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Exclusively breastfed infants at risk for false negative double blind placebo controlled milk challenge

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Key words
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Summary
The double blind placebo controlled food challenge (DBPCFC) is the gold standard for diagnosing cow's milk allergy (CMA). However, false-negative DBPCFC have been reported. We present 2 cases with a false negative DBPCFC in exclusively breastfed infants suspected of CMA. These cases highlight the occurrence of severe allergic reactions of infants who were exclusively breastfed. Several reported causes of a false negative DBPCFC will be discussed. However, there is currently no clear understanding of the cause of a false negative DBPCFC. This paper highlights that a negative outcome of a DBFCFC must be interpreted with caution, because a severe allergic reaction might occur upon re-introduction of cow's milk. Therefore, an additional open food challenge under medical supervision is recommended in exclusively breastfed infants with a negative DBPCFC.

Abbreviations:
AAF: Amino acid formula
CMA: Cow's milk allergy
CMP: Cow's milk protein
DBPCFC: Double-blind placebo controlled food challenge
OFC: Open food challenge
pHF: Partially hydrolysed formula
w-eHF: Whey-based extensively hydrolysed formula
SPT: Skin prick test

Background

Cow's milk allergy (CMA) is a common food allergy in infants (1,2). The double-blind placebo controlled food challenge (DBPCFC) is the gold standard for diagnosing CMA (3). However, false-negative DBPCFC have been reported with a prevalence varying from 3-13% (4-6). We encountered two cases of exclusively breastfed infants who underwent a DBPCFC with a negative outcome, followed by an allergic reaction upon introduction of cow's milk protein (CMP).

Case A

A full term boy with a normal birth weight and APGAR scores had postnatal complications of persisting pulmonary hypertension of the neonate and perinatal sepsis, for which he was managed with surfactant, breathing support and antibiotics. He was exclusively breastfed from birth on an unrestricted maternal diet until 8 weeks of age, with the exception of one bottle of partially hydrolysed cow's milk based infant formula (pHF) in the first week of life. At 6 weeks he developed severe irritability, persistent crying and eczema. CMA was suspected. Since he was taking part in the EuroPrevall Birth Cohort Study, investigations were carried out according to the protocol (7). He

***Table 1** - Skin prick test results before and after DBPCFC for Case A*

	Wheal size before DBPCFC (mm)	Allergen-histamine ratio before DBPCFC	Wheal size after DBPCFC (mm)	Allergen-histamine ratio after DBPCFC
Histamine	3		5.5	
CMP	0	0	3.5	0.64
Fresh w-eHF	N.D.		0	0
Fresh standard infant formula	N.D.		5.5	1
Fresh semi-skimmed milk	N.D.		6.5	1.2

DBPCFC = double blind placebo controlled food challenge; mm = millimeter; CMP = cow's milk protein; w-eHF = whey-based extensively hydrolysed formula; N.D. = not done

was successfully managed on a maternal CMP elimination diet followed by an amino-acid based formula (AAF), according to protocol (7).

Both skin prick test (SPT) for CMP (ALK-Abelló, Hørsholm, Denmark) and CMP specific IgE measurement (Phadia Diagnostics, Uppsala, Sweden) were negative. At 3 months of age a DBPCFC was performed (7). He developed eczema on the chest at dose 7 and redness/flushed skin around the nose at dose 8 at the placebo-day, and had no symptoms on the active day. The DBPCFC was determined negative. As he had a positive atopic family history, a pHF was introduced (8,9). He immediately developed urticaria, angioedema and wheezing. He was diagnosed with anaphylaxis and managed accordingly. Three weeks later, the SPT was repeated for CMP, fresh whey-based extensively hydrolyzed formula (w-eHF), fresh standard infant formula and fresh semi-skimmed milk, and showed allergen-histamine ratios of 0.64, 0.1 and 1.2 respectively (**table 1**). Specific IgE for cow's milk was 8.17 kU/L. As the parents refused a second DBPCFC, an open food challenge (OFC) was carried out to confirm CMA. After a dose of 3 mg CMP (equivalent of 90.4 µl cow's milk) (7), he developed urticaria around the mouth and swelling of the right side of the lip. A CMP elimination diet was continued including an AAF to maintain nutritional adequacy (9). Also an adrenaline auto injector was prescribed. He was re-challenged annually and after 3 years he became tolerant to cow's milk.

Case B

A full term girl with a normal birth weight and APGAR scores presented with eczema at 3 months. She was exclusively breastfed, had abdominal cramps since birth and she was vomiting on consumption of breast milk. Family history was positive for atopic diseases. On physical examination she had a dry skin and moderate eczema; Scoring Atopic Dermatitis score was 36 out of 103 (objective score 34 out of 83) (10). CMA was suspected. As she was taking part in the EuroPrevall Birth Cohort Study, investigations were carried out according to protocol (7). SPT carried out for CMP (ALK-Abelló, Hørsholm, Denmark) and fresh standard infant formula, w-eHF and semi-skimmed milk were negative. IgE for cow's milk was negative (< 0.35kU/L). Maternal CMP elimination diet was successful. At 4 months AAF was initiated, because of significant reduction in breast milk supply. A DBPCFC was postponed until baby B was willing to drink adequate amounts of AAF, required for testing. The DBPCFC at 6 months of age was negative. Additionally, an OFC was performed with a pHF. Within 1.5 hours after receiving the top dose (251 ml = 4.0 gram CMP) she had erythema and oedema in the face as well as on the arms and legs, while no skin lesions were reported at the start of the OFC. Within 24 hours she also developed diarrhoea. CMA was confirmed, the elimination diet was continued and w-eHF was introduced. Within 2 weeks after introduction of w-eHF she again developed diarrhoea 4-6 times a day, which persisted for 3 months. Stool cultures for Salmonella, Shigella, Yersinia, Campylobacter, and triple faeces test remained negative. She was switched from a w-eHF to a casein-based eHF, however there was no improvement in diarrhoea. At reintroduction of AAF the diarrhoea disappeared. At scheduled follow-up at 18 months, all allergy tests that where carried out, including DBPCFC, were negative and CMP was successfully introduced.

Discussion

Both exclusively breastfed infants had initially a false negative outcome of a DBPCFC and a severe reaction during reintroduction of cow's milk. Our cases are not the first to describe false negative outcomes and several explanations have been discussed (4,5,11). Firstly, the possibility of a masked reaction due to medication was ruled out since both infants were not given any medication, including so called "over the counter medication" (11). Secondly, the dose of challenge food could have been too

small to elicit symptoms (12). In both cases mentioned above the infants reacted to a lower dose compared to the dose used in the DBPCFC. Formula samples were analysed in a laboratory and exchange of active and placebo foods was ruled out. Thirdly, Niggemann and Beyer described a so called short-term specific oral tolerance, which means that the infant develops tolerance for the allergenic food during the increasing doses of the DBPCFC, but loses this tolerance quickly after the DBPCFC (11). Another possibility may be that during the challenge the infants become sensitised, while the actual clinical reaction occurs upon re-introduction of CMP.
We would like to add that exclusive breastfeeding might be a risk factor of having a false negative DBPCFC, especially since all mentioned studies were performed in children of several ages, while we only describe young infants who were exclusively breastfed (4,5,11,12).

Implications

Despite the fact that DBPCFC is being considered as the gold standard for diagnosing CMA, a false negative outcome remains possible. Exclusively breastfed infants are at risk of experiencing a false negative DBPCFC outcome compared to formula-fed infants. This could result in severe allergic reactions occurring when CMP is re-introduced. Therefore, it is recommended that in exclusively breastfed infants an additional OFC with the formula of choice (standard formula or pHF) is performed under medical supervision, rather than introduction of the formula at home (5).
After the occurrence of these severe reactions on re-introduction of CMP we have adapted our protocol accordingly.

Conclusion

We described two cases of exclusively breastfed infants with a severe allergic reaction after a negative DBPCFC. Despite the fact that we are not able to provide a clear explanation for the false negative DBPCFC, an additional OFC with the formula of choice, performed under medical supervision, is necessary in exclusively breastfed infants to avoid severe allergic reactions.

The institute where the work was conducted

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Competing interests

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References

1. Chafen JJ, Newberry SJ, Riedl MA, et al. Diagnosing and managing common food allergies: a systematic review. JAMA. 2010;303:1848-1856.
2. Rona RJ, Keil T, Summers C, et al. The prevalence of food allergy: a meta-analysis. J Allergy Clin Immunol. 2007;120:638-646.
3. Boyce JA, Assa'ad A, Burks AW, et al. Guidelines for the Diagnosis and Management of Food Allergy in the United States: Summary of the NIAID-Sponsored Expert Panel Report. J Allergy Clin Immunol. 2010;126:1105-1118.
4. Caffarelli C, Petroccione T. False-negative food challenges in children with suspected food allergy. Lancet. 2001;358:1871-1872.
5. Niggemann B, Lange L, Finger A, Ziegert M, Muller V, Beyer K. Accurate oral food challenge requires a cumulative dose on a subsequent day. J Allergy Clin Immunol. 2012;130:261-263.
6. Caffarelli C, Romanini E, Caruana P, Street ME, de' AG. Clinical food hypersensitivity: the relevance of duodenal immunoglobulin E-positive cells. Pediatr Res. 1998;44:485-490.
7. Keil T, McBride D, Grimshaw K, et al. The multinational birth cohort of EuroPrevall: background, aims and methods. Allergy. 2010;65:482-490.
8. Host A, Koletzko B, Dreborg S, et al. Dietary products used in infants for treatment and prevention of food allergy. Joint Statement of the European Society for Paediatric Allergology and Clinical Immunology (ESPACI) Committee on Hypoallergenic Formulas and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition. Arch Dis Child. 1999;81:80-84.
9. Kneepkens CM, Brand PL. Clinical practice: Breastfeeding and the prevention of allergy. Eur J Pediatr. 2010;169:911-917.
10. Oranje AP, Glazenburg EJ, Wolkerstorfer A, de Waard-van der Spek FB. Practical issues on interpretation of scoring atopic dermatitis: the SCORAD index, objective SCORAD and the three-item severity score. Br J Dermatol. 2007;157:645-648.
11. Niggemann B, Beyer K. Pitfalls in double-blind, placebo-controlled oral food challenges. Allergy 2007;62:729-732.
12. Bock SA, Atkins FM. Patterns of food hypersensitivity during sixteen years of double-blind, placebo-controlled food challenges. J Pediatr. 1990;117:561-567.