patients with suspected IgE-mediated cow's milk allergy (1, 6, 10, 16), 7 explicitly included only patients with atopic eczema (4, 9, 11, 19, 21, 22, 24), and the remaining studies included mixed populations of patients with various conditions in whom CMA was investigated.

Using the criteria of methodological quality suggested by the QUADAS questionnaire we found that in many studies the spectrum of patients was not representative of the patients who will receive the test in practice. In most studies the results of a reference standard were very likely interpreted with the knowledge of the results of the skin prick test or vice versa. None of the studies reported uninterpretable or intermediate test results. One study reported 8% inconclusive challenge tests but did not report number of inconclusive skin prick tests (23).

The combined sensitivity in these studies was 0.67 (95% CI: 0.64–0.70) and the specificity was 0.74 (95% CI: 0.72–0.77). Skin prick test accuracy was similar when studies in patients with atopic eczema were excluded (16 studies; sensitivity 0.71, 95% CI: 0.68–0.75 and specificity 0.73, 95% CI: 0.70–0.76). In 4 studies that explicitly enrolled patients suspected of immediate reactions to milk sensitivity seemed slightly improved (0.77, 95% CI: 0.68–0.84) on the expense of lower specificity (0.61, 95% CI: 0.52–0.70). We also investigated the influence of child's age on the accuracy of skin prick tests in the diagnosis of CMA. In children suspected of CMA who were on average younger than 12 months sensitivity of skin prick test was lower (0.55, 95% CI: 0.49–0.61 [4 studies]) than in children older than 12 month of age (0.81, 95% CI: 0.77–0.85 [11 studies]). Age seemed not to influence the estimate of specificity (0.75, 95% CI: 0.69–0.80 vs. 0.72, 95% CI: 0.68–0.76). The overall quality of evidence across outcomes was very low.

Benefits and Downsides

In patients with low pretest probability of CMA (~10%) based on the history and presenting symptoms a negative result of skin prick test (ie, diameter < 3 mm) may be helpful in avoiding a burdensome and costly food challenge with cow's milk in around 50% of patients tested. However, when using SPT instead of a food challenge one may expect about 2% children older than 12 months and more than 4% children younger than 12 months being misclassified as not having CMA while they actually would be allergic to cow's milk (false negative results; see evidence profile for question 1). These children will likely be allowed home and have an allergic reaction to cow's milk at home. False negative result may also lead to unnecessary investigations and possible treatments for other causes of symptoms while the real cause (ie, CMA) has been missed.

In patients with an average pretest probability of CMA (~40%; an average rate of positive food challenge tests in the included studies) based on the history and presenting symptoms, skin prick tests would incorrectly classify 15–28% of patients as allergic to cow's milk (while they would actually not be; false positive results) and a food challenge test might be performed regardless. In these patients one might also expect 8–18% false negative results that in some children are likely to lead to performing a food challenge test, but some children would be allowed home and would have an allergic reaction (possibly anaphylactic) to cow's milk at home. This makes skin prick tests unlikely to be useful as a single test allowing avoiding food challenge test in these patients.

In patients with high pretest probability of CMA (~80%) based on the history (eg, an anaphylactic reaction in the past) performing skin prick test may help to avoid the risk and burden of food challenge test in around 50% of patients tested. However, if the skin prick test is used and food challenge is not done, one may expect 5–6% false positive results. These children would be unnecessarily treated with elimination diet and/or formula that might lead to nutritional deficits, there would be unnecessary stress for the family, use of unnecessary preventive measures (eg, carrying epinephrine self injector) and a correct diagnosis of the real cause of symptoms may be delayed.

Other Considerations

In settings where oral food challenges are always performed (because of low testing threshold and high treatment threshold) the use of skin prick tests is redundant given the limited sensitivity and specificity of skin prick test compared with oral food challenge.

Conclusions

In settings where oral food challenge is done routinely and the clinician's thresholds for testing and treatment are such that exclusion and confirmation of CMA always has to be proven by oral food challenge, there is no need to perform a skin prick test.

In settings where clinicians follow a more prudent approach, skin prick test may help to avoid an oral food challenge in selected patients. In patients with a high pretest probability of IgE-mediated CMA a positive SPT result with a cut-off value of ≥3 mm can help to avoid oral food challenge in 49–70% of patients, but the benefit is counterbalanced by a 5–6% risk of falsely classifying a patient as having CMA. In patients with low pretest probability of CMA a negative skin prick test result with a cut-off value of ≥3 mm can allow to avoid oral food challenge in 67–72%, but with a risk of 2–4% false negative results. In patients with an average pretest probability of CMA a skin prick test with a cut-off value of ≥3 mm used as a single diagnostic test is unlikely to reduce the need for oral food challenge.

Therefore, in patients with high or low pretest probability of CMA the net benefit of using a skin prick test instead of oral food challenge with cow's milk is uncertain. In patients with average pretest probability of CMA the net clinical benefit is unlikely.

Clinical Recommendations, Question 1

Recommendation 1.1

In settings where oral food challenge is considered a requirement for making a diagnosis of IgE-mediated CMA, we recommend using oral food challenge with cow's milk as the only test without performing a skin prick test as a triage or an add-on test to establish a diagnosis (strong recommendation/very low quality evidence).

Underlying Values and Preferences. This recommendation places a relatively high value on avoiding resource consumption and the risk of anaphylactic reactions at home in patients who would be misclassified by a skin prick test alone. It places a lower value on anaphylactic reactions in a controlled setting that can be managed by experienced personnel when oral food challenge is performed. This recommendation also places a high value on avoiding any unnecessary treatment in patients who would be incorrectly classified by a skin prick test as allergic to cow's milk.

Remark. This recommendation applies to clinical practice settings. In research settings there may be compelling reasons to perform skin prick tests even though a food challenge test with cow's milk is always being done.

Recommendation 1.2

In settings where oral food challenge is not considered a requirement in all patients suspected of IgE-mediated CMA, in patients with high pretest probability of CMA we suggest using a skin prick test with a cut-off value of ≥3 mm as a triage test to avoid oral food challenge in those in whom the result of a skin prick test turns out positive (conditional recommendation/low quality evidence).

Underlying Values and Preferences. This recommendation places a relatively high value on avoiding burden, resource use and very likely anaphylactic reactions during the oral food challenge test (~50–70% food challenges avoided). It places a lower value on unnecessary treatment of around 1 in 20 patients misclassified as allergic to cow's milk (5–6% false positive results).

Remarks. A high pretest probability of CMA (~80%) can be estimated based on the history and would represent, for instance, patients who experienced an anaphylactic reaction in the past.

Recommendation 1.3

In settings where oral food challenge is not considered a requirement in all patients suspected of IgE-mediated CMA, in patients with an average pretest probability of CMA we suggest using an oral food challenge test with cow's milk as the only test without performing a skin prick test with a cut-off value of ≥3 mm as a triage or an add-on test to establish a diagnosis (strong recommendation/very low quality evidence).

Underlying Values and Preferences. This recommendation places a high value on avoiding resource consumption and the risk of anaphylactic reactions at home in large proportion of patients who would be incorrectly classified by a skin prick test alone. It places a lower value on anaphylactic reactions in a controlled setting that can be managed by experienced personnel when oral food challenge is performed. This recommendation also places a high value on avoiding any unnecessary treatment in patients who would be incorrectly classified by a skin prick test as allergic to cow's milk.

Remarks. An average pretest probability of CMA (~40%) can be estimated based on the

history and presenting symptoms and would represent the majority of situations.

Recommendation 1.4

In settings where oral food challenge is not considered a requirement in all patients suspected of IgE-mediated CMA, in patients with low pretest probability of CMA we suggest using a skin prick test with a cut-off value of ≥3 mm as a triage test to avoid oral food challenge in those in whom the result of a skin prick test turns out negative (conditional recommendation/low quality evidence).

Underlying Values and Preferences. This recommendation places a relatively high value on avoiding burden and resource use with an oral food challenge test (~70% challenges avoided). It places a lower value on avoiding an allergic reaction (possibly a mild one) in around 1 in 25–50 patients misclassified as not having CMA while they would actually be allergic to cow's milk (2–4% false negative results).

Remarks. A low pretest probability of CMA (~10%) can be estimated based on the history and would represent, for instance, patients with unexplained gastrointestinal symptoms (eg, gastroesophageal reflux).

Question 2

Should in vitro specific IgE determination be used for the diagnosis of IgE-mediated CMA in patients suspected of CMA?

Population: patients suspected of CMA
Intervention: in vitro determination of a cow's milk specific IgE
Comparison: oral food challenge

Outcomes:
TP: Children will undergo oral food challenge that will turn out positive with risk of anaphylaxis, albeit in controlled environment; burden on time and anxiety for family; exclusion of milk and use of special formula. Some children with high pretest probability of disease and/or at high risk of anaphylactic shock during the challenge will not undergo challenge test and be treated with the same consequences of treatment as those who underwent food challenge.
TN: Children will receive cow's milk at home with no reaction, no exclusion of milk, no burden on family time and decreased use of resources (no challenge test, no formula); anxiety in the child and family may depend on the family; looking for other explanation of the symptoms.
FP: Children will undergo an oral food challenge which will be negative; 0064t15unnecessary burden on time and anxiety in a family; unnecessary time and resources spent on oral challenge. Some children with high pretest probability of CMA would not undergo challenge test and would be unnecessarily treated with elimination diet and formula that may led to nutritional deficits (eg, failure to thrive, rickets, vitamin D or calcium deficiency); also stress for the family and unnecessary carrying epinephrine self injector which may be costly and delayed diagnosis of the real cause of symptoms.
FN: Children will be allowed home and will have an allergic reaction (possibly anaphylactic) to cow's milk at home; high parental anxiety and reluctance to introduce future foods; may lead to multiple exclusion diet. The real cause of symptoms (ie, CMA) will be missed leading to unnecessary investigations & treatments.
Inconclusive results: the child would repeat serum IgE that may be distressing for the child and parents; increased cost of testing; alternatively child may undergo food challenge.
Complications of a test: can cause discomfort of blood test and bleeding that can cause distress and parental anxiety; food challenge may cause anaphylaxis and exacerbation of other symptoms.
Resource utilization (cost): sIgE is an expensive test and requires time for phlebotomy, but does not add time to the medical consultation.
TP - true positive (being correctly classified as having CMA); TN - true negative (being correctly classified as not having CMA); FP - false positive (being incorrectly classified as having CMA); FN - false negative (being incorrectly classified as not having CMA); these outcomes are always determined compared with a reference standard (ie, food challenge test with cow's milk).

Outcomes: Question 2

Outcome	Importance
TP	8
TN	7
FP	6
FN	8
Inconclusive results	5
Complications of a test	4
Cost	4

Summary of Findings

We did not find any systematic review of diagnosis of CMA with determining the cow's milk specific immunoglobulin E (IgE) in serum.

We found 25 studies that examined the role of cow's milk specific IgE in comparison to oral food challenge in patients suspected of CMA (1, 2, 4, 6–8, 10, 12, 17–22, 26–36). Seventeen studies used CAP-RAST or FEIA technique of which 13 used a cut-off threshold of ≥0.35 IU/L (2, 4, 6, 8, 18, 19, 21, 22, 28, 30–32, 35), 2 used a cut-off of ≥0.7 IU/L (10, 33), and 2 did not report a cut-off threshold (12, 34). Five studies used a Phadebas RAST technique (7, 21, 26, 27, 29), one study assessed PRIST RAST (36), one assessed Allercoat EAST (1), and Magic Lite (17).

Using the criteria of methodological quality suggested by the QUADAS questionnaire we found that in many studies the spectrum of patients was not representative of the patients who will receive the test in practice (ie, with suspected IgE-mediated CMA). In most studies the results of a reference standard were very likely interpreted with the knowledge of the results of the cow's milk specific IgE or skin prick test or vice versa. None of the studies reported uninterpretable or intermediate test results. One study reported 8% inconclusive challenge tests but did not report number of inconclusive skin prick tests (23).

We used studies that used UniCAP or CAP-System FEIA to inform this recommendation because these techniques are currently commonly used. Other techniques are either used less frequently because they evolved into the new ones or the studies included only several patients that made any estimates of test accuracy unreliable. The combined sensitivity in the studies of CAP-RAST and FEIA that used a cut-off of ≥0.35 IU/L was 0.72 (95% CI: 0.69–0.75) and the specificity was 0.57 (95% CI: 0.54–0.60). Sensitivity of the cow's milk-specific IgE measurement was lower when studies in patients with atopic eczema were excluded (8 studies; sensitivity 0.62, 95% CI: 0.58–0.67) with little change in specificity (0.62, 95% CI: 0.57–0.66). We further examined the influence of child's age on the accuracy of cow's milk-specific IgE measurement in the diagnosis of CMA. In children suspected of CMA who were on average younger than 12 months sensitivity of cow's milk-specific IgE was higher (0.77, 95% CI: 0.71–0.83; 2 studies) than in children older than 12 month of age (0.52, 95% CI: 0.45–0.58; 6 studies) with an reverse difference in specificity (0.52, 95% CI: 0.45–0.59 in children < 12 months versus 0.71, 95% CI: 0.64–0.77 in children > 12 months).

The combined sensitivity in the studies of CAP-RAST and FEIA that used a cut-off of ≥0.7 IU/L was 0.58 (95% CI: 0.52–0.65) and the specificity was 0.76 (95% CI: 0.70–0.81) (see evidence profile 4 for question 2) (6, 10, 20, 33).

Two studies also estimated the accuracy of cow's milk specific IgE with a threshold of 2.5 IU/L (6), 3.5 IU/L (20), and 5.0 IU/L (6). The sensitivity in the studies of CAP-RAST and FEIA that used a cut-off of ≥2.5 IU/L was 0.48 (95% CI: 0.35–0.60) and the specificity was 0.94 (95% CI: 0.88–0.98) (see evidence profile 5 for question 2). The sensitivity in the studies of CAP-RAST and FEIA that used a cut-off of ≥3.5 IU/L was 0.25 (95% CI: 0.17–0.33) and the specificity was 0.98 (95% CI: 0.94–1.00) (see evidence profile 6 for question 2) (20). Further increase of the cut-off of to 5.0 IU/L did not improve the accuracy (sensitivity: 0.30 [95% CI: 0.19–0.42), specificity: 0.99 (95% CI: 0.94–1.00)] (6). The overall quality of evidence across outcomes was very low.

Benefits and Downsides

In patients with low pretest probability of CMA (~10%) based on the history and presenting symptoms a negative result of cow's milk-specific IgE measurement (ie, < 0.35 IU/L) may help to avoid a burdensome and costly food challenge with cow's milk in around 49–69% of patients tested. However, when using IgE measurement with a cut-off value of ≥0.35 IU/L instead of a food challenge one may expect about 2% children younger than 12 months and almost 5% children older than 12 months being misclassified as not having CMA while they actually would be allergic to cow's milk (2–5% false negative results; see evidence profiles for question 2). These children will likely be allowed home and have an allergic reaction to cow's milk at home. False negative result may also lead to unnecessary investigations and possible treatments for other causes of symptoms while the real cause (ie, CMA) has been missed.

In patients with average pretest probability of CMA (~40%; an average rate of positive food challenge tests in the included studies) based on the history and presenting symptoms, measurement of cow's milk-specific IgE in serum with a threshold of ≥0.35 IU/L would incorrectly classify 17–29% of patients as allergic to cow's milk (while they would actually not be allergic; false positive results) most likely leading to perform-

ing a food challenge test anyway. In these patients one might also expect 9–19% false negative results that in some children are likely to lead to performing a food challenge test, but some children would be allowed home and would have an allergic reaction (possibly anaphylactic) to cow's milk at home. This makes the measurement of milk-specific IgE with a cut-off value of ≥0.35 IU/L unlikely to be useful as a single test allowing us to avoid food challenge testing in these patients. However, measurement of cow's milk-specific IgE with a threshold of 2.5 IU/L in patients with average pretest probability of CMA may help to avoid an oral food challenge in 20% of tested patients with an associated 3% risk of incorrectly classifying a patient as having CMA. In these patients with average initial probability of CMA, using a threshold of 3.5 IU/L one may avoid oral food challenge in 10% of tested patients and expect 1% false positive results. However, the above estimates of test accuracy with cut-offs of 2.5 and 3.5 IU/L are based on one study each and were performed in children younger than 12 months. The guideline panel considered them as not reliable enough to make recommendations based on these thresholds.

In patients with high pretest probability of CMA (~80%) based on the history (eg, an anaphylactic reaction in the past) determination of cow's milk-specific IgE in serum can help to avoid the risk and burden of food challenge test in around 47–70% of patients tested. However, if milk-specific IgE with a cut-off value of ≥0.35 IU/L is used and food challenge is not done, one may expect 6% false positive results in children older than 12 months and close to 10% false positive results in children younger than 12 months. These children would be unnecessarily treated with elimination diet and/or formula that might lead to nutritional deficits, there would be unnecessary stress for the family, use of unnecessary preventive measures (eg, carrying epinephrine self injector) and a correct diagnosis of the real cause of symptoms may be delayed.

In patients with high pretest probability of CMA measurement of cow's milk-specific IgE in serum with a threshold of 0.7 IU/L may help to avoid the oral food challenge in 50% of tested patients, with an associated 5% risk of incorrectly classifying a patient as having CMA. In these patients, using a threshold of 2.5 IU/L one may avoid oral food challenge in around 40% of tested patients and expect 1% false positive results. Setting the threshold of 3.5 IU/L one may avoid oral food challenge in 20% of tested patients and expect 0.4% false positive results. However, as mentioned above, the estimates of test accuracy with cut-offs of 2.5 and 3.5 IU/L are based on one study each and were performed in children younger than 12 months. The guideline panel considered them as not reliable enough to make recommendations based on these thresholds.

Other Considerations

The use of milk-specific IgE measurements in settings where oral food challenges are always performed is redundant given the limited sensitivity and specificity of IgE measurement compared with oral food challenge.

Conclusions

In patients suspected of CMA the net benefit of measuring cow's milk-specific IgE instead of oral food challenge with cow's milk is uncertain. The quality of the supporting evidence is very low.

In settings where the oral food challenge is done routinely and the clinician's thresholds for testing and treatment are such that exclusion and confirmation of CMA always has to be proven by oral food challenge, there is no need to perform cow's milk-specific IgE measurements.

In settings where clinicians follow a more prudent approach, determination of the concentration of milk-specific IgE may help to avoid an oral food challenge in selected patients.

In patients with low pretest probability of CMA a negative result of milk-specific IgE with a threshold of ≥0.35 IU/L can allow to avoid oral food challenge in 49–69% of tested patients with an associated risk of 2–5% false negative results.

In patients with average pretest probability of CMA determination of milk-specific IgE with a threshold of ≥0.35 IU/L as a single diagnostic test is unlikely to reduce the need for oral food challenge.

In patients with a high pretest probability of CMA a positive milk-specific IgE result with a threshold of ≥0.35 IU/L may help to avoid oral food challenge in 47–70% patients tested (those that tested positive) with associated 6–10% risk of false positive results.

Clinical Recommendations, Question 2

Recommendation 2.1

In practice settings where an oral food challenge is a requirement in all patients suspected of IgE-mediated CMA, we recommend using oral food

challenge with cow's milk as the only test without measuring a cow's milk-specific IgE level as a triage or an add-on test to establish a diagnosis (strong recommendation/low quality evidence).

Underlying Values and Preferences. This recommendation places a relatively high value on avoiding resource consumption and the risk of anaphylactic reactions at home in patients who would be misclassified by milk-specific IgE test alone. It places a lower value on anaphylactic reactions in a controlled setting that can be managed by experienced personnel when oral food challenge is performed. This recommendation also places a high value on avoiding any unnecessary treatment in patients who would be incorrectly classified by milk-specific IgE measurement as allergic to cow's milk.

Remark. This recommendation applies to clinical practice settings. In research settings there may be compelling reasons to perform skin prick tests even though a food challenge test with cow's milk is always being done.

Recommendation 2.2

In settings where oral food challenge is not a requirement, in patients with a high pretest probability of IgE-mediated CMA we suggest using cow's milk-specific IgE with a threshold of 0.7 IU/L to avoid oral food challenge if a result of milk-specific IgE turns out positive (conditional recommendation/low quality evidence).

Underlying Values and Preferences. This recommendation places a relatively high value on avoiding burden, resource use and very likely anaphylactic reactions during the oral food challenge test (food challenges would be avoided in 50% of patients with milk-specific IgE results ≥0.7 IU/L). It places a lower value on unnecessary treatment of around 1 in 20 patients misclassified as allergic to cow's milk (5% false positive results).

Remarks. A high pretest probability of CMA (~80%) can be estimated based on the history and would represent, for instance, patients who experienced an anaphylactic reaction in the past.

Recommendation 2.3

In settings where oral food challenge is not a requirement in all patients suspected of IgE-mediated CMA, in patients with an average pretest probability of IgE-mediated CMA we suggest using an oral food challenge test with cow's milk as the only test without measuring milk-specific IgE as a triage or an add-on test to establish a diagnosis (conditional recommendation/low quality evidence).

Underlying Values and Preferences. This recommendation places a high value on avoiding resource consumption and the risk of anaphylactic reactions at home in large proportion of patients who would be incorrectly classified by a milk-specific IgE test alone. It places a lower value on anaphylactic reactions in a controlled setting that can be managed by experienced personnel when oral food challenge is performed. This recommendation also places a high value on avoiding any unnecessary treatment in patients who would be incorrectly classified by a milk-specific IgE test as allergic to cow's milk.

Remarks. An average pretest probability of CMA (~40%) can be estimated based on the history and presenting symptoms and would represent the majority of clinical situations. Using higher cut-off values (eg, 2.5 IU/L) might be of benefit; however, we believe the available evidence does not allow us to make a recommendation to support any recommendation.

Recommendation 2.4

In practice settings where oral food challenge is not a requirement in all patients suspected of IgE-mediated CMA, in patients with low pretest probability of IgE-mediated CMA we suggest using milk-specific IgE measurement with a cut-off value of ≥0.35 IU/L as a triage test to avoid oral food challenge in those in whom the result of milk-specific IgE turns out negative (conditional recommendation/low quality evidence).

Underlying Values and Preferences. This recommendation places a relatively high value on avoiding burden and resource use with an oral food challenge test (~50–70% food challenges avoided). It places a lower value on avoiding an allergic reaction (possibly a mild one) in around 1 in 20–50 patients misclassified as not having CMA (2–5% false negative results).

Remarks. A low pretest probability of CMA (~10%) can be estimated based on the history and would represent, for instance, patients with unexplained gastrointestinal symptoms (eg, gastroesophageal reflux).

Question 3

Should in vitro specific IgE determination be used for the diagnosis of CMA in patients suspected of CMA and a *positive result of a skin prick test*?

Population: patients suspected of CMA with a positive skin prick test

Intervention: in vitro specific IgE determination

Comparison: oral food challenge

Outcomes:

TP: The child will undergo oral food challenge that will turn out positive with a risk of anaphylaxis, albeit in controlled environment; burden on time and anxiety for family; exclusion of milk and use of formula; some children with high pretest probability (based on history, clinical presentation and positive result of SPT) may receive treatment without performing food challenge with same consequences as those in whom challenge test was performed.

TN: The child will undergo oral food challenge that will turn out negative; burden on time and anxiety for family.

FP: The child will undergo an oral food challenge which will be negative; 0064t16unnecessary burden on time and anxiety in a family; unnecessary time and resources spent on oral challenge.

FN: The child will undergo oral food challenge which will turn out positive with risk of anaphylaxis, albeit in controlled environment; burden on time and anxiety for family; exclusion of milk and use of special formula.

Inconclusive results: repeated measurement of sIgE that can cause discomfort of blood test and bleeding which can cause distress and parental anxiety.

Complications of a test: can cause discomfort of blood test and bleeding which can cause distress and parental anxiety; food challenge may cause anaphylaxis and exacerbation of other symptoms.

Resource utilization (cost): sIgE is an expensive test and requires time for phlebotomy, but does not add time to the medical consultation.

TP - true positive (being correctly classified as having CMA); TN - true negative (being correctly classified as not having CMA); FP - false positive (being incorrectly classified as having CMA); FN - false negative (being incorrectly classified as not having CMA); these outcomes are always determined compared with a reference standard (ie, food challenge test with cow's milk).

Outcomes: Question 3

Outcome	Importance
TP	7
TN	6
FP	6
FN	7
Inconclusive results	4
Complications of a test	4
Cost	4

Summary of Findings

We did not find any systematic review of diagnosis of CMA with in vitro specific IgE or SPT.

We found 15 studies that examined the role of milk-specific IgE measurement and SPT in comparison to oral food challenge alone in patients suspected of CMA (1, 2, 4, 6–8, 10, 12, 17–22, 31). Only 3 of these studies reported results of using skin prick test and cow's milk specific IgE measurement together (8, 17, 21). All used a threshold for SPT of 3 mm. All 3 studies used different methods of determination of milk-specific IgE.

One study reported no negative results, all patients had either true or false positive results of SPT and milk-specific IgE combined and 4 results were discordant (8). The pooled sensitivity and specificity from the remaining 2 studies including 36 patients were 0.71 (95% CI: 0.29–0.96) and 0.93 (95% CI: 0.77–0.99). Discordant results of skin prick test and milk-specific IgE were observed in 28% of patients.

Using the criteria of methodological quality suggested by the QUADAS questionnaire we found that one study enrolled only patients with atopic eczema and the selection criteria were not described, in all studies the results of the tests were most likely interpreted with the knowledge of the other tests. The overall quality of evidence across outcomes was very low.

Benefits and Downsides

In patients with low pretest probability of CMA (~10%) based on the history and presenting symptoms, who have a positive result of a skin prick test, measurement of cow's milk-specific IgE is unlikely to be of benefit. It can help to avoid a food challenge in only 10% of patients tested (those with positive results of both tests) with an associated risk of 5% false positive results (see

evidence profile for question 3 in Appendix 2: Evidence profiles: diagnosis of CMA).

In patients with average pretest probability of CMA (~40%; an average rate of positive food challenge tests in the included studies) based on the history and presenting symptoms, who have a positive result of a skin prick test, measurement of cow's milk-specific IgE in serum can help to avoid a food challenge with cow's milk in around 22% of patients tested (those with positive results of both tests). However, when relying on a positive result of both skin prick test and milk-specific IgE measurement instead of a food challenge in these patients one may still expect about 3% of patients being misclassified as having CMA while they actually would not be allergic to cow's milk.

In patients with high pretest probability of CMA (~80%) based on the history (eg, an anaphylactic reaction in the past) positive results of both skin prick test and cow's milk-specific IgE measurement may help to avoid a burdensome and costly food challenge with cow's milk in around 42% of patients tested (those with positive results of both tests). However, when relying on a positive result of both skin prick test and milk-specific IgE measurement instead of a food challenge one may still expect about 1% of patients being misclassified as having CMA while they actually would not be allergic to cow's milk.

A negative result of milk-specific IgE in patient with a positive skin prick test is likely to lead to performing an oral food challenge test regardless (28% of tests were discordant).

Conclusions

In patients with low initial probability of CMA, who have a positive result of a skin prick test, the net benefit of measuring cow's milk specific IgE instead of oral food challenge with cow's milk is unlikely.

In patients with average and high initial probability of CMA, who have a positive result of a skin prick test, the net benefit of measuring cow's milk specific IgE instead of oral food challenge with cow's milk is uncertain. Positive results of both skin prick test and milk-specific IgE can help to avoid an oral food challenge in 22% of patients with average initial probability of CMA and in 42% of those with high initial probability of CMA. However, this benefit is counterbalanced by a risk of falsely classifying a patient as having CMA (3% in patients with initial average probability of CMA and 1% in those with high initial probability of CMA).

In patients suspected of CMA, who have a positive result of a skin prick test, a negative result of milk-specific IgE is likely to lead to performing food challenge test.

Clinical Recommendations, Question 3

Recommendation 3.1

In patients with a low initial probability of IgE-mediated CMA, who have a positive result of skin prick test (≥3 mm), we suggest oral food challenge rather than measuring cow's milk-specific IgE level with a cut-off value of ≥0,35 IU/L (conditional recommendation/low quality evidence).

Underlying Values and Preferences. This recommendation places a relatively high value on avoiding unnecessary treatment in patients who would be misclassified by milk-specific IgE test alone. It places a lower value on anaphylactic reactions in a controlled setting that can be managed by experienced personnel when oral food challenge is performed.

Recommendation 3.2

In patients with a an average or high initial probability of IgE-mediated CMA, who have a positive result of skin prick test (≥3 mm), we suggest measurement of cow's milk-specific IgE with a cut-off value of ≥0.35 IU/L to avoid food challenge test in those in whom the result of milk-specific IgE turns out positive (conditional recommendation low quality evidence).

Underlying Values and Preferences. This recommendation places a relatively high value on avoiding resource consumption and burden of food challenge test (~20% food challenges would be avoided in patients with average initial probability of CMA and ~40% in those with high initial probability). It places a lower value on unnecessary treatment of small proportion of patients who would be misclassified as having CMA (3% false positive results in patients with average initial probability of CMA and 1% in those with high initial probability).

Remarks. An average pretest probability of CMA (~40%) can be estimated based on the history and presenting symptoms and would represent the majority of situations.

A high pretest probability of CMA (~80%) can be estimated based on the history and would represent, for instance, patients who experienced an anaphylactic reaction in the past.

Question 4

Should in vitro specific IgE determination be used for the diagnosis of CMA in patients suspected of CMA and a *negative result of a skin prick test*?

Population: patients suspected of cow's milk allergy (CMA) with a negative skin prick test
Intervention: in vitro specific IgE
Comparison: oral food challenge

Outcomes:
TP: The child will undergo oral food challenge that will turn out positive with a risk of anaphylaxis, albeit in controlled environment; burden on time and anxiety for family; exclusion of milk and use of formula.
TN: The child will ingest cow's milk at home with no reaction, no exclusion of milk, no burden on family time and decreased use of resources (no challenge test, no formula); anxiety in the child and family may depend on the family; looking for other explanation of the symptoms.
FP: The child will undergo an oral food challenge that will be negative; unnecessary burden on time and anxiety in a family; unnecessary time and resources spent on oral challenge. Some children with high 0064t17pretest probability of CMA may not undergo challenge test and would be unnecessarily treated with elimination diet and formula that may lead to nutritional deficits (eg, failure to thrive, rickets, vitamin D or calcium deficiency); also stress for the family and unnecessary carrying epinephrine self injector that may be costly and delayed diagnosis of the real cause of symptoms.
FN: The child will be allowed home and will have allergic reactions (possibly anaphylactic) to cow's milk at home; high parental anxiety and reluctance to introduce future foods; may lead to multiple exclusion diet. The real cause of symptoms (ie, CMA) will be missed leading to other unnecessary investigations and treatments.
Inconclusive results: repeated measurement of sIgE that can cause discomfort of blood test and bleeding that can cause distress and parental anxiety.
Complications of a test: can cause discomfort of blood test and bleeding which can cause distress and parental anxiety; food challenge may cause anaphylaxis and exacerbation of other symptoms.
Resource utilization (cost): sIgE is an expensive test and requires time for phlebotomy, but does not add time to the medical consultation.

TP - true positive (being correctly classified as having CMA); TN - true negative (being correctly classified as not having CMA); FP - false positive (being incorrectly classified as having CMA); FN - false negative (being incorrectly classified as not having CMA); these outcomes are always determined compared with a reference standard (ie, food challenge test with cow's milk).

Outcomes: Question 4

Outcome	Importance
TP	7
TN	5
FP	5
FN	7
Inconclusive results	4
Complications of a test	4
Cost	4

Summary of Findings (Similar to Question 3)

We did not find any systematic review of diagnosis of CMA with in vitro specific IgE or SPT. We found 15 studies that examined the role of milk-specific IgE measurement and SPT in comparison to oral food challenge alone in patients suspected of CMA (1, 2, 4, 6–8, 10, 12, 17–22, 31). Only 3 of these studies reported results of using skin prick test and cow's milk specific IgE measurement together (8, 17, 21). All used a threshold for SPT of 3 mm. All 3 studies used different methods of determination of milk-specific IgE.

One study reported no negative results, all patients had either true or false positive results of SPT and milk-specific IgE combined and 4 results were discordant (8). The pooled sensitivity and specificity from the remaining 2 studies including 36 patients were 0.71 (95% CI: 0.29–0.96) and 0.93 (95% CI: 0.77–0.99). Discordant results of skin prick test and milk-specific IgE were observed in 28% of patients.

Using the criteria of methodological quality suggested by the QUADAS questionnaire we found that one study enrolled only patients with atopic eczema and the selection criteria were not described, in all studies the results of the tests were most likely interpreted with the knowledge of the other tests. The overall quality of evidence across outcomes was very low.

Benefits and Downsides

In patients with low initial probability of CMA (~10%) based on the history and presenting

symptoms, who have a negative result of a skin prick test (ie, diameter of < 3 mm), measurement of cow's milk-specific IgE with a cut-off value of 0.35 IU/L may help to avoid a food challenge with cow's milk in about 62% of patients. However, despite a negative result of both skin prick test and milk-specific IgE measurement one may still expect about 2% of patients being misclassified as not having CMA while they actually do (false negative results; see evidence profile for question 3). These children will likely be allowed home and have an allergic reaction to cow's milk at home. False negative result may also lead to unnecessary investigations and possible treatments for other causes of symptoms while the real cause (ie, CMA) has been missed.

In patients with average and high pretest probability of CMA (>40%) based on the history and presenting symptoms, who have a negative result of a skin prick test (ie, diameter of < 3 mm), measurement of cow's milk-specific IgE in serum with a cut-off value of 0.35 IU/L is unlikely to be of benefit. In patients with an average initial probability of CMA one would be able to avoid a food challenge with cow's milk in about 47% of patients with a risk of about 8% false negative results. In patients with a high initial probability of CMA one would be able to avoid a food challenge with cow's milk in about 30% of patients, but a risk of incorrectly classifying a patient as not having CMA would be high (about 17% false negative results). A positive result of milk-specific IgE in patient with a negative skin prick test is likely to lead to performing an oral food challenge test regardless.

Conclusions

In patients with low initial probability of CMA, who have a negative result of a skin prick test, the net benefit of measuring cow's milk specific IgE instead of oral food challenge with cow's milk is uncertain. Negative results of both skin prick test and milk-specific IgE can help to avoid an oral food challenge in about 60% of patients. However, this benefit is counterbalanced by approximately a 2% risk of falsely classifying a patient as not having CMA.

In patients with average or high initial probability of CMA, who have a negative result of a skin prick test, the net benefit of measuring cow's milk specific IgE instead of oral food challenge is unlikely.

In patients suspected of CMA, who have a negative result of a skin prick test, a positive result of milk-specific IgE is likely to lead to performing food challenge test.

Clinical Recommendations, Question 4

Recommendation 4.1

In patients with a low initial probability of IgE-mediated CMA, who have a negative result of a skin prick test, we recommend measuring cow's milk-specific IgE level as a triage test to avoid food challenge test in those in whom the result of milk-specific IgE turns out negative (strong recommendation/low quality evidence).

Underlying Values and Preferences. This recommendation places a relatively high value on avoiding burden and resource use with an oral food challenge test (around 60% tests avoided). It places a lower value on avoiding an allergic reaction (possibly a mild one) in around 1 in 50 patients misclassified as not having cow's milk allergy (false negative result).

Remarks. A low pretest probability of CMA (~10%) can be estimated based on the history and would represent, for instance, patients with unexplained gastrointestinal symptoms (eg, gastroesophageal reflux).

Recommendation 4.2

In patients with an average initial probability of IgE-mediated CMA, who have a negative result of a skin prick test, we suggest oral food challenge rather than measuring cow's milk-specific IgE level (conditional recommendation/low quality evidence).

Underlying Values and Preferences. This recommendation places a relatively high value on avoiding resource consumption and the risk of anaphylactic reactions at home in patients who would be misclassified as not having CMA by skin prick test and milk-specific IgE tests. It places a lower value on anaphylactic reactions in a controlled setting that can be managed by experienced personnel when oral food challenge is performed.

Remarks. An average pretest probability of CMA (~40%) can be estimated based on the history and presenting symptoms and would represent the majority of situations.

Recommendation 4.3

In patients with a high initial probability of IgE-mediated CMA, who have a negative result of a skin prick test, we recommend oral food challenge rather than measuring cow's milk-specific

IgE level (strong recommendation/low quality evidence).

Underlying Values and Preferences. This recommendation places a relatively high value on avoiding resource consumption and the risk of anaphylactic reactions at home in a large proportion of patients who would be misclassified as not having a CMA by skin prick test and milk-specific IgE tests. It places a lower value on anaphylactic reactions in a controlled setting that can be managed by experienced personnel when oral food challenge is performed.

Remarks. A high pretest probability of CMA (~80%) can be estimated based on the history and would represent, for instance, patients who experienced an anaphylactic reaction in the past.

Question 5

Should *allergen microarrays* or *component resolved diagnostics* be used for the diagnosis of IgE-mediated CMA in patients suspected of CMA?

Population: patients suspected of CMA
Intervention: allergen microarrays or component-resolved diagnostics
Comparison: oral food challenge

Outcomes:
TP: The child will undergo oral food challenge that will turn out positive with a risk of anaphylaxis, albeit in controlled environment; burden on time and anxiety for family; exclusion of milk and use of formula.
TN: The child will receive cow's milk at home with no reaction, no exclusion of milk, no burden on family time, and decreased use of resources (no challenge test, no formula); anxiety in the child and family may depend on the family; looking for other explanation of the symptoms.
FP: The child will undergo an oral food challenge that will be negative; unnecessary burden 0064t18on time and anxiety in a family; unnecessary time and resources spent on oral challenge.
FN: The child will be allowed home and will have an allergic reaction (possibly anaphylactic) to cow's milk at home; high parental anxiety and reluctance to introduce future foods; may lead to multiple exclusion diet. The real cause of symptoms (ie, CMA) will be missed0064t19 leading to unnecessary investigations and treatments.
Inconclusive results: the child would have SPT done and subsequent testing or treatment would depend on its results (see Question 1).
Complications of a test: can cause discomfort of blood test and bleeding that can cause distress and parental anxiety; food challenge may cause anaphylaxis and exacerbation of other symptoms.
Resource utilization (cost): a very expensive test, but it does not add time to the medical consultation.
TP - true positive (being correctly classified as having CMA); TN - true negative (being correctly classified as not having CMA); FP - false positive (being incorrectly classified as having CMA); FN - false negative (being incorrectly classified as not having CMA); these outcomes are always determined compared with a reference standard (ie, food challenge test with cow's milk).

Outcomes: Question 5–Should Component-Resolved Diagnostics Be Used for the Diagnosis of IgE-Mediated CMA?

Outcome	Importance
TP	6
TN	5
FP	5
FN	6
Inconclusive results	4
Complications of a test	4
Cost	5

Summary of Findings

We did not find any systematic review of the microarrays or component-resolved diagnostics used for the diagnosis of CMA.

We found 4 studies that examined the role of cow's milk allergen-specific IgE measurement with microarrays (18, 37–39). Two of these studies did not use a reference standard (37, 38) and one did not report any data on test accuracy (39) These 3 studies used a home-made allergen chip. One study used a commercially available allergen microarray, however, it was custom modified for the purpose of this study (18). This study also examined the role of component-resolved diagnostics in comparison to oral food challenge in patients suspected of CMA using an allergen microarray. We did not identify any study of unmodified commercially available allergen microarray compared with the oral food challenge test used for the diagnosis of CMA.

In the study that used customized allergen microarray in children suspected of IgE-mediated cow's milk allergy estimated sensitivity was 0.60 (95% CI: 0.43–0.74) with specificity of 0.84 (95% CI: 0.69–0.93).

Conclusions, Question 5

Any clinical benefit resulting from using allergen microarrays in the diagnosis of CMA is currently unknown.

Clinical Recommendations, Question 5

Recommendation 5.1

We suggest that allergen microarrays are used only in the context of well designed and executed studies that investigate the accuracy of commercially available allergen microarrays compared with oral food challenge with cow's milk in patients suspected of IgE-mediated CMA.

Recommendation 5.2

We suggest that more well designed and executed studies of component-resolved diagnostics compared with oral food challenge with cow's milk are performed in patients suspected of IgE-mediated CMA.

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Section 10: Oral Food Challenge Procedures in the Diagnosis of CMA

Overview

The oral food challenge (OFC) is considered the

a. Confirmation of suspicion of cow's milk allergy (CMA)
b. periodical follow-up of the condition and monitoring of the resolution of CMA
c. Assessment of tolerance in SPT-positive breast-fed infants suspected of CMA who have not yet ingested cow's milk (CM) proteins
d. Assessment of tolerance of cross-reactive foods (beef, mare's milk, donkey's milk, etc)
e. Evaluation of CM reactivity in persons with multiple dietary restrictions, usually because of subjective complaints
f. Exclusion of possible immediate reactions to milk in chronic conditions such as atopic dermatitis or allergic eosinophilic esophagitis
g. Evaluation of the tolerance threshold to CM proteins

A double-blind, placebo-controlled food challenge (DBPCFC) is the method of choice for research and delayed reaction settings. It should be performed in the face of an open challenge with uncertain outcome. In all the other situations, challenges can be performed openly. Except when dealing with delayed allergic reaction (chronic diarrhea, colitis, allergic proctocolitis, gastroesophageal reflux) without CM-specific IgE, OFCs with CM

must be performed in a hospital setting. Low-risk challenges in cooperative patients are appropriate for the office setting.

However, all challenge procedures carry a certain risk and are labor-, time-consuming, and costly. OFC is essential for planning avoidance regimens, reduce of the risk of inadvertent exposure, and validate efforts to avoid CM. Negative OFC expands dietary options and thereby nutrition and quality of life. It is also cost-sparing and reduces the use of special formula.

Introduction

The diagnosis of CMA can be achieved with certainty only after direct observation of clinical events after milk ingestion. In fact, the common tests to identify CM sensitization (at cutaneous level or using specific IgE determination) have no absolute accuracy (1). They can return often falsely positive in children who tolerate milk, or conversely can be negative even in the presence of a delayed, non-IgE mediated, CMA. The OFC and in particular the DBPCFC is considered today, according to the literature, the "gold standard" for diagnosing food allergies (2, 3), able to minimize false positive diagnoses. Such a specific diagnosis will prevent unnecessary and potentially deleterious dietary restrictions when a suspected CMA is not present. Unfortunately, in the world not all children can avail themselves of the OFC in milk allergy evaluation (4, 5). Resources for the practical planning and carrying-out of OFCs are available through many scientific societies (6–8) and lay organizations (9).

Definitions

OFC

OFCs with cow's milk are in vivo diagnostic tests performed to definitely confirm a preliminary suspicion of CMA. OFCs can be performed in 3 different ways:

a. Open, where everyone is aware that milk is brought to the child that day
b. Single-blinded, where the pediatrician is aware of the content but child and parents do not
c. DBPCFC when neither the pediatrician nor the child or parents know the day when milk will be administered.

Positive/Negative OFC

An OFC resulting in a clinical reaction is defined a "positive" or "failed" challenge, whereas an OFC without a clinical reaction is termed a "negative" or "passed" challenge. For the purpose of this document, the authors chose to use positive and negative terminology. A positive challenge will give indication of the tolerated dose, if any, thus allowing the planning of elimination diets with complete or partial exclusion of CM proteins.

Immediate and Delayed Reactions After OFC

According to the majority of authors, allergic reactions are defined as immediate when occurring within 2 hours after administration of the intake of milk, delayed when appearing after more than 2 hours (10, 11) (see also *Mechanisms*). Some authors evaluated delayed reactions occurring up to 7, (12) 9, (13) or 14 days (14). Within those periods, however, the diagnosis of delayed reaction may be difficult because when the child returns home, multiple environmental factors (infections, dietary factors, emotional, casual contacts, sports-related physical activity) may impinge diagnostic interpretation. Frequently, immediate and delayed symptoms are present concomitantly in the same child (15).

Indications for OFCs

The AAAAI work group (6) recently re-evaluated the indications for an OFC to be performed, adding some not contained in previous statements including the European statement. Specifically for cow's milk, this panel agrees that the after should be indications to a diagnostic challenge:

- Initial diagnosis of CMA after acute reactions
- Evaluation of the tolerance threshold to CM proteins
- Periodical follow-up of the condition and monitoring of the resolution of CMA
- Assessment of tolerance in SPT-positive breast-fed infants which have not yet directly taken CM proteins
- Exclusion of possible immediate reactions to milk in chronic conditions such as atopic dermatitis or allergic eosinophilic esophagitis
- Evaluation of CM reactivity in persons with multiple dietary restrictions, usually because of subjective complaints

- Assessment of tolerance to cross-reactive foods (beef, equine milks, etc)
- Assessment of the effect of food processing on food tolerability, eg, beef tolerated in cooked form.

OFC is a complex test, requiring several hours for both the pediatrician, his or her staff and the family, and not without risks for the patient. Given the frequency of suspected CMA, indications for performing an oral food challenge should be weighed carefully. Furthermore, although it is considered for years the gold standard in diagnosis of CMA, there are still many controversial issues about which children must undergo an OFC, and what is the best way to perform the study.

Open Challenge

This is the simplest procedure, requiring less commitment to the pediatrician, the patients and their families and thus lowering costs for the health facilities. After a thorough physical examination, the linchpin for a comparative assessment of pre- and postchallenge, CM is administered openly in increasing doses up to the dose liable to be responsible for symptoms. Clinical observation will be carried-out for about 2 hours after the last dose of milk for immediate reactions and, after discharge, an appointment should be scheduled in the clinic for observation of delayed reactions. Given its simplicity, open challenge can be considered a reasonable first choice to evaluate an adverse reaction to milk. However, it has been shown even in children that up to half of positive open challenges are not reproduced in DBPCFC (1).

Single-Blinded Challenge

Single-blind is a procedure in which the pediatrician is aware of which food is given to the child at that moment. It is used less than open or DBPCFC, because it entails in principle the same difficulties found with a DBPCFC, but is a bit less reliable as it introduces the possible bias of subjective interpretation by observer. Single-blind OFC may be conducted with or without placebo, depending on the physician's judgment of the potential for subjective symptoms and the patient's anxiety (6). In case of immediate reactions, it will consist of 2 sessions, one with CM and one with placebo, completed on one day with at least a 2-hour period separating the 2 sessions, or on separate days. If 2 foods are tested on the same day, the sequence of the foods is not revealed to the child. We must underline that this option is valid only when delayed symptoms can be excluded in advance. For patients reporting delayed onset of symptoms, sessions of blinded OFC should be separated by several days or weeks (16, 17). In patients suspected of having a psychologic response, the *verum* might be tested first. In this case, a negative challenge will spare a second day of procedure. If symptoms develop, CM should be retested for reproducibility in a DBPCFC (3, 7).

After a negative blind challenge, CM would be administered openly: this recommendation is based on the possibility of detecting a reaction to an open feeding in children with delayed CM reactions (18).

Double-Blind, Placebo-Controlled Food Challenge (DBPCFC)

A DBPCFC is the oral administration, usually on different days, of placebo and increasing amounts of milk. First used in 1973 by May (19) in the assessment of allergic reactions to foods in children with bronchial asthma, the DBPCFC is now the test of choice in the diagnosis of CMA. In this procedure, only personnel who prepared the test is aware of the food offered at the time: CM (*verum*) or placebo. Such personnel, not in contact with either the child or the family or the doctor, is the only one to prepare the meals and, in principle, to decide the randomization. The randomization code is prepared in closed envelopes. A major problem in the preparation of the placebo is the avoidance of possibly sensitizing foods. In general, for milk challenges the use of amino acid mixtures make the test safe from misinterpretations. If another placebo is used, the absence of sensitization should be tested by SPT. To enhance masking of appearance and flavor, it is necessary that the amount of placebo in the verum is approximately half the cow's milk. On completion of the challenges, the code is broken, and results are discussed with the patient or parent. Placebo reactions are infrequent, but possible (20).

Open or Blinded? General Indications

The choice of the procedure has to be done according to the indications listed in Table 10-1 (general indications) and Table 10-2 (indications according to clinical history). Challenges should not be performed in general when a negative skin test, undetectable serum milk-specific IgE level, and no history of convincing symptoms of

immediate CMA make the condition very unlikely. In these cases, gradual home introduction of milk may be attempted. For those patients who have a history of convincing immediate allergic reactions to milk (within 2 hours) or who present with a history of anaphylaxis, even in the setting of negative laboratory and skin tests, a physician-supervised OFC is needed to confirm or refute allergy to this food.

Table 10-1. Open or Blinded? General Indications

	Method of choice for scientific protocols
DBPCFC	Method of choice for delayed reactions with chronically developing symptoms Mandatory for subjective symptoms After an uncertain OFC
Open milk challenge	For evaluation of immediate symptoms in IgE-mediate CMA When the probability of a negative OFC is high (in this case, consider a SBPCFC using placebo first) A negative DBPCFC should be followed by an open-OFC

Preliminary Evaluation of CM Sensitization

In DRACMA, specific recommendations are made for allergy evaluation using SPT, APT, and/or specific IgE determinations. Whatever test is done, it should be remembered that serum CM-specific IgE levels and sizes of SPT wheals do not predict the severity of the clinical reactions (3, 27).

These guidelines for deciding when to perform an OFC on the basis of the results of serum CM-specific IgE and SPT are constantly evolving and need to be frequently updated according to new evidence.

Diagnostic Elimination Diet

A trial elimination diet may be helpful to determine if a disorder with frequent or chronic symptoms is responsive to dietary manipulation. Trial elimination diets are diagnostic and therapeutic procedures that may be used in children with presumed CMA (see section on *Diagnostic Elimination Diets*) (28, 29).

Clinical Assessment

To undergo challenge procedures, the patient must be well, without intercurrent fever episodes, vomiting, diarrhea, nor seasonal rhinitis and/or asthma (30). Atopic dermatitis should be stabilized in the weeks preceding the OFC, and not subject to significant fluctuations that would make the test difficult to interpret. A 10-point increase in postchallenge SCORAD is considered the minimum threshold for defining a significant worsening of atopic dermatitis (31). The child should discontinue antihistamine therapies long enough to get a normal histamine skin reactivity (32), and at least for 72 hours before OFC (11).

OFC Benefits

The benefits of a positive OFC include a conclusive diagnosis of CMA demonstrating the need for continued counseling in strict avoidance of cow's milk, reduction of the risk of inadvertent exposures, reduction of anxiety about the unknown, and validation of the patients and families efforts to avoid the food. It allows accurate prescription of elimination diet. A positive OFC may induce fear of reactions, thus leading to closer monitoring of avoidance. The benefits of a negative OFC include expansion of the diet and improvement of the patient's nutrition and quality of life. This can spare unnecessary health expenses and reduce the use of special formula.

Table 10-2. Open or Blinded? Indications According to Clinical History

Clinical Situation	Indication	Challenge Type	Setting
CMA anaphylaxis21	Not indicated at diagnosis Verify every 12 months for assessment of tolerance onset	Open	Hospital
Generalized, important allergic reaction in a single organ (such as urticaria, angioedema, or vomiting, or respiratory symptoms) occurred immediately (within 2 hours after ingestion) with positive CM IgE tests (22)	Not indicated at diagnosis Verify every 9–12 months, depending on age, for assessment of tolerance onset	Open	Hospital
Clinical history of Food Protein Enterocolitis from cow's milk with at least one previous episode, both in presence and absence of CMA-specific IgE (6)	Not indicated at diagnosis Verify every after 18–24 months, for assessment of tolerance onset	Open	Hospital
Moderate to severe atopic dermatitis (AD) resistant to properly done topical therapy for a reasonable period in presence of IgE antibodies to CM. AD of any entity, whether associated with the occurrence of other possible allergic symptoms (rhinitis, asthma, diarrhoea, vomiting, etc.) both in the presence and absence of specific IgE to milk (23)	Indicated	DBPCFC	Hospital

Table 10-2. (Continued)

Clinical Situation	Indication	Challenge Type	Setting
Clinical situation not suggestive and/or clinical response not immediate (eg. Atopic dermatitis) when patient or her family are convinced of the existence of CMA and thus inclined to interpret any clinical signs as related to cow's milk ingestion(24)	Indicated	DBPCFC	Hospital
First introduction of cow's milk in CM-sensitized children	Indicated	Open	Hospital
Reintroduction of cow's milk excluded from the diet for several months on a mere detection of specific IgE in the absence of a suggestive clinical history(25)	Indicated	Open	Hospital
Clinical subjective symptoms (nausea, abdominal pain, itching, oral, etc.) after CM ingestion(7,26)	Indicated	DBPCFC	Hospital
Clinical picture of delayed allergic reaction (chronic diarrhea, colitis, allergic proctocolitis, gastroesophageal reflux) without CM-specific IgE(6)	Indicated	Open	Home

Table 10-3. The OFC With Milk: Methodological Details

Authors	Dose	Intervals	Placebo	Method	Time of Reaction
Bock SA50 Sicherer SH3 Sicherer SH51 Rancé F52	Total of 100 mL of fresh milk The powdered forms with a weight of 8 to 10 g are approximately equivalent to 100 mL of skim milk	Doses at 10- to 15-minute intervals for ~90 minutes followed by a larger, meal-size portion of milk a few hours later	Not specified		
Chapman JA8	7 doses with increasing doses, eg, 1, 4, 10, 20, 20, 20, and 25% of the total	?	Not specified		
Niggemann B11	7 doses: 0,1; 0,3; 1, 3, 10, 30, 100 mL	Each 20'	Neocate SHS, Liverpool, United Kingdom		
Sporik R53	day 1: one drop inside lip, 0.5, 2.5, 5, 10, 20, and 30 mL day 2: 30, 60 and 120 mL day 3: normal volumes of milk, ie, more then 450 mL per day	At 30 minutes intervals	Open	Open challenge with CM	I & D (up to 1 week)
Saarinen KM54	Up to 160 mL drops of CM placed on the volar side of the wrist, the cheek and the lips, followed by CM formula given orally in quantities of 1, 10, 50, and 100 mL. The next day, infants without symptoms continued to receive the formula at home up to 186 mL	30 ± 60 minutes	Open at the out-patient clinic	Open challenge with CMF	I & D (up to 5 days)
Majamaa H55	On the first day, rising doses of the placebo or test formula (1, 5, 10, 50, and 100 mL) challenge period 1 week. Challenge started in the hospital, continued at home	The doses were given at approximate 30-minute intervals until milk intake appropriate for the age was reached	Neocate (SHS Int. Ltd., Liverpool, UK)	DBPCFC or open challenge with CMF	I & D (up to 7 days)
Roehr CC46	Up to 143 mL Successive doses (0.1, 0.3, 1.0, 3.0, 10.0, 30.0, and 100.0 mL) of fresh pasteurized CM containing 3.5% fat, soy milk, and wheat powder (Kröner; total amount of 10 g of wheat protein) were administered	Time interval between doses 20 minutes	Neocate; SHS, Liverpool, UK	DBPCFC with CM	I: 2 hours. D: 48 hours
Eigenmann PA56	Up to 10g powder (77 mL reconstituted formula). The food was given in graduated servings, up to a total corresponding to 10 g of dehydrated food	The time interval between doses was 60 ± 80 minutes	Not reported	Challenge (either open or DBPC) with dehydrated CM	NR
Klemola T45	Not reported	Not reported	Extensively hydrolyzed formula Soy formula Amino acid formula		I: within 2 hours D: within 5 days
Bahna SL14	If high risk history: one drop of CM:water 1:100, then one drop of undiluted CM, then 10 drops, 10 mL, 100 mL	Each hour	Not reported		
Roehr CC46	Up to 143 mL Successive doses (0.1, 0.3, 1.0, 3.0, 10.0, 30.0, and 100.0 mL) of fresh pasteurized CM containing 3.5% fat, soy milk, and wheat powder (Kröner; total amount of 10 g of wheat protein) were administered	Time interval between doses 20 minutes	Neocate; SHS, Liverpool, UK	DBPCFC with CM	I: 2 hours. D: 48 hours

Table 10-3. (Continued)

Authors	Dose	Intervals	Placebo	Method	Time of Reaction
Eigenmann PA56	Up to 10g powder (77 mL reconstituted formula) The food was given in graduated servings, up to a total corresponding to 10 g of dehydrated food	The time interval between doses was 60 ± 80 minutes minutes	Not reported	Challenge (either open or DBPC) with dehydrated CM	NR
Klemola T45	Not reported	Not reported	Extensively hydrolyzed formula Soy formula Amino acid formula		I: within 2 hours D: within 5 days
Bahna SL14	If high risk history: one drop of CM:water 1:100, then one drop of undiluted CM, then 10 drops, 10 mL, 100 mL	Each hour	Not reported		

OFC Limitations

Challenge procedures are risky, labor- and time-consuming, and costly. Before performing a challenge, procedural details, risks and benefits must be discussed with the patient and his or her family (3). Immediate systemic reactions can be severe. They are unpredictable on the basis of sensitization, but an association can be found between clinical history of severe symptoms and symptoms after OFC (33, 34). Similarly, a number of risk factors for more severe reactions have been suggested: unstable or severe asthma, progressively more severe reactions, reactions to small quantities of cow's milk or treatment with beta-adrenergic antagonists (6). To minimize these risks, venous access should be maintained during CM challenges, in particular when a severe systemic reaction seems possible. In Europe it has been recommended that for young children intravenous access should be applied only in selected cases (7). These recommendations take into account the fact that deaths from anaphylaxis are more frequently described after the age of 5 years. Given these considerations, it is essential that be conducted under the observation of a team with specific expertise in pediatric allergy and supplied with all equipment and drugs for emergency treatment (35).

OFCs are more standardized for IgE- than for non-IgE-mediated reactions; in the latter case, the observation should be prolonged for an extended period of time. Thus, a diagnostic elimination diet is generally prescribed and sensitization tests are usually carried-out before DBPCFC. The state of the art CMA work-up uses the informed prescription of DBPCFC and various diagnostic tests according to clinical context. The combination of prechallenge test in DRACMA is object of GRADE evaluation (see section on *GRADE Assessment of CMA Diagnosis*).

OFCs In Children With Previous Anaphylactic Reaction

A recent anaphylactic reaction to cow's milk contraindicates OFCs except in the after situations:

- If the severe reaction occurred immediately after simultaneous introduction of many foods at the same time: typical example is the introduction of the first solid meal including CM proteins (and many other putative food allergens) in a breast-fed
- For the assessment of tolerance to cow's milk after a reasonable period from previous anaphylactic reaction.

In these cases, the hospital setting with ICU availability is mandatory.

OFC Setting

The challenges are generally labor-intensive and carry some risk to the patient. Anyone who performs such challenges on children and adults with suspected CM allergies must have the background and equipment to recognize symptoms of allergy and to treat anaphylactic reactions (36). The first step is to consider whether the test can be performed at home or needs to be under direct physician supervision. There are many specific issues that must be considered in this particular decision. In general, whenever there is an even remote potential for an acute and/or severe reaction, physician supervision is mandatory. This decision for a supervised challenge includes, but is not limited to, a history of prior significant reactions and/or positive tests for IgE to milk (3). The ideal setting is hospital, both at an in-patient and out-patient level (37). When there is a very high risk for a severe reaction but OFC is required, challenges preferably should be done in the

intensive care unit. Low-risk challenges in cooperative patients are appropriate for the office setting.

Times and doses can vary according to clinical history. For a suspected FPIES, the procedure should be administered with intravenous access with prolonged observation. For immediate reactions, a limited observation time can ensure appropriate diagnostic accuracy. In delayed forms, longer observation periods will be necessary. Challenges requiring exercise to precipitate symptoms need to be performed where suitable exercise equipment is available (38).

Challenge Preparation: Vehicles and Masking

Evidence indicates that processing, including heating (and presumably drying), has no effect on the allergenicity of cows' milk (39). Thus, liquid whole milk, nonfat dry milk, and infant formula have been used as challenge materials in various clinics (40). For the placebo to be used, it is relevant that eHF, safe for most of cows' milk-allergic infants, can determine occasional allergic reactions in exquisitely allergic infants (41–44). In general cow's milk hydrolysate or soy formula are supported as placebo in the literature (45) and amino acid formula are considered an advance in clinical and research contexts (46, 47). When challenges are done using dehydrated cow's milk in capsules, lactose is used as placebo. However, the "capsule" is not the ideal presentation as it escapes the oral phase and lactose has been associated with reactivity in CM-allergic children (48, 49).

Challenge Procedure

In absence of comparative studies between different challenge protocols, there is no universal consensus on timing and doses for milk challenge administration. The consensus documents published in this field (6, 7) report some example of procedures, but the suggestion to individualize doses and times based on the clinical history remains valid (57, 58). Initial doses has been suggested to be 0.1 mL,(7) but can vary according to the risk of reaction and type of milk allergy (IgE vs. non-IgE-mediated) (6). Labial CM challenges have been suggested as a safe starting point for oral challenges by some researchers. This procedure begins with placing a drop of milk on the lower lip for 2 minutes and observing for local or systemic reactions in the ensuing 30 minutes (59).

Given these observations, this panel recommends the after for milk challenges in IgE-mediated CMA:

1. Total dose should be calculated according to the maximum consumed per serving or based on the total weight of the patient (6);
2. Use the same type of milk the patient will be consuming everyday in case of negative challenge;
3. Chose the least allergenic placebo possible, with preference for the type of milk the patient will be administered everyday in case of positive challenge;
4. Start with a dose clearly under the expected threshold dose, for example, the amount that the patient reacted to previously;
5. In general, one drop, or a 0.1 mL dose, is suitable for starting, but in high-risk cases one drop of CM:water 1:100 can be used;
6. Give a dose every 20–30 minutes; this will minimize the risk of severe allergic reaction and allow precise identification of the lowest provoking dose;
7. Increase the doses using a logarithmical modality, for instance: 0.1, 0.2, 0.5, 1.5, 4.5, 15, 40, and 150 mL (total 212 mL (60)); or 0.1, 0.3, 1.0, 3.0, 10, 30, and 100 mL (total 145 mL (61)); or 0,1; 0,3; 1, 3, 10, 30, and 100 mL (total 144 mL (11, 46));
8. To minimize the possibilities of identification, dilute the *verum* with the placebo 50:50 when administering CM;
9. Administer a placebo sequence in identical doses on a separate day;
10. Discontinue the procedure on first onset of objective symptoms or if no symptom develop after challenge;
11. Consider only reactions occurring within 2–3 hours after stopping the procedure;
12. Complete a negative procedure with open administration of CM.

For delayed reactions, the same rules apply except:

Rule 4: start with a 0.1 mL dose.
Rule 5: does not apply.
Rule 6: the interval in that case should be calculated according to the clinical history.
Rule 11: consider reactions occurring within 24–48 hours after stopping the procedure.

Challenge Interpretation

An OFC with milk should be stopped at the first onset of objective symptoms (62). Even mild

objective signs, such as a few skin wheals in the absence of gastrointestinal or respiratory symptoms, may not be diagnostic of CMA and can be contradicted by a subsequent DBPCFC (63, 64). For this reason, during OFCs skin contact with milk must be carefully avoided. Subjective symptoms include itching, nausea or dysphagia, sensation of respiratory obstruction, dyspnoea, change in behavior, prostration, headache, or refusal of milk.

Objective symptoms include:

- Generalized urticaria
- Erythematous rash with itching and scratching
- Vomiting or abdominal pain
- Nasal congestion
- Repetitive sneezing
- Watery rhinorrhea
- Rhino-conjunctivitis
- Changes in tone of voice
- Stridor
- Laryngospasm
- Inspiratory stridor
- Cough and/or wheezing
- Abnormal pallor
- Change in behavior (62)
- Increased heart rate by at least 20% (this can occur by anxiety)
- Decreased blood pressure by more than 20%
- Collapse
- Anaphylaxis

Sometimes subjective symptoms may be the harbinger of an incipient allergic reaction (6). If the child is able to ingest milk without any reaction, the challenge may be considered negative for immediate reaction, but at least 24–48 hours are necessary to exclude the possibility of delayed reactions.

Laboratory Data for OFC Interpretation

Attempts to use laboratory studies to validate the results of OFCs have a long history. Serum tryptase and urinary 1-methylhistamine have been evaluated as parameters for monitoring oral milk challenges in children, but their accuracy characteristics are lacking (65). Decreases in peripheral blood eosinophils and increases in serum eosinophil cationic protein (ECP), 8 to 24 hours after a positive challenge have been suggested as indicating a positive food challenge (66), but this finding has not been reproduced (67). FENO values are not predictive and not related to the occurrence of a positive reaction during cow's milk challenges in infants, suggesting that a positive reaction may not result from eosinophilic activation (68). Infants with atopic eczema and CMA exhibit markedly increased systemic pro-allergenic IL-4 responses on intestinal antigen contact (69, 70). While a failed oral challenge with cow's milk is associated with increase in both ECP and tumor necrosis factor (TNF)-α, allergic infants with delayed intestinal manifestations show an elevation of fecal TNF-α (71). These observations, however, are of scarce utility for diagnostic judgment.

Delayed Reactions Interpretation

A protocol for two-stage DBPCFC has been proposed to clarify delayed type CMA in patients presenting with predominantly gastrointestinal symptoms from 2 hours and up to 6 days after milk exposure. This procedure is able to differentiate immediate-type IgE-dependent, or delayed-type IgE-independent CMA (72). In non-IgE-mediated food protein-induced enterocolitis syndrome, in which there is a low risk for immediate reactions in the first hour, with symptoms usually starting within 1 to 4 hours after milk ingestion, the entire portion of the challenge may be administered gradually over a period of 45 minutes and divided into 3 smaller portions (6, 73).

After the Challenge . . .

A negative "remission" challenge ends up with the open reintroduction of cow's milk and dairy products. This represents for the patient an important step toward a "normal" personal and social life. However, many patients do not of themselves ingest the food and pursue an "unofficial" elimination diet. Reasons include fears of persistence of CMA, recurrent pruritus or nonspecific skin rashes after ingesting milk (74). After a negative challenge, however, a patient with CMA should not be lost to medical monitoring, to prevent such untoward eliminations, and to reassess possible minor complaints (eg, gastrointestinal) associated with CMA.

References, Section 10

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