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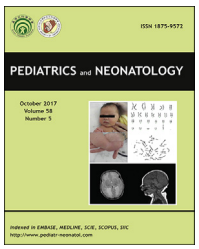

Original Article

Prognostic accuracy of clinical signs and diagnostic tests in cow's milk allergy in newborns

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Key Words
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Background: The aim of the study was to explore the correlation between clinical signs and confirmatory tests for cow's milk allergy (CMA) in the neonatal period and their relation to family history and the occurrence of food allergies in the postneonatal period.
Methods: The medical documentation of 361 newborns with suspected CMA and exclusion of other comorbidities was analyzed. The correlations between clinical signs and methods to confirm CMA [elevated levels of total immunoglobulin E (IgE) and/or specific IgE for cow's milk, improvement after the introduction of a cow's milk-free diet and positive challenge procedure] were studied. In 90 children, the data were additionally analyzed in relation to outcome (at the age of 1–11 years), evaluated by questionnaires, which inquired about signs and symptoms of food allergy, methods of CMA confirmation, and the presence of other food allergies.
Results: There was a positive correlation between exanthema and confirmed CMA in the neonatal period ($R = 0.184$; $p = <0.001$; $n = 361$), and hematochezia and confirmed CMA in the neonatal ($R = 0.203$; $p < 0.001$; $n = 361$) and postneonatal period ($R = 0.215$; $p = 0.042$; $n = 90$). Additional food allergies in the postneonatal period were positively correlated with neonatal CMA ($R = 0.275$; $p = 0.009$; $n = 90$). No correlation was found between a positive family history of food allergies and CMA in the neonatal ($R = -0.66$; $p = 0.398$; $n = 165$) and postneonatal periods ($R = 0.00$; $p = 1.000$; $n = 116$).
Conclusion: Neonatal exanthema and hematochezia were the predominant clinical signs in neonates with CMA. Allergies to other food allergens appeared more frequently in children with CMA in the neonatal period. Neonatal CMA did not occur more frequently in families with food allergies.

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1. Introduction

Food allergy is estimated to affect approximately 4–6% of young children in the United States.[1] The prevalence of self-reported food allergy in European adult and children populations is reported to be 5.9% and 6.9%, respectively.[2] The prevalence of challenge-proven food allergy in European general and children population is 0.9%.[2] The most common food allergy in children is allergy to cow's milk proteins. In different European countries, the incidence of challenge-proven cow's milk allergy (CMA) in children in the first 2 years ranges from 0.0% to 4.2%.[2,3]

The diagnosis of CMA can be very challenging in the neonatal period because of nonspecific and diverse clinical signs and symptoms since the clinical presentation of CMA depends on the pathophysiological mechanism.[4] Immunoglobulin E (IgE)-mediated hypersensitivity occurs within minutes after exposure to cow's milk (CM) proteins and presents with signs and symptoms on the skin, in the gastrointestinal tract, and, rarely, in the respiratory system. Delayed cell-mediated hypersensitivity manifests itself within hours, mostly with gastrointestinal tract signs and symptoms.[4]

The only method that can definitively confirm or exclude CMA is the challenge procedure, but this is rarely implemented in the neonatal period because of possible complications. Therefore, the diagnosis of CMA in this period is usually based on the determination of the serum IgE concentration or the efficacy of an elimination diet.[5]

These specific limitations of CMA diagnostics in the neonatal period are often the reason for under- or overdiagnosis of CMA, leading to under- and overtreatment of this disease, along with important complications and unnecessary discomfort. Therefore, the aim of our study was to explore the correlation between clinical signs and confirmatory tests for CMA in the neonatal period and their relation to family history and the occurrence of food allergies in the postneonatal period.

2. Patients and methods

In the retrospective part of the study, we analyzed the medical documentation of 361 newborns, hospitalized in the Department of Neonatology, University Children's Hospital Ljubljana, Slovenia, between 2002 and 2014, in whom CMA was suspected based on clinical signs and symptoms. All had undergone investigations for confirmation of CMA after exclusion of structural, infectious, and metabolic etiologies of their problems. Term infants up to the age of 28 days and premature infants up to 44 weeks' postmenstrual age were included. The inclusion criteria for suspected CMA included expression of at least one of the following signs/symptoms, according to the Roma III criteria[6–8]: frequent vomiting or regurgitation, generalized exanthema, failure to thrive, hematochezia (positive quick stool test for the detection of blood), abdominal colic, colitis and diarrhea, and the group of "Other" including sleepiness, sleeplessness, tearfulness, restlessness, irritability, tremor, slow feeding, nausea, abdominal pain, meteorism, constipation, short obstructive apnea after feeding, transient muscular hypo- or hypertonia after feeding, and localized edema.

The exclusion criteria included insufficient medical data on the clinical picture, imaging, laboratory, and microbiology results, and the presence of any other comorbidity with the exception of prematurity. We collected data comprising birth measurements, gestational age, chronological age at which signs or symptoms started, family history of food allergy, and methods for confirmation of CMA: levels of total IgE and specific IgE for CM, positive challenge procedure, improvement after the introduction of a CM-free diet, and the age at its introduction.

Blood samples were obtained using standard venepuncture using Vacutainer tubes (Becton Dickinson, Heidelberg, Germany). Serum was collected and stored at −80°C until assayed. The determination of serum total and specific IgE was carried out on an automated immunological analyzer IMMULITE 2000 (Siemens Healthcare GmbH, Erlangen, Germany), using chemiluminescent competitive immunoassay method. For the determination of total serum IgE and specific IgE, we used reagents, calibrators, and controls L2KIE2 and L2KUN6 (Siemens Healthcare GmbH), respectively.

Confirmed CMA was defined as elevated total IgE (higher than 2.0 kU/L) and/or specific cow's milk protein IgE (higher than 0.35 kU/L) and/or a positive result of the challenge procedure and/or improvement in clinical signs after the introduction of a CM-free diet.

In the prospective part of the study, a questionnaire was sent to all patients included in the retrospective analysis. The questionnaire included questions concerning the postneonatal period: signs and symptoms, method for confirmation of CMA, age at confirmation, duration of CMA, the need for a CM-free diet, and the presence of other food allergies. Only patients in whom diagnostic evaluation of CMA was performed and structural, infectious, and metabolic causes were excluded entered into further analysis. The confirmation of CMA was performed by the same methods as those described for the neonatal period. The cutoff value of total IgE was adjusted for age.[9] If more than one food allergy was found, then total IgE level was excluded from the diagnostic criteria, and CMA was confirmed only by elevated specific cow's milk protein IgE (higher than 0.35 kU/L) and/or a positive result of the challenge procedure and/or improvement in clinical signs after the introduction of a CM-free diet.

We received correctly completed questionnaires from 90 of 361 participants. The data were statistically analyzed using the statistics software SPSS (Statistical Package for the Social Sciences, version 20.0; SPSS Inc., Chicago, IL, USA). The differences between groups were analyzed by Fisher's exact test, and the correlation between variables was assessed using Spearman's analysis.

The National Ethics committee approved the study in December 2014.

3. Results

A total of 361 newborns with suspected CMA were included in the study. In the neonatal period, CMA was confirmed in 110 (30.5%) and excluded in 251 (69.5%) newborns. The group consisted of 206 boys (57.1%) and 155 girls (42.9%). Their mean gestational age was 38.8 ± 2.7 weeks (range,

24–42 weeks), and the mean birth weight was 3187 ± 681 g (range 645–4690 g). The mean chronological age at which clinical signs appeared was 15.9 ± 14.6 days (range, 3–140 days).

Fifty-nine (16.3%) newborns were born prematurely. In 48 (81.3%) of them clinical signs of possible CMA appeared after and in 11 (18.7%) prior to postconceptional age of 37 weeks. In the latter group, the earliest testing for possible CMA was performed at postconceptional age of 36 weeks.

Ninety completed questionnaires (25% response rate) were returned by parents of 57 (63.3%) boys and 33 (36.7%) girls at the age of 1–11 years. Figure 1 presents the distribution of patients according to the time of confirmation of CMA.

The age of patients at the onset of clinical signs and symptoms in the neonatal period was 1–90 days (mean, 15.9 ± 15.5 days). In patients with confirmed CMA in the neonatal or postneonatal period, the signs and symptoms of CMA or confirmatory measures persisted for 1–95.3 months (mean, 18.6 ± 20.3 months). The overall duration of CMA was 3–96 months (mean, 20.7 ± 21.4 months). At the time of the study, CMA was still present in three (8.8%) patients.

In the majority of patients with excluded CMA, the signs and symptoms were attributed to gastrointestinal functional disorders, such as infantile colic, infant regurgitation ("physiological reflux"), functional diarrhea ("irritable bowel syndrome"), infant dyschezia, and functional intestinal constipation. No specific treatment was needed in this group. The signs and symptoms initially reported gradually ceased, and no long-term problems persisted. The minority of patients with excluded CMA had gastroesophageal reflux disease and needed specific treatment.

3.1. Clinical picture

Clinical signs and symptoms in neonates with suspected, confirmed, and excluded CMA in the neonatal period are presented in Figure 2. Exanthema ($p = 0.001$) and hematochezia ($p < 0.001$) presented significantly more frequently in the group with confirmed CMA in the neonatal period.

The frequency of category "Other" was high, but the frequency of individual sign or symptom was low. There were no statistically significant differences in frequencies of these signs and symptoms between the groups with confirmed and excluded CMA.

In both neonatal and postneonatal periods, the most sensitive clinical signs for identifying patients with CMA were exanthema and vomiting. Hematochezia and abdominal colic were found to be the most specific signs in children with confirmed CMA in the neonatal period, whereas abdominal colic and colitis were the most specific in those with confirmed CMA in the postneonatal period (Table 1).

3.2. Confirmatory tests

In the neonatal and postneonatal periods, CMA was most frequently confirmed by elevated total IgE (71.8%) and/or cow's milk protein-specific IgE (75.0%). In the neonatal period, total IgE and specific IgE for CM proteins were

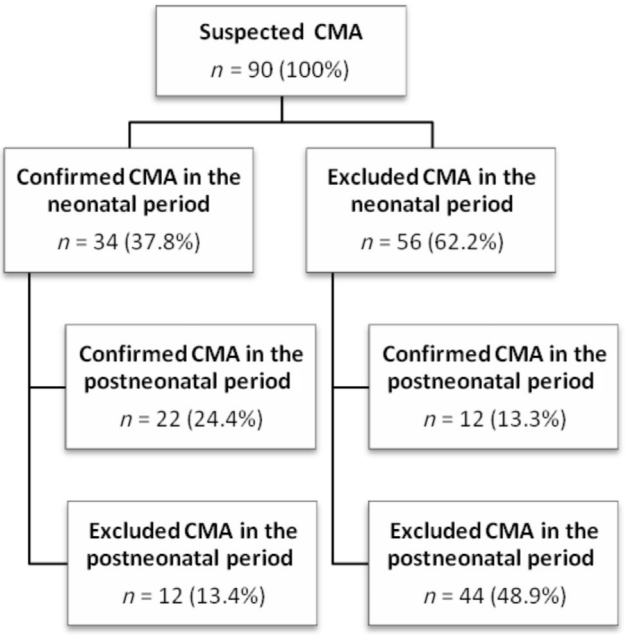

Figure 1 Distribution of patients with suspected, confirmed, and excluded cow's milk allergy (CMA) in the neonatal and postneonatal period.

determined in all 361 patients. Total IgE was elevated in 76 (21.1%) and specific IgE for CM protein in 30 (8.3%): in 27 (7.5%) patients total IgE and specific CM protein-IgE were found to be elevated; only total IgE was elevated in 49 (13.6%) and only specific CM protein-IgE in three (0.8%) patients. Improvement in clinical signs after the introduction of a CM-free diet was found in 91 newborns, and a challenge procedure was performed in four. The sensitivity, specificity, and the predictive values of diagnostic tests for CMA in the neonatal period are presented in Table 2.

We found a positive correlation between exanthema and confirmed CMA in the neonatal period ($R = 0.184$; $p = <0.001$; $n = 361$), as well as elevated total and/or specific IgE ($R = 0.175$; $p = 0.001$; $n = 361$) and the effect of a CM-free diet ($R = 0.193$; $p = <0.001$; $n = 361$). We also demonstrated a positive correlation between hematochezia and confirmed CMA in the neonatal ($R = 0.203$; $p = <0.001$; $n = 361$) and postneonatal periods ($R = 0.215$; $p = 0.042$; $n = 90$), as well as between hematochezia and the effect of a CM-free diet in the neonatal period ($R = 0.232$; $p = <0.001$; $n = 361$).

3.3. Additional food allergies and family history

Additional food allergies in the postneonatal period were present in 21 (23.3%) patients: in 18 (85.7%) to eggs, in four (19.0%) to peanuts, and in six (28.6%) to other food allergens. There was a positive correlation between additional food allergies in the postneonatal period and confirmed CMA in the neonatal period ($R = 0.275$; $p = 0.009$; $n = 90$), but there was no correlation between additional food allergies and confirmed CMA in the postneonatal period ($R = 0.170$; $p = 0.109$; $n = 90$). Seventy-two (43.6%) children from the neonatal group ($n = 165$) and nine (10.0%) children from the postneonatal group had a positive

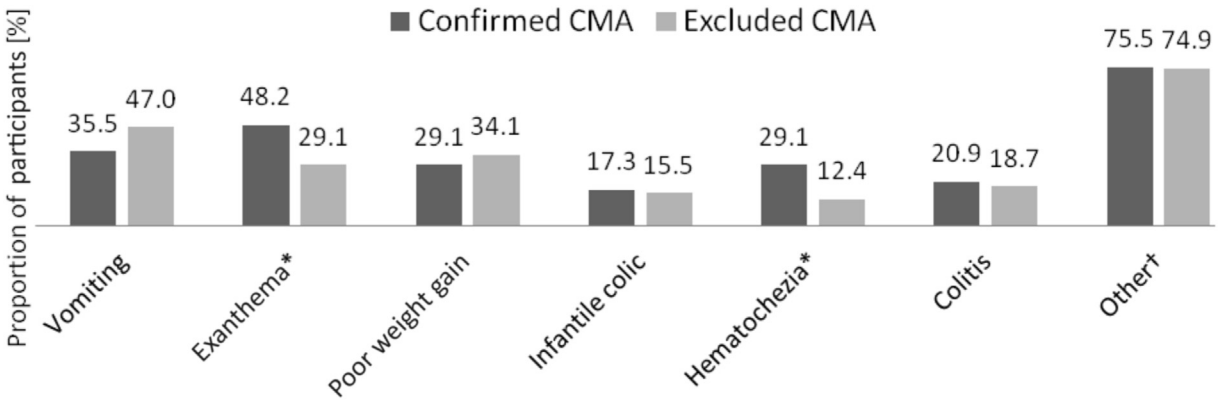

Figure 2 Clinical signs and symptoms in neonates with confirmed and excluded cow's milk allergy (CMA) in the neonatal period. * The higher incidence of exanthema ($p = 0.001$) and hematochezia ($p < 0.001$) in the group with confirmed CMA is statistically significant. † Other—sleepiness, sleeplessness, tearfulness, restlessness, irritability, tremor, slow feeding, nausea, abdominal pain, meteorism, constipation, short obstructive apnea after feeding, transient muscular hypo- or hypertonia after feeding, localized edema.

Table 1 Sensitivity, specificity, and predictive values of clinical signs and symptoms of cow's milk allergy in the neonatal ($n = 361$) and postneonatal period ($n = 90$).

Clinical sign, symptom	Sensitivity NP; PNP (%)	Specificity NP; PNP (%)	Positive predictive value NP; PNP (%)	Negative predictive value NP; PNP (%)
Vomiting	36; 32	53; 54	25; 30	65; 57
Exanthema	48; 35	71; 71	43; 43	76; 65
Poor gain weight	29; 29	66; 68	27; 36	68; 61
Abdominal colic	17; 15	84; 86	33; 39	70; 62
Hematochezia	29; 18	88; 80	51; 35	74; 62
Colitis or diarrhea	21; 24	81; 84	33; 47	70; 64
Other	76; 74	25; 25	31; 37	70; 61

NP = neonatal period; Other = sleepiness, sleeplessness, tearfulness, restlessness, irritability, tremor, slow feeding, nausea, abdominal pain, meteorism, constipation, short obstructive apnea after feeding, transient muscular hypo- or hypertonia after feeding, localized edema; PNP = postneonatal period.

Table 2 Sensitivity, specificity, and the predictive values of diagnostic tests for cow's milk allergy in the neonatal period ($n = 90$).

Diagnostic test	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Elevated total IgE and; or specific cow's milk protein IgE	41	84	61	70
Elimination of the allergen from the diet	27	89	60	67
Challenge procedure	12	98	80	65

IgE = immunoglobulin E.

family history of allergies. No correlation was found between a positive family history of allergies and CMA in the neonatal ($R = -0.66$; $p = 0.398$; $n = 165$) and postneonatal periods ($R = 0.00$; $p = 1.000$; $n = 116$).

4. Discussion

The clinical picture of CMA in the neonatal period is diverse, comprising a wide variety of different signs and symptoms in various combinations, which can overlap with clinical signs and symptoms of other etiologies. Despite the fact that the first part of our study was retrospective, clearly defined inclusion criteria, a good overview from the medical documentation, and a long observational period throughout childhood make our results relevant to everyday clinical practice and a platform for further studies.

We showed that the most sensitive clinical sign of neonatal CMA was exanthema, which was also present in 84% of children with CMA in a similar study.[3] Diffuse

exanthema occurs primarily in the context of allergic reactions, although other pathophysiological mechanisms, such as cytokine-derived toxic exanthema or infection, can be involved, especially in newborns.[10] Other etiologies can be differentiated from CMA by clinical or laboratory methods.

The main CM proteins that can trigger an allergic reaction are casein and whey proteins.[5] In susceptible infants, exanthema generally occurs following ingestion of CM, but it has been demonstrated that CMA may also present as contact urticaria if the newborn's skin comes into contact with CM. This so-called "finger test" could provide additional confirmation of CMA.[11]

Contrary to the results of other studies that showed no significant specificity of abdominal colic,[12–15] we demonstrated this clinical sign to be the most specific for CMA. Although the etiology of abdominal colic is multifactorial, some studies have shown that even without confirmation of CMA, the introduction of partially or extensively hydrolyzed CM leads to improvement in the clinical picture, probably because of the favorable effect of hydrolyzed formula on intestinal motility and digestion.[7,13] When CMA is suspected in a child with abdominal colic, some authors recommend the introduction of a CM-free diet only in the presence of additional clinical signs. Although we defined abdominal colic by the Roma III criteria,[8] the interpretation of abdominal colic as a symptom remains inter- and intra-observer variable and all these "confounding" factors could influence the results of our study.[7,13,14]

We also demonstrated a positive correlation between hematochezia and CMA in both the neonatal and postneonatal periods. In susceptible neonates, CM proteins cause allergic non-IgE-mediated inflammation of the intestinal mucosa (most commonly proctitis or proctocolitis), followed by fine mucosal ulceration and hemorrhage, which can appear as bloody filaments in the stool.[16] Neonatal hematochezia is most commonly described as a sign of idiopathic neonatal transient colitis, which resolves without treatment.[16–19] Differentiation of this entity from CMA is difficult; some authors suggest endoscopic examination and rectal mucosal biopsy as the first-choice method and the introduction of a CM protein-free diet and challenge testing for definitive confirmation.[16] Others suggest that CMA is confirmed by improvement after a CM protein-free diet, demonstration of eosinophilia in the peripheral blood, and endoscopic findings.[17,19] As rectal endoscopic examination was not performed in any of our patients, the incidence of hematochezia and its correlation with CMA could be overestimated.

In our study, serum IgE levels were the most commonly used method for confirmation of CMA. The specificity of neonatal serum IgE was higher (84%) in our study compared to a similar study, where it was 53%.[20] The method is simple, fast, easy to perform, and far less risky than a challenge procedure.[3] However, IgE-mediated hypersensitivity is only one of the possible mechanisms of CMA, and evaluation of serum IgE can elucidate only patients with this type of hypersensitivity. Moreover, there are other concerns about confirming CMA only by the determination of serum IgE, because in the neonatal period the humoral immune response is immature and the production of all immunoglobulin classes, especially IgE, is significantly reduced. Within the first months of life, there is a gradual increase in the total IgE level, but there are still no clearly defined normal values of total IgE in premature newborns.[4,9,20,21] Although premature newborns were included in our study, their number was not high and only a small proportion of them developed clinical signs of possible CMA prior to postconceptional age of 37 weeks. Furthermore, the earliest evaluation for possible CMA was performed at postconceptional age of 36 weeks, when no important immunological differences between term and preterm newborns are expected.

The most specific test for the detection of CMA in newborns was the challenge procedure. Both the challenge procedure and an elimination diet reveal CMA, regardless of its pathophysiological mechanism (either IgE- or cell-mediated hypersensitivity), and are superior to the IgE level.[4] Despite its high specificity, the challenge procedure is rarely used for confirmation of CMA because it carries a high risk of triggering severe allergic reactions.[5] When interpreting the results of our study, we should also be aware that IgE was tested in all children whereas other methods were used only rarely.

As in some other studies, we also failed to demonstrate a correlation between CMA and a positive family history of food allergies.[22–24] However, in our study neonatal CMA was associated with other food allergies later in life. Hypersensitivity to one food allergen in the neonatal period may lead to hypersensitivity to other food allergens later in life, as the epidemiologic risk factors usually remain the same in a susceptible individual.[2,25,26]

An important factor affecting the incidence of CMA in newborns of atopic parents and the incidence of other food allergies later in life could be breastfeeding. The fact that we did not differentiate between breast- and formula-fed infants is one of the limitations of our study. Human milk contains several components with active immunological and anti-infective functions (antibodies, growth factors, cytokines, antimicrobial compounds, and specific immune cells), and these components importantly compensate for the newborn's immunological immaturity and modulate the baby's immune response.[22] The most abundant human milk protein is IgA, which, among other effects, inhibits abnormal immune activation caused by antigens and microorganisms, amplifies the digestive system epithelial barrier, and consequently protects against food allergy. There is evidence that a mother's atopy does not affect the quantity of IgA in her breast milk.[24] This could at least partly explain our finding of a lack of correlation between CMA in newborns and food allergies in their atopic parents.

Aside from not differentiating between preterm and term infants and those being breast- and formula-fed, another limitation of our study is also a relatively low participants' response rate. However, we think that the large sample size to some extent compensates for this issue.

In the majority of our patients with excluded CMA in the neonatal and postneonatal periods, clinical signs and symptoms were attributed to gastrointestinal functional disorders. They manifest as different transient signs and symptoms that originate in developmental changes of the digestive tract.[7,8,27] In fact, gastrointestinal functional disorders, gastroesophageal reflux disease, and CMA are the

most common causes of many gastrointestinal and other signs and symptoms that were also used as inclusion criteria in this study.[27]

5. Conclusion

Based on our results, we would recommend that neonates with exanthema and/or hematochezia, in whom other possible etiologies have been excluded, be tested for CMA. Early diagnosis of CMA is important because other nutritional allergies appear more frequently in children with CMA in the neonatal period.

Conflicts of interest

The authors have indicated they have no personal financial relationships relevant to this article to disclose.

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