy from participating in Oral Food Challenges and possible solutions

Elaine Hsu, MPH[1], Lianne Soller, PhD[1], Christopher Mill, MPH[1], Elissa Michele Abrams, MD, FRCPC[2], and Edmond S. Chan, MD, FRCPC[1]; [1]Division of Allergy and Immunology, Department of Pediatrics, University of British Columbia, BC Children's Hospital, Vancouver, BC, Canada, [2]Section of Allergy and Immunology, Department of Pediatrics, University of Manitoba, Winnipeg, MB, Canada.

RATIONALE: Oral food challenge (OFC) is the gold standard for diagnosing food allergy. However, OFCs are often not performed for various reasons, including resistance from children and parents. We conducted focus groups with parents of food-allergic children to determine barriers preventing them from OFC participation, and potential solutions.

METHODS: Parents of children with physician-diagnosed food allergies (recruited online through a Vancouver area support group) were invited to participate in a two-hour focus group on OFC barriers and solutions. Focus groups were audio-recorded, transcribed, and analyzed to determine the most common barriers and solutions.

RESULTS: Seventeen parents (82.3% female, 76.4% post-secondary educated, 76.4% Caucasian) participated in two focus groups (which had 20 spaces total) in June 2017. Barriers to participating in OFCs included fear of a severe reaction or of needing to use epinephrine, logistical issues such as scheduling, lack of information on what to expect with the procedure itself, as well as lack of understanding of the risks/benefits of an oral challenge regardless of outcome. Solutions included providing more information and education for parents and children, offering psychological support pre- and post-OFC, and conducting OFCs in hospitals instead of community clinics.

CONCLUSIONS: This is the first Canadian study to describe parental OFC barriers and solutions. A limitation was selection of parents from a specific city who join support groups, which might not be representative of other allergy parents. Further research should be conducted to determine the most effective strategies to make OFCs more accessible to families.

826 Investigating Parents Experiences in Re-introducing Baked Milk Foods in Children with Cow's Milk Allergy

Panagiota Athanasopoulou[1], Heather Mackenzie[1,2], Elena Deligianni[3], Tara Dean[1], and Carina Venter[1,4]; [1]School of Health Sciences and Social Work, University of Portsmouth, Portsmouth, [2]Graduate School, University of Portsmouth, Portsmouth, [3]Department of Medicine, Imperial College London, London, [4]Section of Pediatric Allergy & Immunology, Children's Hospital Colorado, School of Medicine, University of Colorado, Colorado.

RATIONALE: Baked milk challenges and milk ladders (ML) are currently recommended to determine the development of tolerance to baked milk in children with IgE and Non-IgE-mediated Cow's Milk Allergy (CMA). However, there is still relatively little known about the parental perceptions of the gradual reintroduction of baked milk. This is the first qualitative study to explore parents' experiences, understanding and their level of satisfaction in using milk in baked goods and the impact/outcomes of these products in the management of their children's milk allergy.

METHODS: Twenty two semi-structured individualised phone interviews were conducted with mothers (UK residents) of children (n=7; IgE-mediated CMA and n=15; Non-IgE-mediated CMA, 15months-8years) who followed or completed a ML (recruited via social networking sites and analysed using thematic analysis).

RESULTS: It emerged that mothers of children following the ML experienced: 1)Confusion when there was not an explicit nationally recommended ML that all healthcare professionals (HCPs) adhere to. 2) Restricted healthcare support due to limited communication between parents and HCPs/lack of counselling or follow ups. 3)Dissatisfaction with the lack of healthy food choices or alternative food options in each stage of the ML. 4)Concerns about how to recognize potential symptoms of a baked milk reaction. 5)Uncertainty regarding the time spent on each stage of the ML. 6)Insecurity around home reintroduction in case of severe reaction.

CONCLUSIONS: Gradual milk reintroduction is usually a long process and mothers need an improved ML based on national recommendation, and local healthcare support, to ensure the efficacy and safety of this process.

MONDAY

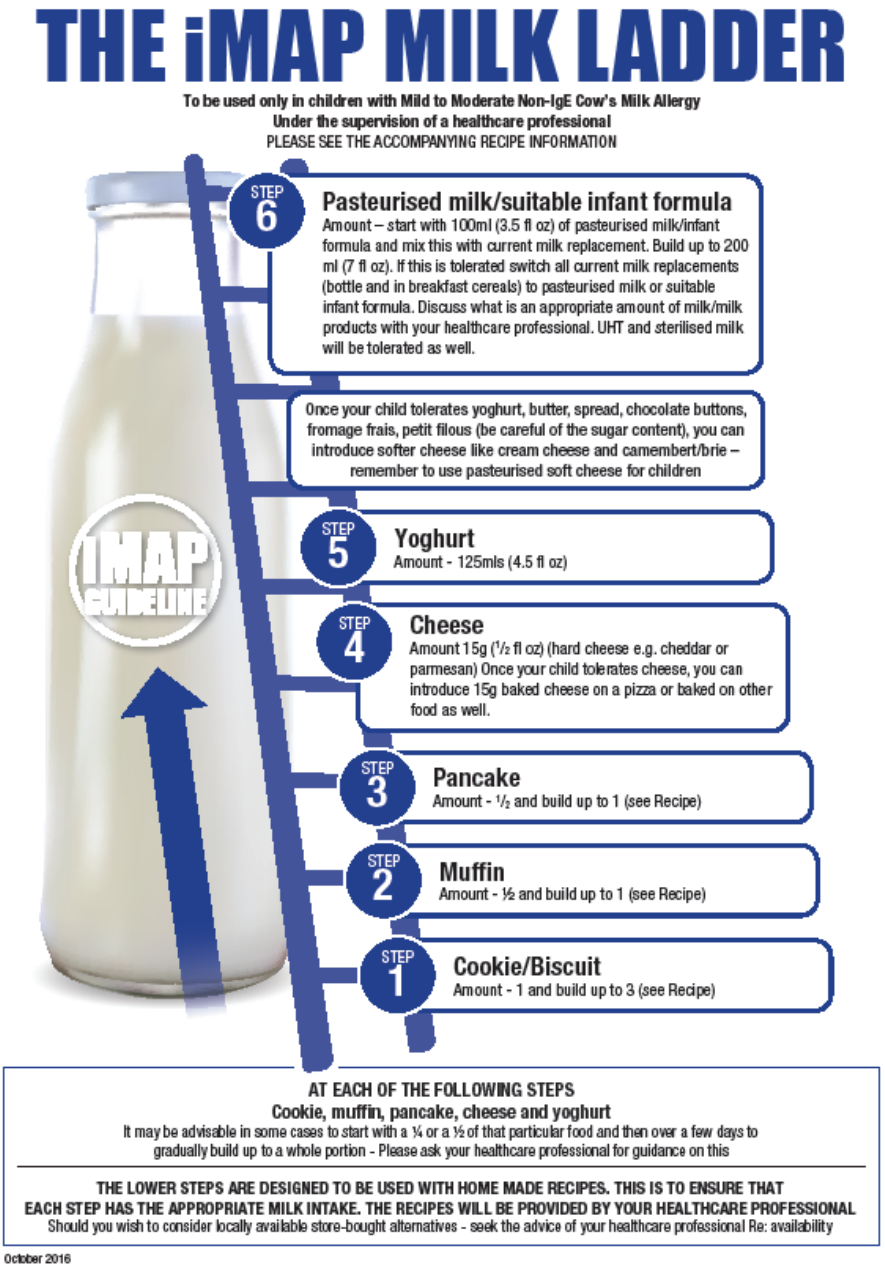
Appendix 5: Used search terms for PubMed and Web of Science

	Search Terms for Pub Med	Search terms for Web of Science
Keywords		
Baked milk challenges	baked[All Fields] AND ("milk, human"[MeSH Terms] OR ("milk"[All Fields] AND "human"[All Fields]) OR "human milk"[All Fields] OR "milk"[All Fields] OR "milk"[MeSH Terms]) AND ("Challenge (Atlanta Ga)"[Journal] OR "challenge"[All Fields])	Baked or milk or challenges
Food challenges protocol	("food"[MeSH Terms] OR "food"[All Fields]) AND ("Plan Parent Chall"[Journal] OR "challenges"[All Fields]) AND protocol[All Fields]	Challenge or protocol or “food protocol”
Food challenges	("food"[MeSH Terms] OR "food"[All Fields]) AND ("Plan Parent Chall"[Journal] OR "challenges"[All Fields])	Food or challenge
Milk tolerance	("milk, human"[MeSH Terms] OR ("milk"[All Fields] AND "human"[All Fields]) OR "human milk"[All Fields] OR "milk"[All Fields] OR "milk"[MeSH Terms]) AND ("immune tolerance"[MeSH Terms] OR ("immune"[All Fields] AND "tolerance"[All Fields]) OR "immune tolerance"[All Fields] OR "tolerance"[All Fields] OR "drug tolerance"[MeSH Terms] OR ("drug"[All Fields] AND "tolerance"[All Fields]) OR "drug tolerance"[All Fields])	Milk or tolerance or “immune tolerance” or immune
Baked milk tolerance	baked[All Fields] AND ("milk, human"[MeSH Terms] OR ("milk"[All Fields] AND "human"[All Fields]) OR "human milk"[All Fields] OR "milk"[All Fields] OR "milk"[MeSH Terms]) AND ("immune tolerance"[MeSH Terms] OR ("immune"[All Fields] AND "tolerance"[All Fields]) OR	Baked or milk or tolerance or immune “immune tolerance”

	"immune tolerance"[All Fields] OR "tolerance"[All Fields] OR "drug tolerance"[MeSH Terms] OR ("drug"[All Fields] AND "tolerance"[All Fields]) OR "drug tolerance"[All Fields])	
Heated milk tolerance	heated[All Fields] AND ("milk, human"[MeSH Terms] OR ("milk"[All Fields] AND "human"[All Fields]) OR "human milk"[All Fields] OR "milk"[All Fields] OR "milk"[MeSH Terms]) AND ("immune tolerance"[MeSH Terms] OR ("immune"[All Fields] AND "tolerance"[All Fields]) OR "immune tolerance"[All Fields] OR "tolerance"[All Fields] OR "drug tolerance"[MeSH Terms] OR ("drug"[All Fields] AND "tolerance"[All Fields]) OR "drug tolerance"[All Fields])	Heated or milk or tolerance or immune or “immune tolerance”
Cow’s milk epitope	cow's[All Fields] AND ("milk, human"[MeSH Terms] OR ("milk"[All Fields] AND "human"[All Fields]) OR "human milk"[All Fields] OR "milk"[All Fields] OR "milk"[MeSH Terms]) AND ("epitopes"[MeSH Terms] OR "epitopes"[All Fields] OR "epitope"[All Fields])	“Cow milk” or milk or epitopes
Cow’s milk	cow's[All Fields] AND ("milk, human"[MeSH Terms] OR ("milk"[All Fields] AND "human"[All Fields]) OR "human milk"[All Fields] OR "milk"[All Fields] OR "milk"[MeSH Terms])	Cow or “cow milk” or milk
Cow’s milk allergy	cow's[All Fields] AND ("milk hypersensitivity"[MeSH Terms] OR ("milk"[All Fields]	cow or“cow milk” or “milk allergy” or “milk hypersensitivity” or allergy or hypersensitivity
Biomarkers and milk tolerance	("biological markers"[MeSH Terms] OR ("biological"[All Fields] AND "markers"[All Fields]) OR "biological markers"[All Fields] OR "biomarkers"[All Fields]) AND ("milk, human"[MeSH Terms] OR ("milk"[All Fields] AND "human"[All Fields]) OR "human	Biomarkers or milk or tolerance or “biological markers” or “milk tolerance”or immune or “immune tolerance”

	milk"[All Fields] OR "milk"[All Fields] OR "milk"[MeSH Terms]) AND ("immune tolerance"[MeSH Terms] OR ("immune"[All Fields] AND "tolerance"[All Fields]) OR "immune tolerance"[All Fields] OR "tolerance"[All Fields] OR "drug tolerance"[MeSH Terms] OR ("drug"[All Fields] AND "tolerance"[All Fields]) OR "drug tolerance"[All Fields])	

Appendix 6: The iMAP guidelines – Milk ladder



Appendix 7: HCP's online questionnaire

Questionnaire: Use of baked milk challenges & milk ladders in clinical practice:

A worldwide survey of healthcare professionals

Section 1: Questions 1-7 refer to your clinical practice

1. What is your professional background?

- GP
- Allergist/Immunologist
- Paediatric Allergist
- Paediatrician with special interest in Allergy
- Dietitian
- Other *(please specify)*:

2. Where do you work?
(select all that apply)

- Primary care/Community
- Secondary care/Hospital
- Tertiary Care/Specialist centre
- Private Practice
- Other *(please specify)*:

3. In which country do you practice?

4. Do you consider yourself to be an Allergy Specialist in your particular health care professional group?

- No
- Yes

If yes, please tick your training: *(select all that apply)*

- Speciality in Allergology/Immunology

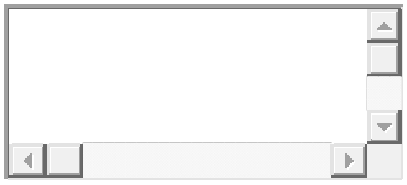
☐ PG Cert in Allergy

☐ PG Dip in Allergy

☐ MSc in Allergy

☐ PhD in Allergy ☐ Practical experience

☐ Other *(please specify)*:



5. What percentage of your weekly work load is spent on dealing with food allergies or intolerances?

○ <50%

○ >50%

6. Do you see infants/children with:

○ IgE-mediated CMA

○ Non-IgE mediated CMA

○ Both

○ Not applicable

7. Do you see adults with:

- IgE-mediated CMA
- Non-IgE mediated CMA
- Both
- Not applicable

Section 2: Questions 8-9 refer to the initial diagnosis of Cow's Milk Allergy

For this survey *Milk Challenge* is the term used for deliberate exposure to Cow's Milk for the purposes of an initial diagnosis of Cow's Milk Allergy usually after a 2-4 week period of avoidance.

8. Do you use baked milk challenges to confirm the *initial* diagnosis of Cow's Milk Allergy?

	Yes	**No**
a. IgE-mediated CMA		
b. Non-IgE mediated CMA		

9. If **No**, which type of milk do you use to confirm the diagnosis of CMA? *(select all that apply)*

- Formula for infants
- Pasteurized milk for infants
- Pasteurized milk for older children /adults
- Other *(please specify)*:

Section 3: Questions 10 to 14 are related to the use of 'Baked Milk Food Challenges' to test the development of tolerance to Cow's Milk

These questions refer to baked milk challenges where one food is introduced in increasing doses usually performed over a period of one day e.g. eating the same cake in increasing amount. These types of challenges are often used to test for resolution of Cow's Milk Allergy

after an extended period of avoidance and usually contain only one food given over a period of a few hours.

10. Do you use baked milk food challenges to determine tolerance in?

	No	**Yes**
a. IgE-mediated CMA	○	○
b. Non-IgE mediated CMA	○	○

11. If **Yes**, what kind of milk containing products do you use for these challenges? *(select all that apply)*

- ☐ Cake
- ☐ Biscuits
- ☐ Waffle
- ☐ Pizza
- ☐ Other *(please specify)*:

12. Where do these challenges take place?

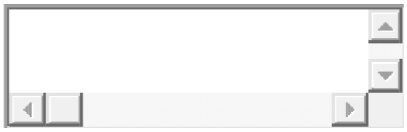
	In hospital/a medical setting	**At home/outside the medical setting**	**Both**	**Not applicable**
a. IgE-mediated CMA	○	○	○	○
b. Non-IgE mediated CMA	○	○	○	○

13. What clinical guidelines do you follow when making the decision to carry out these challenges in hospital or a medical setting versus at home or outside the medical setting?

Please list in the applicable box:

a. In hospital/a medical setting

b. At home/outside the medical setting



14. Please tick the symptoms that your patients have experienced during these challenges in hospital/a medical setting or at home/outside the medical setting? (As many as appropriate)

Hospital/medical setting

IgE- mediated CMA **Non-IgE mediated** ○

Urticaria o Gastro-oesophageal reflux

○ Angioedema o Diarrhoea

○ Runny nose and eyes o Abdominal pain
○ Nausea o Constipation
○ Vomiting o Atopic eczema ○ Diarrhoea
o Colic
○ Wheezing o Food aversion ○ Anaphylaxis
o Blood and/or mucus in the stool
○ Other o Other

Home/outside medical setting

IgE- mediated CMA **Non-IgE mediated**

○ Urticaria o Gastro-oesophageal reflux ○ Angioedema o Diarrhoea ○ Runny nose and eyes o Abdominal pain
○ Nausea o Constipation
○ Vomiting o Atopic eczema ○ Diarrhoea
o Colic
○ Wheezing o Food aversion ○ Anaphylaxis
o Blood and/or mucus in the stool
○ Other o Other

Section 4: Questions 15-18 refer to 'Graded Reintroduction of Milk Containing Food'

Graded reintroduction of milk containing food *is the term used for gradual reintroduction (increasing the amount of milk protein and decreasing the level of heat denaturation) of cow's milk after an extended period of avoidance. This reintroduction is also referred to as the 'Milk Ladder' and usually contains a variety of food such as biscuits, cakes, muffin or pizza, given over a period of days, weeks or months.*

15. Do you use graded reintroduction of milk containing food in determining tolerance to Cow's Milk?

- Yes
- No

16. If **No**, please click *not applicable*
If **Yes**, where does the graded reintroduction of milk containing food take place?

	In hospital/a medical setting	**At home/outside the medical setting**	**Both**	**Not applicable**
a. IgE-mediated CMA	○	○	○	○
b. Non-IgE mediated CMA	○	○	○	○

17. What clinical guidelines do you follow when making the decision to carry out a graded reintroduction of milk containing food in hospital/a medical setting versus at home/outside of the medical setting?

a. In hospital/a medical setting

b. At home/outside the medical setting

18. Please tick the symptoms that your patients have experienced during a graded reintroduction of milk containing food in hospital/ a medical setting or at home/outside the medical setting? (as many as appropriate)

Hospital/medical setting

IgE- mediated CMA **Non-IgE mediated** ○

Urticaria o Gastro-oesophageal reflux

○ Angioedema o Diarrhoea ○ Runny nose

and eyes o Abdominal pain ○ Nausea

o Constipation ○ Vomiting o Atopic

eczema ○ Diarrhoea o Colic

○ Wheezing o Food aversion ○ Anaphylaxis

o Blood and/or mucus in the stools

○ Other o Other

Home/outside medical setting

IgE- mediated CMA **Non-IgE mediated**

○ Urticaria o Gastro-oesophageal reflux

○ Angioedema o Diarrhoea ○ Runny nose

and eyes o Abdominal pain ○ Nausea

o Constipation

○ Vomiting o Atopic eczema ○

Diarrhoea o Colic

○ Wheezing o Food aversion ○ Anaphylaxis

o Blood and/or mucus in the stools

○ Other o Other

Section 5: Questions 19-20 refer to your personal opinions

19. Do you consider the home/outside the medical setting as a safe place to carry out:

	Baked milk food	**Graded reintroduction of milk containing**	**Both**	**Not safe**

	challenges		food	
a. IgE-mediated CMA	○	○	○	○
b. Non-IgE mediated CMA	○	○	○	○

20. Do you think that the parents are more anxious when food challenges take place:

	At hospital/a medical setting	At home/outside the medical setting	Both	Not applicable	Please list any comments:
a. Baked milk food challenges	○	○	○	○	
b. Graded reintroduction of milk containing food	○	○	○	○	

21. Can you send us an example of your protocol on baked milk challenges and graded reintroduction of milk containing food in hospital/at home/both?

○ No

○ Yes If Yes, please email to Yiota.Athanasopoulou@port.ac.uk

Appendix 8: SFEC ethical review – HCP's study

Panagiota Athanasopoulou
School of Health Sciences & Social Work
University of Portsmouth

yiota.athanasopoulou@port.ac.uk **Science Faculty Ethics Committee**
Science Faculty Office
University of Portsmouth
St Michael's Building
White Swan Road
PORTSMOUTH
PO1 2DT

023 9284 3379 ethics-sci@port.ac.uk

6 July 2018
ETHICAL REVIEW – NO ETHICAL OPINION POSSIBLE – BUT INCLUDE STUDY IN PHD THESIS

Study Title: Use of baked milk challenges and milk ladders in clinical practice: a worldwide survey of healthcare professionals

Reference Number: SFEC 2018-070 Date Submitted: 29 June 2018

Thank you for submitting your application to the Science Faculty Ethics Committee (SFEC) for retrospective ethical review. Your application has been reviewed by SFEC and cannot issue a retrospective ethical opinion for work already completed.

However, given the information previously provided and in discussions we have had with your current (new) 1st supervisor, Dr Mackenzie, SFEC was of the view that the failure to submit for ethical review prior to the study starting (6 years ago), was a combination of factors relating to conflicting advice from NHS / HRA regarding this as a user evaluation which would not require ethical review (the same advice would be provided today), and unclear (and unrecorded) advice from the then newly forming SFEC. These clearly indicate that this was a genuine oversight / misunderstanding, and primarily not of your making.

Accordingly, it was the view of SFEC, that this study be included in the PhD thesis, and the reference number given here, should be used when submitting the thesis for examination.

Good luck completing your thesis, at your viva, and for the future.

Dr Jim House
Chair, Science Faculty Ethics Committee

Information:

Dr Heather Mackenzie - PhD 1st Supervisor
Holly Shawyer - Faculty Administrator

Statement of compliance

SFEC is constituted in accordance with the Governance Arrangements set out by the University of Portsmouth

Appendix 9: Invitation email to Health Professional Organisations

School of Health Sciences
University of Portsmouth
James Watson West
2 King Richard I Road
Portsmouth PO1 2FR
United Kingdom

Secretary General
European Academy of Allergy and Clinical Immunology

Friday 21 February 2014

Dear …….

Re: Request regarding survey on baked milk challenges

One of our PhD students, Yiota Athanasopoulou, who is a dietitian by background, at the University of Portsmouth-UK, is investigating the use of baked milk challenges in clinical practice worldwide.

She has developed a very short questionnaire of 22 questions, which should take no longer than 10mins to complete.

I am writing to you to ask if there is a possibility that e.g the European Academy of Allergy and Clinical Immunology can send this e-mail link out to its members or if a link to the survey could be placed on the EAACI website.

We are very happy to share the results of this survey with EAACI in the newsletter or anywhere else where it may be of interest.
https://www.survey.bris.ac.uk/portsmouth/sbmc

Please do not hesitate to contact me, should you have any questions.

Regards

Carina Venter
Secretary: Allied Health Board of the EAACI
Senior Lecturer, University of Portsmouth

Appendix 10: Statistical analysis of HCPs survey

In this appendix is reported the statistics analysis that indicates if there is an association between:

- The type of milk challenges (BMC or ML), that healthcare professionals use to identify the development of baked milk tolerance in IgE or non-IgE mediated CMA and the setting (hospital or home), that a BMC or ML challenge/introduction is performed (chapter 4, 4.2 and 4.3 table)
- Safety of a milk challenge setting in IgE/non-IgE mediated CMA (chapter 4, table 4.4)
- Differences between symptoms and setting of a BMC or ML in IgE and non-IgE CMA (chapter 4, table 4.5 and table 4.6).
- Parental anxiety according to HCPs' reports and the setting of milk challenges (chapter 4, table 4.7)
- Differences across countries regarding the setting of BMC and ML in IgE and non-IgE mediated CMA (chapter 4, table 4.8 and 4.9)

1. **Type (BMC/ML) and setting (hospital/home) that healthcare professionals use to introduce baked milk in children with IgE and non-IgE mediated CMA (chapter 4, 4.2 and 4.3 tab1e)**

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
BMC_Setting * Ige_NonIge_Mediated	182	99.5%	1	0.5%	183	100.0%

BMC_Setting * Ige_NonIge_Mediated Crosstabulation

			Ige_NonIge_Mediated		
			Ige_mediated	NonIge_mediated	Total
BMC_Setting	Hospital	Count	52	8	60
		% within BMC_Setting	86.7%	13.3%	100.0%
		% within Ige_NonIge_Mediated	55.3%	9.1%	33.0%
		% of Total	28.6%	4.4%	33.0%
	Home	Count	8	52	60
		% within BMC_Setting	13.3%	86.7%	100.0%
		% within Ige_NonIge_Mediated	8.5%	59.1%	33.0%
		% of Total	4.4%	28.6%	33.0%

	Both	Count	34	28	62
		% within BMC_Setting	54.8%	45.2%	100.0%
		% within Ige_NonIge_Mediated	36.2%	31.8%	34.1%
		% of Total	18.7%	15.4%	34.1%
Total		Count	94	88	182
		% within BMC_Setting	51.6%	48.4%	100.0%
		% within Ige_NonIge_Mediated	100.0%	100.0%	100.0%
		% of Total	51.6%	48.4%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	64.987[a]	2	.000
Likelihood Ratio	72.497	2	.000
Linear-by-Linear Association	11.827	1	.001
N of Valid Cases	182		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 29.01.

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
ML_Setting * Ige_NonIge_Med	149	81.4%	34	18.6%	183	100.0%

ML_Setting * Ige_NonIge_Med Crosstabulation

			Ige_NonIge_Med		
			Ige_med	NonIge_med	Total
ML_Setting	Hospital	Count	19	3	22
		% within ML_Setting	86.4%	13.6%	100.0%

		% within Ige_NonIge_Med	27.1%	3.8%	14.8%
		% of Total	12.8%	2.0%	14.8%
	Home	Count	23	57	80
		% within ML_Setting	28.7%	71.3%	100.0%
		% within Ige_NonIge_Med	32.9%	72.2%	53.7%
		% of Total	15.4%	38.3%	53.7%
	Both	Count	28	19	47
		% within ML_Setting	59.6%	40.4%	100.0%
		% within Ige_NonIge_Med	40.0%	24.1%	31.5%
		% of Total	18.8%	12.8%	31.5%
Total		Count	70	79	149
		% within ML_Setting	47.0%	53.0%	100.0%
		% within Ige_NonIge_Med	100.0%	100.0%	100.0%
		% of Total	47.0%	53.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	27.366[a]	2	.000
Likelihood Ratio	29.083	2	.000
Linear-by-Linear Association	.464	1	.496
N of Valid Cases	149		

2. Statistical analysis of HCPs responses regarding the safety of milk challenges in IgE and non-IgE CMA(chapter 4, Table 4.4)

MC_SAFETY * Ige_NonIge Crosstabulation

			Ige_NonIge		
			Ige	Non Ige	Total
MC_SAFE	Safe_home	Count	30	72	102

TY		% within MC_SAFETY	29.4%	70.6%	100.0%
		% within Ige_NonIge	31.3%	81.8%	55.4%
		% of Total	16.3%	39.1%	55.4%
	Nonsafe_home	Count	66	16	82
		% within MC_SAFETY	80.5%	19.5%	100.0%
		% within Ige_NonIge	68.8%	18.2%	44.6%
		% of Total	35.9%	8.7%	44.6%
Total		Count	96	88	184
		% within MC_SAFETY	52.2%	47.8%	100.0%
		% within Ige_NonIge	100.0%	100.0%	100.0%
		% of Total	52.2%	47.8%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	47.524[a]	1	.000		
Continuity Correction[b]	45.499	1	.000		
Likelihood Ratio	50.203	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	47.266	1	.000		
N of Valid Cases	184				

3. Differences between symptoms and setting of a BMC or ML in IgE and non-IgE CMA (chapter 4, table 4.5 and 4.6).

- **Association between IgE-mediated CMA symptoms and a BMC setting (hospital/home)**

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent

Symptoms (Ige) * Setting of BMC	537	100.0 %	0	0.0%	537	100.0 %

Symptoms (Ige) * Setting of BMC Crosstabulation

Count

		Setting of BMC		
		Hospital	Home	Total
Symptoms (Ige)	Urticaria	68	33	101
	Vomiting	56	34	90
	Angioedema	50	11	61
	Runny nose and eyes	50	14	64
	Nausea	49	17	66
	Wheezing	40	4	44
	Diarrhoea	35	43	78
	Anaphylaxis	33	0	33
Total		381	156	537

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	57.260[a]	7	.000
Likelihood Ratio	65.993	7	.000
Linear-by-Linear Association	.501	1	.479
N of Valid Cases	537		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 9.59.

- **Association between IgE-mediated CMA symptoms and ML setting (hospital/home)**

Case Processing Summary

Cases					
Valid		Missing		Total	
N	Percent	N	Percent	N	Percent

Symptoms (IgE) * Setting of ML	304	56.6%	233	43.4%	537	100.0%

Symptoms (IgE) * Setting of ML Crosstabulation

Count

		Setting of ML		
		Hospital	Home	Total
Symptoms (IgE)	Urticaria	34	26	60
	Vomiting	25	23	48
	Angioedema	24	10	34
	Runny nose and eyes	20	13	33
	Nausea	21	23	44
	Wheezing	16	5	21
	Diarrhoea	18	36	54
	Anaphylaxis	10	0	10
Total		168	136	304

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	27.185[a]	7	.000
Likelihood Ratio	31.344	7	.000
Linear-by-Linear Association	.743	1	.389
N of Valid Cases	304		

a. 1 cells (6.3%) have expected count less than 5. The minimum expected count is 4.47.

- **Association between non - IgE-mediated CMA symptoms and a BMC setting (hospital/home)**

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Symptoms (Non Ige) * Setting of BMC	331	61.6%	206	38.4%	537	100.0%

Symptoms (Non Ige) * Setting of BMC Crosstabulation

Count

		Setting of BMC		
		Hospital	Home	Total
Symptoms (Non Ige)	Atopic Eczema	20	44	64
	Abdominal pain	19	42	61
	Diarrhoea	16	38	54
	Gastro-oesoph reflux	13	33	46
	Colic	8	23	31
	Food aversion	7	18	25
	Constipation	6	44	50
Total		89	242	331

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	7.105[a]	6	.311
Likelihood Ratio	8.055	6	.234
Linear-by-Linear Association	5.009	1	.025
N of Valid Cases	331		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 6.72.

- **Association between non - IgE-mediated CMA symptoms and ML setting (hospital/home)**

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Symptoms (Non Ige) * Setting of ML	237	44.1%	300	55.9%	537	100.0%

Symptoms (Non Ige) * Setting of ML Crosstabulation

Count

		Setting of ML		
		Hospital	Home	Total
Symptoms (Non Ige)	Atopic Eczema	8	38	46
	Abdominal pain	5	33	38
	Diarrhoea	7	36	43
	Gastro-oesoph reflux	5	31	36
	Colic	3	18	21
	Food aversion	5	13	18
	Constipation	5	30	35
Total		38	199	237

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	2.392[a]	6	.880
Likelihood Ratio	2.146	6	.906
Linear-by-Linear Association	.038	1	.846
N of Valid Cases	237		

a. 2 cells (14.3%) have expected count less than 5. The minimum expected count is 2.89.

4. Statistical analysis of HCPs responses regarding parental anxiety and milk challenge settings (hospital/home) (chapter 4, Table 4.7)

MC_setting_parentalanxiety * MC_type Crosstabulation

			MC_type		
			Baked milk challenge	Milk ladder	Total
MC_setting_parentalanxiety	Hospital	Count	18	17	35
		% within MC_setting_parentalanxiety	51.4%	48.6%	100.0%
		% within MC_type	20.0%	19.8%	19.9%
	Home	Count	25	27	52

		% within MC_setting_parentalanxiety	48.1%	51.9%	100.0%
		% within MC_type	27.8%	31.4%	29.5%
	Both	Count	47	42	89
		% within MC_setting_parentalanxiety	52.8%	47.2%	100.0%
		% within MC_type	52.2%	48.8%	50.6%
Total		Count	90	86	176
		% within MC_setting_parentalanxiety	51.1%	48.9%	100.0%
		% within MC_type	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	.296[a]	2	.863
Likelihood Ratio	.296	2	.863
Linear-by-Linear Association	.071	1	.790
N of Valid Cases	176		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 17.10.

Statistical analysis milk challenge setting preferences across HCPs' country of residence in IgE and non-IgE mediated CMA (table 4.8 and 4.9)

BMC setting preferences across HCPs' country of residence in IgE mediated CMA

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
BMC_Settings_IgE_mediated * Country	96	51.6%	90	48.4%	186	100.0%

BMC_Settings_IgE_mediated * Country Crosstabulation

			Country			
			USA	UK	Rest	Total
BMC_Settings_IgE_mediated	Hospital	Count	10	18	26	54
		% within BMC_Settings_IgE_mediated	18.5%	33.3%	48.1%	100.0%
		% within Country	55.6%	43.9%	70.3%	56.3%
	Home	Count	2	5	1	8
		% within BMC_Settings_IgE_mediated	25.0%	62.5%	12.5%	100.0%
		% within Country	11.1%	12.2%	2.7%	8.3%
	Both	Count	6	18	10	34
		% within BMC_Settings_IgE_mediated	17.6%	52.9%	29.4%	100.0%
		% within Country	33.3%	43.9%	27.0%	35.4%
Total		Count	18	41	37	96
		% within BMC_Settings_IgE_mediated	18.8%	42.7%	38.5%	100.0%
		% within Country	100.0%	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	6.305[a]	4	.178
Likelihood Ratio	6.706	4	.152
Linear-by-Linear Association	1.430	1	.232
N of Valid Cases	96		

a. 3 cells (33.3%) have expected count less than 5. The minimum expected count is 1.50.

BMC setting preferences across HCPs' country of residence in non- IgE mediated CMA

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
BMC_Settings_nonIgE_mediated * Country_	89	47.8%	97	52.2%	186	100.0%

BMC_Settings_nonIgE_mediated * Country_ Crosstabulation

			Country_			
			USA	UK	Rest	Total
BMC_Settings_nonIgE_mediated	Hospital	Count	1	2	5	8
		% within BMC_Settings_nonIgE_mediated	12.5%	25.0%	62.5%	100.0%
		% within Country_	6.3%	4.5%	17.2%	9.0%
	Home	Count	10	26	17	53
		% within BMC_Settings_nonIgE_mediated	18.9%	49.1%	32.1%	100.0%
		% within Country_	62.5%	59.1%	58.6%	59.6%
	Both	Count	5	16	7	28
		% within BMC_Settings_nonIgE_mediated	17.9%	57.1%	25.0%	100.0%
		% within Country_	31.3%	36.4%	24.1%	31.5%
Total		Count	16	44	29	89
		% within BMC_Settings_nonIgE_mediated	18.0%	49.4%	32.6%	100.0%
		% within Country_	100.0%	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	4.157[a]	4	.385
Likelihood Ratio	3.946	4	.413
Linear-by-Linear Association	1.570	1	.210
N of Valid Cases	89		

a. 3 cells (33.3%) have expected count less than 5. The minimum expected count is 1.44.

ML setting preferences across HCPs' country of residence in IgE mediated CMA

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
ML_Settings_IgE_mediated * Country_1	69	37.1%	117	62.9%	186	100.0%

ML_Settings_IgE_mediated * Country_1 Crosstabulation

			Country_1			
			USA	UK	Rest	Total
ML_Settings_IgE_mediated	Hospital	Count	5	6	8	19
		% within ML_Settings_IgE_mediated	26.3%	31.6%	42.1%	100.0%

		% within Country_1	35.7%	19.4%	33.3%	27.5%
	Home	Count	7	7	8	22
		% within ML_Settings_IgE_mediated	31.8%	31.8%	36.4%	100.0%
		% within Country_1	50.0%	22.6%	33.3%	31.9%
	Both	Count	2	18	8	28
		% within ML_Settings_IgE_mediated	7.1%	64.3%	28.6%	100.0%
		% within Country_1	14.3%	58.1%	33.3%	40.6%
Total		Count	14	31	24	69
		% within ML_Settings_IgE_mediated	20.3%	44.9%	34.8%	100.0%
		% within Country_1	100.0%	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	8.716[a]	4	.069
Likelihood Ratio	9.181	4	.057
Linear-by-Linear Association	.117	1	.733
N of Valid Cases	69		

a. 2 cells (22.2%) have expected count less than 5. The minimum expected count is 3.86.

ML setting preferences across HCPs' country of residence in non- IgE mediated CMA

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
ML_Settings_NonIgE_mediated * Country_2	80	43.0%	106	57.0%	186	100.0%

ML_Settings_NonIgE_mediated * Country_2 Crosstabulation

			Country_2			
			USA	UK	Rest	Total
ML_Settings_NonIgE_mediated	Hospital	Count	1	1	1	3
		% within ML_Settings_NonIgE_mediated	33.3%	33.3%	33.3%	100.0%
		% within Country_2	6.7%	2.6%	3.8%	3.8%
	Home	Count	11	25	22	58
		% within ML_Settings_NonIgE_mediated	19.0%	43.1%	37.9%	100.0%
		% within Country_2	73.3%	64.1%	84.6%	72.5%
	Both	Count	3	13	3	19
		% within ML_Settings_NonIgE_mediated	15.8%	68.4%	15.8%	100.0%
		% within Country_2	20.0%	33.3%	11.5%	23.8%
Total		Count	15	39	26	80
		% within ML_Settings_NonIgE_mediated	18.8%	48.8%	32.5%	100.0%
		% within Country_2	100.0%	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	4.624[a]	4	.328
Likelihood Ratio	4.788	4	.310

Linear-by-Linear Association	.515	1	.473
N of Valid Cases	80		

a. 4 cells (44.4%) have expected count less than 5. The minimum expected count is .56.

Appendix 11: Advertisement poster for the qualitative study

Reference No: SFEC 2016-046 Date: 08/03/16 Version: No1

COWS MILK ALLERGY

Milk Ladder study

The milk ladder is a process that is used to test if a child with Cow's Milk Allergy is able to tolerate small amounts of milk that has been highly heated in baked foods such as milk malted biscuits, cakes, muffins, pizza.

The University of Portsmouth is running a research study to find out the impact of a milk ladder plan into children's diet at the time that they are still allergic to "raw" milk. The aim is to understand the parents' perceptions and opinions on the usefulness of the milk ladder process for the dietetic management of cow's milk allergy.

Have you followed a milk ladder plan to test your child's milk tolerance

Then you might be interested in taking part in a skype/or telephone interview that will last approximately 30 minutes. The researcher, Yiota Athanasopoulou is interviewing parents of children diagnosed with cow's milk allergy in order to collect information about their views on milk ladder.

If you are interested in taking part, please email Yiota and she will send you an invitation leaflet with further details about the study. The contact details are:

Ms Yiota Athanasopoulou
PhD Researcher
School of Health Sciences
University of Portsmouth
Email:yiota.athanasopoulou@port.ac.uk

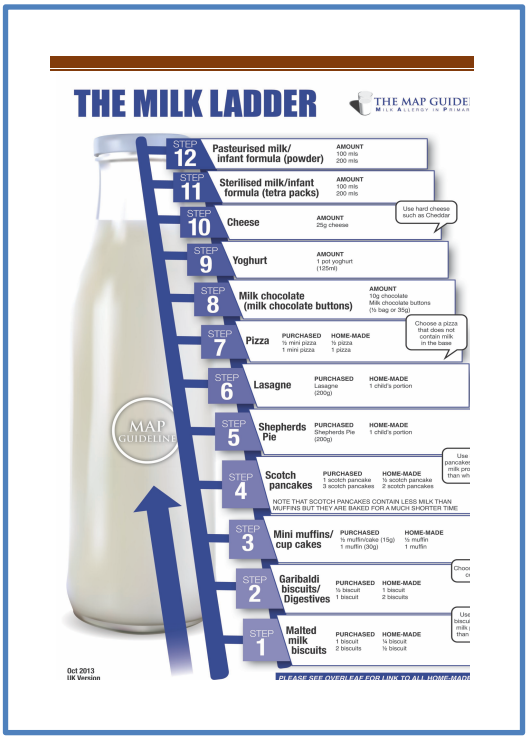
1

Appendix 12: Participant information Sheet

Reference No: SFEC 2016-046 *8/03/2016* *Version 1*

Participant Information Sheet

Title of Study: *Investigating parents' experiences in re-introducing baked milk foods in children with CMA in UK- A qualitative study*



We would like to invite you to take part in our study research, looking at the experience of parents regarding home-use of baked milk foods (e.g. malted biscuits, cakes, muffins) or a milk ladder plan in their children who have still allergy to whole milk. As a parent/carer your opinions and thoughts would be of great values to us. Before you make a decision, it is important to understand why this research is being done, and what it would involve for you. If you wish, you can talk with others about this study before deciding whether to take part. Please read this leaflet carefully and do not hesitate to contact the lead researcher if you need further information about this study.

What is this project all about?

A milk ladder is a process that is used to test if your child is able to tolerate small amounts of milk that has been highly heated in baked foods such as milk malted biscuits, cakes, muffins, etc. However, we do not have enough evidence on the use of milk ladders in the dietary management of children with cow's milk allergy at home. We are interested in finding out what is the impact or outcome of a milk ladder into children's diet at the time that they are still allergic to "raw" milk. Parents will be interviewed to express their personal and their child's experience about the usefulness of a milk ladder plan in the management of cow's milk allergy.

Contact details:

PhD Researcher:

Yiota.Athanasopoulou@port.ac.uk

Why have I been invited to take part?

You are being asked to take part in this project because you have used a milk ladder plan/reintroduced baked milk containing foods into your child's diet.

What would taking part involve?

If you decide to take part in this study you will be asked to be interviewed ONCE through telephone/skype call at a date and time of your convenience. We anticipate the interview to last 30 mins, depending on how much you wish to share. The interview will be anonymised and audio-recorded. The questions that you will be asked are reported at the last section of this informed sheet.

Are there any expenses?

You will not be charged for telephone/skype call. As a gesture of thanks every participant will get a

£10 gift voucher for their time commitment and contribution towards this research study.

Do I have to take part?
It is completely **up to you** to decide whether to join the study. If you decide not to take part, this will have no affect at all on you or on your child's care in any way. If you decide to take part and later change your mind, you can withdraw any time.

What will happen if I take part?
You will be asked to share your experience with us about the use of baked milk foods into your child's diet. If you agree to take part, you will be contacted by the lead researcher to arrange a mutually convenient day and time for the interview. Before the interview, you will be asked to sign a consent form that you are happy to take part.

What are the benefits of taking part in this study?
The aim of this research is to understand parents' and children's experience on the graded introduction of baked milk products into the diet of children diagnosed with cow's milk allergy in the UK, from the parents' perspective. It is hoped to interview 15 parents who all had to introduce the baked milk products at home and gather their perspectives. Although there is no direct benefit to you, we hope that taking part in this study will help you get a deeper understanding of this dietary management. We hope that the information derived from all participants will help other researchers, healthcare professionals and allergy services to provide optimal care to children with cow's milk allergy and contribute to an effective dietary management and treatment with the use of baked milk products into the children's diet in the future. At the end of the research study the results will be written up in a report (thesis) and articles may be written for journals and magazines. Talks may also be given about the research findings.

What are the disadvantages of taking part in this study?
We do not anticipate any risk to you from participating in this study. You will be required to give up some time to be interviewed at a date and time convenient to you. Although you will be free to select the topics and share as much as you like with the researcher, it is possible you may find some topics upsetting. You are free to have some time out or stop the conversation

during the interview and withdraw from the study at any time.

Will my taking part in the study be kept confidential?
Yes, everything discussed during the telephone/skype interview for the purpose of the study will be made anonymous and all information collected will be kept confidential. Your contact details will be kept by the lead researcher (so we can get in touch with you) but will not be shared or passed on to anyone else. You will be given a unique code for the purpose of data analysis and reporting. You will have the chance to check the accuracy of data held about you, and correct any errors if you wish. The data will be stored in locked cabinets at the university and any electronic records will be password protected. We will remove any personal identifiable information, when we report the study so those reading it will not know who said what. The researcher will be the only person who can access the cabinet and the electronic records. It is possible that some of the data collected may also be looked at by the academic supervisors to check that the study is being carried out correctly. The researcher has a duty of confidentiality to you as a research participant.

However, if the researcher finds out during the conversation that you or your child is being harmed or exploited, it is their duty to pass on this information to a relevant agency in confidence and after discussion with you.

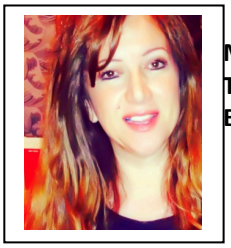
Who is organising the study?
This is an independent research study carried out as part of a Doctorate studentship sponsored by the Faculty of Science at the University of Portsmouth.

Who has reviewed the study?

This study was reviewed by the Faculty of Science Ethics Committee, University of Portsmouth.

What will happen next?
If you decide to take part in the study, please fill in the Reply Slip, sign the Consent Form and send them back by email to yiota.athanasopoulou@port.ac.uk

What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the lead researcher, who will be happy to answer your questions:



PhD researcher
Ms Yiota Athanasopoulou,
Tel; 02392 844434
Email:yiota.athanasopoulou@port.ac.uk

If you remain unhappy and wish to make a formal complaint, you can do this by contacting:
Dr Chris Markham
Head of School
Health Sciences and Social Work
Tel: 02392 842893
Email: **chris.markham@port.ac.uk**

Thank you for taking the time to read the information sheet and looking forward to hearing from you soon.

Appendix 13: Participants' demographic questions

Reference No: SFEC 2016-046 *8/03/2016* *Version 1*

Study Title: Investigating parents' experiences in re-introducing baked milk foods in children with CMA in UK- A qualitative study

Demographic Questions

Date:
Venue: Skype/telephone interview
Parents' details:
1. Gender:
Male □
Female □

2. Age: ……

3. What is the highest level of education you have completed (or still in progress)?
Postgraduate degree □
Undergraduate degree □
College □
High school graduate □
Less than high school □
Other (please specify)……

4. Are you currently employed?
Full time □
Part time □
Stay at home/homemaker □
Other (please specify)……..

Your child's details
1. Age:

2. Gender:
Male □
Female □

3. Diagnosis:
IgE mediated Cow's Milk Allergy □
Non-IgE mediated Cow's Milk Allergy □

4. Method of CMA diagnosis:
Positive milk challenge □
Diagnostic Skin Prick Test □
Serum specific IgE results(blood tests) □
Clinical history of symptoms □

5. Other conditions :
Asthma □
Rhinitis □
Eczema □
Please specify any others……..

6. Other Food Allergies
Egg □
Wheat □
Peanut □
Tree nuts □
Shellfish □
Please specify any others……..

Appendix 14: Mothers' consent form

Reference No: SFEC 2016-046 *8/03/2016* *Version 1*

Consent Form & Reply Slip

Consent Form

Participant Identification Number:

Study Title: ***Investigating parents' experiences in re-introducing baked milk foods in children with CMA in UK- A qualitative study***

Please read each of the following statements carefully and tick each box if you agree and then sign below

Please tick box

1. I confirm that I have read and understand the information sheet dated……. (version) for the above study and have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary. If I decide at any time that I no longer wish to take part in this project I can notify the researcher involved and withdraw immediately without giving any reason. ☐

3. I understand that my participation is confidential and my name will be removed from the interviews and I will be identified by a unique participant identification number rather than my name. ☐

4. I understand that my interview will be audio recorded. ☐

5. I understand that my records will be Confidential and anonymised and will be stored securely. ☐

6. I agree to take part in this study. ☐

Participant's Signature: **Date:**

Reply Slip

If you are interested in this study please complete date, time and type that you prefer to book your scheduled interview:

Your name: ……
Your e-mail: ……

Date(s) of Interview: ……..
Time(s) of Interview: ……
Type of interview:
Skype □ Please provide your skype detail:……
……..
Telephone □ Please provide your telephone number:……
……..

I agree for the researcher to keep my personal details in order to contact me:
Yes □ No □
Thank you for taking the time to complete the consent form and reply slip. Please return them by email at yiota.athanasopoulou@port.ac.uk or post to Yiota Athanasopoulou, PhD researcher, SHSSW, University of Portsmouth, James Watson Building, 2 King Richard 1st Road, Portsmouth, PO1 2FR in the stamped envelope provided.

Appendix 15: SFEC approval letter

Yiota Anthanasopoulou
School of Health Sciences & Social Work
University of Portsmouth
yiota.athanasopoulou@port.ac.uk

Science Faculty Ethics Committee

Science Faculty Office
University of Portsmouth
St Michael's Building
White Swan Road
PORTSMOUTH
PO1 2DT

T: 023 9284 3379
ethics-sci@port.ac.uk

Date 25th May 2016

fAVOURABLE ETHICAL OPINION WITH MINOR CONDITIONS

Study Title: **Investigating parents' experiences in re-introducing baked milk foods in children with CMA in UK- A qualitative study**
Reference Number: **SFEC 2016-046** (Please quote this in any correspondence)

Thank you for **submitting your application to the Science Faculty Ethics Committee (SFEC) dated 09th May 2016**, in accordance with current procedures[1].

I am pleased to inform you that SFEC was content to grant a favourable ethical opinion of the above research on the basis described in the submitted documents listed at Annex A, and subject to standard general conditions[2] and the following minor conditions/recommendations:

1. *Please ensure you communicate to participants that once interviews have been completed, they cannot withdraw their interview data.*
2. *Please ensure you communicate to participants that this is a PhD project.*
3. *Please adjust the milk ladder graphic on your PIS. It is difficult to read and parts of it are cut off.*
4. *State clearly where the 10GBP voucher can be used.*
5. *Please clarify how participants will sign the consent form or will consent be obtained through the reply slip.*

There is no requirement for you to confirm these conditions have been met in writing to the committee.

Please note that the favourable opinion of SFEC does not grant permission or approval to undertake the research. Management permission or approval must be obtained from any

host organisation, including the University of Portsmouth or supervisor, prior to the start of the study.

Wishing you every success in your research

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[1] Procedures for Ethical Review, Science Faculty Ethics Committee, University of Portsmouth, October 2012 (to be updated).
[2] After ethical review – Guidance for researchers (Please read).

Yours sincerely,

Dr Simon Kolstoe
Vice Chair, Science Faculty Ethics Committee

Information:
Professor Tara Dean – Supervisor
Mrs Holly Shawyer - Faculty Administrator

Statement of compliance

SFEC is constituted in accordance with the Governance Arrangements set out by the University of Portsmouth

After Ethical Review

If unfamiliar, please consult the advice After Ethical Review[2] which gives detailed guidance on reporting requirements for studies with a favourable opinion, including, notifying substantial amendments, notification of serious breaches of the protocol, progress reports and notifying SFEC of the end of the study.

Feedback

You are invited to give your view of the service that you have received from the Faculty Ethics Committee. If you wish to make your views known please contact the administrator at ethics-sci@port.ac.uk

NNEX A Documents reviewed

The documents ethically reviewed for this application (SFEC 2016-046)

Document	*Version*	*Date*
A - Ethics Application090516	1	09/05/2016
B - PhD phase 2 supporting docs090516	1	09/05/2016

ANNEX B - After ethical review - Guidance for researchers Guidance for researchers

1. This document sets out important guidance for researchers with a favourable opinion from a University of Portsmouth Ethics Committee. Please read the guidance carefully. A failure to follow the guidance could lead to the committee reviewing and possibly revoking its opinion on the research.

2. It is assumed that the research will commence within 1 year of the date of the favourable ethical opinion or the start date stated in the application, whichever is the latest.

3. The research must not commence until the researcher has obtained any necessary management permissions or approvals – this is particularly pertinent in cases of research hosted by external organisations. The appropriate head of department should be aware of a member of staff's research plans.

4. If it is proposed to extend the duration of the study beyond that stated in the application, the Ethics Committee must be informed.

5. If the research extends beyond a year then an annual progress report must be submitted to the Ethics Committee.

6. When the study has been completed the Ethics Committee must be notified.

7. Any proposed substantial amendments must be submitted to the Ethics Committee for review. A substantial amendment is any amendment to the terms of the application for ethical review, or to the protocol or other supporting documentation approved by the Committee that is likely to affect to a significant degree:
   (a) the safety or physical or mental integrity of participants
   (b) the scientific value of the study
   (c) the conduct or management of the study.

7.1 A substantial amendment should not be implemented until a favourable ethical opinion has been given by the Committee.

8. Researchers are reminded of the University's commitments as stated in the Concordat to Support Research Integrity viz:

- maintaining the highest standards of rigour and integrity in all aspects of research
- ensuring that research is conducted according to appropriate ethical, legal and professional frameworks, obligations and standards
- supporting a research environment that is underpinned by a culture of integrity and based on good governance, best practice and support for the development of researchers
- using transparent, robust and fair processes to deal with allegations of research misconduct should they arise
- working together to strengthen the integrity of research and to reviewing progress regularly and openly

9. In ensuring that it meets these commitments the University has adopted the UKRIO Code of Practice for Research. Any breach of this code may be considered as misconduct and may be investigated following the University Procedure for the Investigation of Allegations of Misconduct in Research. Researchers are advised to use the UKRIO checklist as a simple guide to integrity.

Appendix 16: CCHMC's Institutional Review Board (IRB) Approval letter

Institutional Review Board - Federalwide Assurance #00002988

Cincinnati Childrens Hospital Medical Center

Date: 12/7/2016
From: CCHMC IRB
To: Principal Investigator:Allergy & ImmunologyAmal Assa'ad
Study ID: 2015-4861
Re: Study Title: Factors Affecting Oral Food Challenge Outcomes in Children

The above referenced protocol and all applicable additional documentation provided to the IRB were reviewed and **RE-APPROVED** using an **EXPEDITED** review procedure set forth in 45 CFR 46.110(b)(1), Category(ies)(see below) on 12/6/2016.

For research involving children, the Committee determined that this research presents:

☐ No greater than minimal risk.

Please note the following requirements:

Consent Requirements

Per 45 CFR 46.116 the IRB has waived the requirement to obtain informed consent for all adult participants.

Parental Permission Requirements

Per 45 CFR 46.116 the IRB has waived the requirement to obtain parental permission from the parent(s) (or guardian) of all child participants. NOTE: If your research is subject to FDA regulations it is not eligible for this waiver of parental permission.

Assent Requirements

Per 45 CFR 46.116 the IRB has waived the requirement to obtain assent from all child participants. NOTE: If your research is subject to FDA regulations it is not eligible for this waiver of assent.

HIPAA Requirements

Per 45 CFR 164.512 the IRB has granted a waiver from the requirement to obtain an authorization for the use and/or disclosure of protected health information (PHI). **This study will be due for continuing review at least 30 days before** 12/5/2017.

Study Documents

Factors Affecting Oral Food Challenge Outcomes in Children

AMENDMENTS: The principal investigator is responsible for notifying the IRB of any changes in the protocol, participating investigators, procedures, recruitment, consent forms, FDA status, or conflicts of interest. Approval is based on the information as submitted. New procedures cannot be initiated until IRB approval has been given. If you wish to change any aspect of this study, please submit an Amendment via ePAS to the IRB, providing a justification for each requested change.

CONTINUING REVIEW: The investigator is responsible for submitting a Continuing Review via ePAS to the IRB at least 30 days prior to the expiration date listed above. Please note that study procedures may only continue into the next cycle if the IRB has reviewed and granted re-approval prior to the expiration date.

UNANTICIPATED PROBLEMS: The investigator is responsible for reporting **unanticipated problems** promptly to the IRB via ePAS according to current reporting policies.

STUDY COMPLETION: The investigator is responsible for notifying the IRB by submitting a Request to Close via ePAS when the research, including data analysis, has completed.

Research Categories

5. Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for nonresearch purposes (such as medical treatment or diagnosis). (NOTE: Some research in this category may be exempt from the HHS regulations for the protection of human subjects. 45 CFR 46.101(b)(4). This listing refers only to research that is not exempt.)

Please note: This approval is through the IRB only. You may be responsible for reporting to other regulatory officials (e.g. VA Research and Development Office, UC Health - University Hospital). Please check with your institution and department to ensure you have met all reporting requirements.

Statement regarding International conference on Harmonization and Good Clinical Practices: The Institutional Review Board is duly constituted (fulfilling FDA requirements for diversity), has written procedures for initial and continuing review of clinical trials; prepares written minutes of convened meetings, and retains records pertaining to the review and approval process; all in compliance with requirements defined in 21 CFR Parts 50, 56 and 312 Code of Federal Regulations. This institution is in compliance with the ICH GCP as adopted by FDA/DHHS.

Appendix 17: Data transfer agreement

DATA USE AGREEMENT FOR RESEARCH

This Data Use Agreement ("Agreement") is entered into by and between Children's Hospital Medical Center located at 3333 Burnet Avenue, Cincinnati, OH 45229 ("CHMC") and the University of Portsmouth ("Site") and effective as of the date of the last signature ("Effective Date").

WHEREAS, CHMC and Site have been collaborating on a research project as further described in the protocol titled "Factors Affecting Oral Food Challenge Outcomes in Children" (the "Purpose");

WHEREAS, CHMC will send data (the "Data") to the Site for further analysis as described in the protocol;

WHEREAS, Site would like to use and analyze the Data and CHMC would like to provide the Data solely for the Purpose; and

WHEREAS, Site agrees to use the Data solely for the Purpose and Site agrees to the conditions contained in this Agreement regarding the use of the Data.

NOW, THEREFORE, CHMC and Site agree as follows:

1. Use of Data. Site agrees to comply with all applicable laws governing the use of the Data, including without limitation, laws regarding the privacy or protection of personal or medical information as required by the provisions of the Health Insurance Portability and Accountability Act of 1996 ("HIPAA") and regulations promulgated thereunder and the protection of human subjects. Site agrees it will: (a) not use or further disclose the Data in a manner that would violate the requirements of HIPAA if such use or disclosure were made by CHMC itself; (b) not use or further disclose the Data other than as permitted by this Agreement or as otherwise required by law; (c) use appropriate safeguards to prevent the use or disclosure of the Data other than as provided for by this Agreement; and (d) not use the Data to identify or contact any Study subject. The rights and obligations of this paragraph shall survive the expiration or termination of this Agreement.

2. Site Responsibilities. Site accepts responsibility for ensuring that all research related to the Purpose is conducted in full compliance with this Agreement, all applicable laws and any requirements of Site's IRB or equivalent. Site will ensure that its' IRB or equivalent is established and conducted in full compliance with all applicable laws.

3. Confidential Information. In furtherance of the conduct of the Purpose, it may be necessary for the parties hereto to disclose proprietary, trade secret, and/or other confidential information ("Confidential Information") to one another. All such Confidential Information will remain the property of the party disclosing same. Each party agrees that any such Confidential Information disclosed to him or to her, or to it or its employees, agents or contractors, will be used only in connection with the legitimate purposes of this Agreement, will be disclosed only to those who have a need to know it

and are obligated to keep same in confidence, and will be safeguarded with reasonable care. The foregoing confidentiality obligations will not apply when, after and to the extent the Confidential Information disclosed: (i) is now, or hereafter becomes, generally available to the public through no fault of the receiving party or its employees, agents, or contractors; (ii) was already in possession of the receiving party without restriction as to confidentiality at the time of disclosure as evidenced by competent written records; (iii) is subsequently received by the receiving party from a third party without restriction and without breaching any confidential obligation between the third party and the disclosing party hereunder; or (iv) is required to be disclosed by applicable law, rule, or court order, in which case receiving party will promptly notify disclosing party of such required disclosure, take all reasonable steps to limit the scope of such disclosure, and provide disclosing party with an opportunity to comment on such proposed disclosure. The foregoing obligations of confidentiality and use will continue for five (5) years after the termination of this Agreement.

4. Transfer and Use of Data. Site will not transfer the Data without the express written consent of CHMC.

5. Permitted Users. Site agrees to use and allow access to Data only to personnel who are so authorized for such access by that party's Institutional Review Board (IRB) ("Permitted Users") or the equivalent. Site agrees that they will not disclose, or allow access to Data to anyone other than Permitted Users except as required by law.

6. Reporting. Site agrees to report in writing to CHMC any unauthorized use or disclosure of Data that they become aware of within five (5) business days of discovery. Should Site receive a request to use or disclose Data as required by law, that party shall notify the other party promptly of any request or subsequent use or disclosure of the Data.

7. Term and Termination. This Agreement becomes effective when signed below by all parties, on the date of the last signature. Either party may terminate this Agreement upon thirty (30) days written notice to the other. Rights and obligations which by their nature should survive or which this Agreement expressly states will survive, will remain in full force and effect following termination or expiration of this Agreement. The parties will cooperate with each other during and following termination or expiration of this Agreement to comply with all applicable laws, rules, and regulations.

8. Publicity. Neither party will use the name of the other party in any publicity, advertising, or news release without the prior written approval of the authorized representative of the other party.

9. Miscellaneous. This Agreement constitutes the sole and complete agreement between the parties and replaces all other written and oral agreements relating to the Purpose or Data. This Agreement may only be modified by a written agreement signed by all of the parties hereto. This Agreement may be executed in one or more counterparts, each of which will be deemed to be an original copy of the Agreement, and all of which, when taken together, will be deemed to constitute one and the same Agreement. Signatures to this Agreement transmitted by fax, by electronic mail in "portable document format" (".pdf"),

or by any other electronic means intended to preserve the original graphic and pictorial appearance of the Agreement, will have the same effect as physical delivery of the paper document bearing the original signature.

IN WITNESS WHEREOF, the parties authorized representatives have signed below.

Children's Hospital Medical Center

By: Joan Gates
Name: Joan Gates
Title: Senior Counsel
Date: 4/14/16

University of Portsmouth

By: T. Dean
Name: Professor Tara Dean
Title: Professor, Dean of Faculty of Science
Date: April 14th, 2016

Appendix 18: University of Portsmouth SFEC Ethical Review-USA study

Panagiota Athanasopoulou
School of Health Sciences and Social Work
University of Portsmouth

Yiota.Athanasopoulou@port.ac.uk

Science Faculty Ethics Committee
Science Faculty Office
University of Portsmouth
St Michael's Building
White Swan Road
P0ORTSMOUTH
PO1 2DT
United Kingdom

T: + 44 (0)23 9284 3379 ethics-sci@port.ac.uk

14 December 2017

ADVISING OF EXTERNAL ETHICAL FAVOURABLE OPINION

Study Title: Factors Affecting Oral Food Challenge Outcomes in Children

UoP Reference Number: SFEC External ethics - Other - 2017-003.

Institutional Review Board Cincinnati Childrens Hospital Medical Center: 2015-4861

Date Submitted: 30 November 2017

Thank you for advising the Science Faculty Ethics Committee (SFEC) for research you are conducting under the favourable opinion of an external ethical review body and international data exchange agreement (as detailed at Annex A). All appears to be well, and there is no need to undertake a secondary ethical review at UoP, although the standard general conditions for research (*See Annex B)* still apply.

If your research extends to data collected after 12 December 2017, then please ensure that an updated favourable ethical opinion is obtained first from the Cincinnati Childrens Hospital Medical Center Institutional Review Board. It is not thought that this is the intention, but please bear in mind this date in case the data period is extended.

Wishing you every success in your research.

Dr Jim House

Chair, Science Faculty Ethics Committee

Annexes

A - Documents reviewed
B - After ethical review - Guidance for researchers

Information:

Carina Venter - PhD Supervisor
Dr Heather Mackenzie - PhD Supervisor
Rose Barrand - Faculty Administrator
External - orcra@cchmc.org

Statement of compliance

SFEC is constituted in accordance with the Governance Arrangements set out by the University of Portsmouth

After Ethical Review

If unfamiliar, please consult the advice After Ethical Review (Annex B), which gives detailed guidance on reporting requirements for studies with a favourable opinion, including, notifying substantial amendments, notification of serious breaches of the protocol, progress reports and notifying SFEC of the end of the study.

Feedback

You are invited to give your view of the service that you have received from the Science Faculty Ethics Committee. If you wish to make your views known please contact the administrator at ethics-sci@port.ac.uk

ANNEX A **Documents reviewed**

The documents ethically reviewed for this application

Document	*Version*	*Date*
A - Email notification to SFEC 30.11.17	n/a	30 Nov 2017
B - Cover letter from UoP PhD student_Panagiota Athanasopoulou	n/a	30 Nov 2017
C - IRB approval of continuing review	n/a	30 Nov 2017
D - Letter from PI, Cincinnati Children's Hospital Medical Centre(Amal Assa'ad)	m/a	30 Nov 2017
E - IRB_Factors Affecting Oral Food Challenge Outcomes in Children	V1	30 Nov 2017
F - Data transfer agreement between CCHMC and UoP	n/a	30 Nov 2017

A - 1 ANNEX B - After ethical review - Guidance for researchers

1. This Annex sets out important guidance for researchers with a favourable opinion from a University of Portsmouth Ethics Committee. Please read the guidance carefully. A failure to follow the guidance could lead to the committee reviewing and possibly revoking its opinion on the research.

2. It is assumed that the research will commence within 1 year of the date of the favourable ethical opinion or the start date stated in the application, whichever is the latest.

3. The research must not commence until the researcher has obtained any necessary management permissions or approvals – this is particularly pertinent in cases of research hosted by external organisations. The appropriate head of department should be aware of a member of staff's research plans.

4. If it is proposed to extend the duration of the study beyond that stated in the application, the Ethics Committee must be informed.

5. Any proposed substantial amendments must be submitted to the Ethics Committee for review. A substantial amendment is any amendment to the terms of the application for ethical review, or to the protocol or other supporting documentation approved by the Committee that is likely to affect to a significant degree:

(a) the safety or physical or mental integrity of participants
(b) the scientific value of the study
(c) the conduct or management of the study.

5.1 A substantial amendment should not be implemented until a favourable ethical opinion has been given by the Committee.

6. Researchers are reminded of the University's commitments as stated in the Concordat to Support Research Integrity viz:

- maintaining the highest standards of rigour and integrity in all aspects of research
- ensuring that research is conducted according to appropriate ethical, legal and professional frameworks, obligations and standards
- supporting a research environment that is underpinned by a culture of integrity and based on good governance, best practice and support for the development of researchers
- using transparent, robust and fair processes to deal with allegations of research misconduct should they arise
- working together to strengthen the integrity of research and to reviewing progress regularly and openly.

7. In ensuring that it meets these commitments the University has adopted the UKRIO Code of Practice for Research. Any breach of this code may be considered as misconduct and may be investigated following the University Procedure for the Investigation of Allegations of Misconduct in Research. Researchers are advised to use the UKRIO checklist as a simple guide to integrity.

Appendix 19: Statistical analysis of immune biomarkers and milk challenges

Descriptive statistics was used to calculate the mean of SPT wheal size (mm) measurements and serum milk sIgE levels (KU/I. Frequency statistics was used to assess and classified (positive/negative) the immune biomarkers' results and baked milk challenge outcomes of children with CMA.

Statistical tests

Descriptive statistics test

		Milk challenges outcome	SPT Size Fresh Milk	SPT Size	Allergen MILK	Total IgE	IgECasein_M	IgE b-lact
N	Valid	191	191	191	191	191	191	191
	Missing	86	86	86	86	86	86	86
Mean			2.6230	4.07	8.3532	89.9709	3.3661	2.5972
Std. Deviation		.501	5.16306	6.249	49.45852	173.90277	15.47108	12.57803
Minimum		1	.00	0	.00	.00	.00	.00
Maximum		2	22.00	35	647.00	876.00	120.00	100.00

Frequency statics tests

1. Milk challenge outcomes

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Positive	91	32.9	47.6	47.6
	Negative	100	36.1	52.4	100.0
	Total	191	69.0	100.0	
Missing	System	86	31.0		
Total		277	100.0		

2. SPT Fresh milk

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	positive	53	19.1	27.7	27.7
	negative	138	49.8	72.3	100.0

	Total	191	69.0	100.0	
Missing	System	86	31.0		
Total		277	100.0		

3. SPT milk extract

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Positive	85	30.7	44.5	44.5
	Negative	106	38.3	55.5	100.0
	Total	191	69.0	100.0	
Missing	System	86	31.0		
Total		277	100.0		

4. Milk Allergen

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	positive	109	39.4	57.1	57.1
	negative	82	29.6	42.9	100.0
	Total	191	69.0	100.0	
Missing	System	86	31.0		
Total		277	100.0		

5. Total milk-IgE

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	positive	93	33.6	48.7	48.7
	negative	98	35.4	51.3	100.0
	Total	191	69.0	100.0	
Missing	System	86	31.0		
Total		277	100.0		

6. Milk Casein-IgE

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Negative (≤0.35)	137	49.5	71.7	71.7
	Positive (>0.35)	54	19.5	28.3	100.0
	Total	191	69.0	100.0	

Missing	System	86	31.0		
Total		277	100.0		

7. Milk β-lactoglobulin IgE

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Negative (≤0.35)	146	52.7	76.4	76.4
	Positive (>0.35)	45	16.2	23.6	100.0
	Total	191	69.0	100.0	
Missing	System	86	31.0		
Total		277	100.0		

1. **SPT fresh milk**

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	PPV	95%CI	NPV	95%CI
<1	0.00	0.0 - 4.0	100.00	96.4 - 100.0		1.1		1.1
≤1	49.45	38.8 - 60.1	92.00	84.8 - 96.5	84.9	73.7-91.9	66.7	61.8-71.2
≤2	100.00	96.0 - 100.0	0.00	0.0 - 3.6	1.00			

Area under the ROC curve (AUC)

Area under the ROC curve (AUC)	0.707
Standard Error [a]	0.0297
95% Confidence interval [b]	0.637 to 0.771
z statistic	6.986
Significance level P (Area=0.5)	<0.0001

[a] DeLong et al., 1988
[b] Binomial exact

2. SPT milk extract

Criterion values and coordinates of the ROC curve

Criterion	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95 % CI
<1	0.00	0.0 - 4.0	100.00	96.4 - 100.0		1.00		
≤1	69.23	58.7 - 78.5	78.00	68.6 - 85.7	74.1	65.9-80.9	73.6	66.8 - 79.4
≤2	100.00	96.0 - 100.0	0.00	0.0 - 3.6	1.00			

Area under the ROC curve (AUC)

Area under the ROC curve (AUC)	0.736
Standard Error [a]	0.0320
95% Confidence interval [b]	0.668 to 0.797
z statistic	7.376
Significance level P (Area=0.5)	<0.0001

[a] DeLong et al., 1988
[b] Binomial exact

3. **Milk Allergen**

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI
<1	0.00	0.0 - 4.0	100.00	96.4 - 100.0		1.00		
≤1	68.13	57.5 - 77.5	53.00	42.8 - 63.1	58.9	50.7-62.9	69.6	56.2-72.2
≤2	100.00	96.0 - 100.0	0.00	0.0 - 3.6	1.00			

Area under the ROC curve (AUC)

Area under the ROC curve (AUC)	0.606
Standard Error [a]	0.0351
95% Confidence interval [b]	0.533 to 0.675
z statistic	3.010
Significance level P (Area=0.5)	0.0026

[a] DeLong et al., 1988
[b] Binomial exact

4. Total milk IgE

Criterion	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI
<1	0.00	0.0 - 4.0	100.00	96.4 - 100.0		1.00		
≤1	58.24	47.4 - 68.5	60.00	49.7 - 69.7	57	49.6-64.1	61.2	54.1-67.9
≤2	100.00	96.0 - 100.0	0.00	0.0 - 3.6	1.00			

Area under the ROC curve (AUC)

Area under the ROC curve (AUC)	0.591
Standard Error [a]	0.0358
95% Confidence interval [b]	0.518 to 0.662
z statistic	2.548
Significance level P (Area=0.5)	0.0108

[a] DeLong et al., 1988
[b] Binomial exact

5. Milk Casein – IgE

6. Criterion	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI
≥1	100.00	96.0 - 100.0	0.00	0.0 - 3.6	1.00			
>1	40.66	30.5 - 51.5	83.00	74.2 - 89.8	68.5	56.9-78.2	60.6	59-78.2
>2	0.00	0.0 - 4.0	100.00	96.4 - 100.0		1.00		

Area under the ROC curve (AUC)

Area under the ROC curve (AUC)	0.618
Standard Error [a]	0.0320
95% Confidence interval [b]	0.545 to 0.687
z statistic	3.692
Significance level P (Area=0.5)	0.0002

[a] DeLong et al., 1988
[b] Binomial exact

6. Milk β-lactoglobulin

Criterion	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI
≥1	100.00	96.0 - 100.0	0.00	0.0 - 3.6	1.00			
>1	31.87	22.5 - 42.5	84.00	75.3 - 90.6	64.4	51.4-75.5	57.5	53.5-61.5
>2	0.00	0.0 - 4.0	100.00	96.4 - 100.0		1.00		

Area under the ROC curve (AUC)

Area under the ROC curve (AUC)	0.579
Standard Error [a]	0.0307
95% Confidence interval [b]	0.506 to 0.650
z statistic	2.584
Significance level P (Area=0.5)	0.0098

[a] DeLong et al., 1988
[b] Binomial exact

[2]Area Under the ROC Curve

Appendix 20: UPR16 form – Ethics Review Checklist

FORM UPR16

Research Ethics Review Checklist

Please include this completed form as an appendix to your thesis (see the Research Degrees Operational Handbook for more information

Postgraduate Research Student (PGRS) Information				**Student ID:**	694444
PGRS Name:	Panagiota (Yiota) Athanasopoulou				
Department:	SHSSW	**First Supervisor:**	Heather Makenzie		
Start Date: (or progression date for Prof Doc students)	**31/05/2013**				
Study Mode and Route:	Part-time ☐ Full-time ☒	MPhil ☐ PhD ☒	MD ☐ Professional Doctorate ☐		

Title of Thesis:	An investigation on the use of baked milk foods in children with cow's milk allergy
Thesis Word Count: (excluding ancillary data)	78,598

If you are unsure about any of the following, please contact the local representative on your Faculty Ethics Committee for advice. Please note that it is your responsibility to follow the University's Ethics Policy and any relevant University, academic or professional guidelines in the conduct of your study

Although the Ethics Committee may have given your study a favourable opinion, the final responsibility for the ethical conduct of this work lies with the researcher(s).

UKRIO Finished Research Checklist:
(If you would like to know more about the checklist, please see your Faculty or Departmental Ethics Committee rep or see the online version of the full checklist at: http://www.ukrio.org/what-we-do/code-of-practice-for-research/)

a) Have all of your research and findings been reported accurately, honestly and within a reasonable time frame?	YES ☒ NO ☐
b) Have all contributions to knowledge been acknowledged?	YES ☒ NO ☐
c) Have you complied with all agreements relating to intellectual property, publication and authorship?	YES ☒ NO ☐
d) Has your research data been retained in a secure and accessible form and will it remain so for the required duration?	YES ☒ NO ☐
e) Does your research comply with all legal, ethical, and contractual requirements?	YES ☒ NO ☐

Candidate Statement:	
I have considered the ethical dimensions of the above named research project, and have successfully obtained the necessary ethical approval(s)	
Ethical review number(s) from Faculty Ethics Committee (or from NRES/SCREC):	SFEC 2016-046
If you have *not* submitted your work for ethical review, and/or you have answered 'No' to one or more of questions a) to e), please explain below why this is so:	
Signed *(PGRS)*: 	**Date:** 27/02/20

UPR16 – April 2018