Retrospective Multicenter Survey on Food-Related Symptoms Suggestive of Cow’s Milk Allergy in NICU Neonates

Tokuo Miyazawa¹, Kazuo Itabashi¹ and Takanori Imai¹,²

ABSTRACT
Background: Cow’s milk allergy (CMA) is one of the causes of gastrointestinal symptoms in neonates. A relationship between non-immunoglobulin (Ig) E mediated allergic reactions and CMA in early infancy has been proposed, but the clinical features and pathogenesis have not been established. The objective of this study is to determine the clinical characteristics of the neonates found in the earlier study to have food-related symptoms that suggested CMA.

Methods: A second questionnaire was sent to 53 NICUs, as a follow-up to the earlier study, to collect information on the background, onset age, clinical features, and results of clinical examinations.

Results: The median birth weight was 2614 g and the median gestational age was 36.9 weeks. Symptoms developed within 6 days after birth in 40% of cases. Gastrointestinal symptoms were seen in 90% of cases and were mainly vomiting, bloody stool and abdominal distention. A specific IgE test, a lymphocyte stimulation test, and a fecal eosinophil test were conducted in 88%, 23% and 55% of cases, respectively, and the positive rates were 30%, 84%, and 75%, respectively. An oral food challenge (OFC) test was performed in 26% for confirmation of the diagnosis.

Conclusions: We confirmed that the clinical manifestations of food-related symptoms suggestive of CMA in neonates were distinctly different from those of common immediate type food allergy and were largely affected by underlying factors such as prematurity and gastroenterological surgery. Further OFC-based prospective accumulation of cases of CMA in neonates will be particularly important to reveal the full clinical features of this disease.

KEY WORDS
cow’s milk allergy, gastrointestinal food allergy, gastrointestinal symptoms, lymphocyte stimulation test, neonate

INTRODUCTION
In neonates, gastrointestinal symptoms are common and suggest disorders such as congenital anomaly of the gastrointestinal tract, infection, metabolic disease, and allergic disease. If these symptoms develop after intake of milk formula, involvement of allergic reactions to cow’s milk protein may be suspected. Cow’s milk allergy (CMA) presents with a variety of symptoms and is a common cause of gastrointestinal symptoms such as diarrhea, vomiting, and bloody stool in the neonatal period.¹,² However, these symptoms are not specific to CMA, which makes an accurate diagnosis extremely difficult. A relationship between non-immunoglobulin (Ig) E-mediated delayed type allergic reactions and CMA in early infancy has been proposed, but the clinical features and pathogenesis have not been fully established.³⁻⁴
In the field of neonatal medicine, an oral food challenge (OFC) test for suspected CMA tends to be avoided because of concern regarding induction of severe symptoms, and standardized diagnostic criteria for CMA are not available. Therefore, a definite diagnosis of CMA is rare and epidemiological studies have not been performed due to a lack of accumulation of cases. We previously reported that the incidence of cases with food-related symptoms that suggested CMA was 0.21% (145/69,796) in neonatal care units (NICUs) in Japan and that most NICUs currently make a diagnosis and establish treatment strategies based on observation of the clinical course, rather than performing an OFC. In this study, we retrospectively evaluated clinical data for these identified neonates presenting with food-related symptoms suggestive of CMA.

METHODS
We distributed a primary questionnaire to 263 NICUs to obtain information on the number of hospitalized neonates and the number of cases with food-related symptoms suggestive of CMA between January 2004 and December 2005. Of the 263 NICUs, 145 responded to the questionnaire and 53 of these had at least one case with food-related symptoms suggestive of CMA. These NICUs are certified as educational institutions by the Japan Society of Perinatal and Neonatal Medicine. The inclusion criteria did not require performance of an OFC, because it was assumed that an OFC would be avoided by most neonatologists due to fear of induction of severe symptoms. All the subjects had food-related symptoms that suggested CMA based on the results of clinical examinations and positive results in milk elimination tests: that is, disappearance of symptoms after elimination of milk, and no recurrence of symptoms and normal weight gain after initiation of feeding with therapeutic formula.

A second questionnaire was mailed to the 53 NICUs to request information on background, onset age, clinical features, and the results of clinical examinations for the 145 neonates. Specific IgE levels were all determined by an ImmunoCAP assay (Phadia K. K., Tokyo, Japan). Data were analyzed by Mann-Whitney U test, Fisher exact test and Student t-test. The level of significance was \( p < 0.05 \). Statistical analysis was performed using PASW Statistics ver.18. The study was approved by the Ethics Committee of Showa University School of Medicine and was performed with consideration of all appropriate ethical issues.

RESULTS

PATIENT BACKGROUND
Of the 53 NICUs, 38 (72%) responded to the second questionnaire and data were collected for 111 cases (77%). The median birth weight in the 111 subjects was 2614 g (406-3936 g), the median gestational age was 36.9 weeks (range: 25.3-42.0 weeks), and the male-to-female ratio was 1.5. There were 45 low birth weight infants (41%), including 9 with birth weight <1000 g and 4 with birth weight 1000-1500 g. The incidence of complications was 51% (57/111), including 32 cases (29%) with respiratory disease, 17 (15%) with infection, 13 (12%) with gastrointestinal disease, 6 (5%) with heart disease, and 3 (3%) with a chromosomal aberration; these data include multiple responses. Surgery was performed in 12 cases (11%) before onset, including 10 with gastrointestinal disease (3 with gastrointestinal atresia, 2 with Hirschsprung disease, 2 with congenital diaphragmatic hernia, 1 with idiopathic perforation of the ileum, 1 with necrotising enterocolitis, and 1 with exomphalos) and 2 cases of congenital heart disease (1 of coarctation of the aorta and 1 of univentricular heart complicated by coarctation of the aorta).

ONSET AGE AND FEEDING AT ONSET
The median onset age was 7 days (range: 0-67 days) in 109 cases for which records were available. Of these cases, 44 (40%) developed food-related symptoms within 6 days after birth. The feeding at onset was exclusively breastfeeding in 18 of the 111 cases (16%), complementary feeding in 60 cases (54%), formula feeding in 26 (23%), MCT (medium chain triglycerides) milk in 2 (2%), and breast milk with human milk fortifier in 1 (1%). Onset occurred before starting lactation in 2 cases (2%) and the feeding in another 2 cases (2%) was unknown.

CLINICAL MANIFESTATIONS
Gastrointestinal symptoms occurred in 90% of subjects and were mainly vomiting, bloody stool and abdominal distension (Fig. 1). Failure to thrive and systemic symptoms such as fever, hypothermia and not doing well occurred frequently. A few neonates also presented with shock symptoms, cutaneous symptoms or respiratory symptoms, which are frequently seen in immediate food hypersensitivity.

LABORATORY FINDINGS
Specific IgE for milk allergens were tested in 98 cases (88%). Of these cases, 25 (26%) and 29 (30%) had specific IgE values of >0.70 UA/ml (>class 2) and >0.35 UA/ml (>class 1), respectively. A lymphocyte stimulation test (LST) was performed in 25 cases (23%) and the positive rate was 84% (21/25). Specific IgE was negative in 20 of these 21 cases and not tested in the remaining case; that is, no neonates were identified who were positive in a LST and a specific IgE test. A fecal eosinophil test was performed in 61 cases (55%) and was positive in 46 (75%). Among 98 cases in which food-related symptoms developed within 1 month after birth, an OFC was conducted in 25 (26%) to confirm the diagnosis.
Food-Related Symptoms in Neonates

The results of other common blood tests are shown in Table 1. Some neonates had an increase in CRP similar to that seen in sepsis. Those with high CRP (>4.0 mg/dl, n = 14) had systemic symptoms such as fever (21%) and not doing well (43%), as well as gastrointestinal symptoms. Eosinophilia (>700/μl) was present in about half of the neonates, but there were no differences in clinical features or in the positive rate of fecal eosinophils in neonates with and without eosinophilia.

Table 1 Results of blood tests

<table>
<thead>
<tr>
<th>Item</th>
<th>n</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells (μl)</td>
<td>99</td>
<td>12100</td>
<td>2900-51100</td>
</tr>
<tr>
<td>Eosinophils (μl)</td>
<td>98</td>
<td>814</td>
<td>0-10000</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>100</td>
<td>0.54</td>
<td>0.00-15.00</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>85</td>
<td>30</td>
<td>9-413</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>86</td>
<td>11</td>
<td>1-482</td>
</tr>
<tr>
<td>γGTP (IU/l)</td>
<td>25</td>
<td>179</td>
<td>36-689</td>
</tr>
</tbody>
</table>

The incidence of bloody stool was significantly higher in neonates who developed symptoms within 6 days after birth (early onset group, n = 44) than in those who developed symptoms after 7 days after birth (late onset group, n = 55). In contrast, the late onset group had significantly higher incidences of diarrhea, failure to thrive and cutaneous symptoms (Fig. 3). The positive rates of fecal eosinophils were significantly higher in the early onset group (91% vs. 62%, p < 0.05). The results of the specific IgE test and LST did not differ significantly between the groups.

There were no significant differences in gestational age, birth weight, routine blood tests and stool tests increased gastric residual (67%) and failure to thrive (56%) were significantly higher than those in other groups (p < 0.05). There were no significant differences in the results of the specific IgE test, LST and fecal eosinophil test among the groups.

The incidence of bloody stool was significantly higher in neonates who developed symptoms within 6 days after birth (early onset group, n = 44) than in those who developed symptoms after 7 days after birth (late onset group, n = 55). In contrast, the late onset group had significantly higher incidences of diarrhea, failure to thrive and cutaneous symptoms (Fig. 3). The positive rates of fecal eosinophils were significantly higher in the early onset group (91% vs. 62%, p < 0.05). The results of the specific IgE test and LST did not differ significantly between the groups.

There were no significant differences in gestational age, birth weight, routine blood tests and stool tests increased gastric residual (67%) and failure to thrive (56%) were significantly higher than those in other groups (p < 0.05). There were no significant differences in the results of the specific IgE test, LST and fecal eosinophil test among the groups.

The incidence of bloody stool was significantly higher in neonates who developed symptoms within 6 days after birth (early onset group, n = 44) than in those who developed symptoms after 7 days after birth (late onset group, n = 55). In contrast, the late onset group had significantly higher incidences of diarrhea, failure to thrive and cutaneous symptoms (Fig. 3). The positive rates of fecal eosinophils were significantly higher in the early onset group (91% vs. 62%, p < 0.05). The results of the specific IgE test and LST did not differ significantly between the groups.

There were no significant differences in gestational age, birth weight, routine blood tests and stool tests...
between neonates who were specific IgE-positive (class 1 or higher, \( n = 29 \)) and -negative (\( n = 69 \)) or between the specific IgE-positive group and the LST-positive group (\( n = 21 \)). The LST-positive group had a significantly higher incidence of vomiting and abdominal distension (\( p < 0.05 \)). The incidence of cutaneous symptoms was 17% in the IgE-positive group, compared to 4% and 5% in the IgE-negative and LST-positive groups, respectively, with a significant difference between the IgE-positive and LST-positive groups.

The median onset time in 10 cases in which gastro-
enterolocical surgery was performed prior to onset was 24 days (range: 7-47 days). Five of these cases (50%) showed failure to thrive, significantly higher incidence than that in all other subjects (p < 0.05). Among the 10 cases, all 3 tested for fecal eosinophils were negative, but 5 cases (50%) were positive in a specific IgE test, which was significantly higher than the positive rate in all other subjects (p < 0.05).

**DISCUSSION**

The incidence of gastrointestinal symptoms is high in cases of CMA that develop in early infancy. The results in this study suggest that this tendency is stronger in CMA during the neonatal period. Since these symptoms can also be seen in other diseases and milk-specific IgE is typically not detected in neonates who are allergic to cow's milk, an OFC test should be the sole basis for diagnosis of CMA. However, an OFC has a potential risk of worsening the baby's condition and cannot be performed for all cases, especially after premature birth or in a case with a severe underlying medical condition. Indeed, CMA was mainly suspected only on the basis of a positive milk elimination test in our study. Thus, the neonates in this study had suspected CMA without fulfilling the exact diagnostic criteria for food allergy; therefore, these cases may include neonates with food intolerance or eosinophilic diseases.

Neonates with birth weight <1000 g tended to develop symptoms later than other birth weight groups. In these neonates, the incidence of mild gastrointestinal symptoms, such as abdominal distention or increased gastric residual, was relatively high, and the incidence of bloody stool was very low. Thus, CMA may not be suspected until a neonate has developed failure to thrive. Also, life-threatening complications specific to premature infants, such as respiratory distress syndrome and patent ductus arteriosus, may mimic symptoms of CMA.

A high incidence of CMA after gastroenterological surgery has been reported, and we also found 10 cases in which gastroenterological surgery was performed prior to development of symptoms. Because the recurrence of symptoms was not confirmed by OFC, these symptoms cannot be clearly distinguished from transient symptoms caused by surgical stress. However, interestingly, these cases showed a high positive rate for specific IgE, which suggests their sensitization mechanism may differ from that in cases of common CMA. The mechanistic link between gastroenterological surgery and CMA development is unknown, but may be associated with dysfunction of the gastrointestinal tract resulting from primary diseases, surgical invasion of the gastrointestinal tract, and atrophy in the intestinal mucosa caused by extended fasting before and after surgery. Cases of CMA that developed after a long-term elemental diet for postoperative short bowel syndrome have also been described. This diet is assumed to produce atrophy in the intestinal mucosa, enterocolitis combined with abnormal distension in the remaining gastrointestinal tract, and abnormal proliferation of intestinal flora, thereby resulting in increased protein permeability in the intestinal mucosa.

Most of the cases were receiving formula or complementary feeding at onset, but there were some cases with breastfeeding only. Low levels of proteins in cow's milk and other dairy products consumed by the mother may transfer to breast milk and serve as a causative antigen. Schulmeister et al. detected IgE antibodies against breast milk proteins in >80% of patients with IgE-mediated CMA from infancy through adulthood. A similar cross reaction may be involved in development of CMA in neonates.

Powell and Sicherer et al. proposed the concept of food protein-induced enterocolitis syndrome (FPIES) as a non-IgE-mediated gastrointestinal allergy that develops in early infancy. Typical FPIES is non-IgE mediated, but atypical FPIES with elevated IgE antibodies has also been reported and these patients less easily acquire tolerance for milk with age. In our study, milk-specific IgE was detected in only 30% of cases. Thus, a test for specific IgE alone is likely to be insufficient for diagnosis of CMA in neonates. However, it is possible that CMA with an increased level of IgE antibodies may follow a similar course, and awareness of this issue is important.

Several institutions in Japan have recently reported that the LST is useful for diagnosis of delayed type food allergy. The LST-positive rate was 80% in our study, which supports the utility of this test. However, a LST tended not to be performed for antigen-specific IgE positive cases, and the LST may be viewed as a secondary test that is performed in cases that are antigen-specific IgE-negative. This is because the LST requires a higher blood sample volume and is not currently available on a commercial basis. Several reports have also shown that there is no significant difference in the results of the LST between IgE-mediated and non-IgE-mediated CMA. Further evaluation of the sensitivity and specificity of the LST is needed in a large population.

Tests for fecal eosinophils are commonly conducted for cases with food-related gastrointestinal symptoms suggestive of CMA and the positive rate may reach 70%, which suggests that this is a useful test to screen CMA and to evaluate the disease condition and the effects of treatment. However, the results should be used carefully because the test may be positive even in normal neonates in early infancy. High levels of inflammatory reactions and liver dysfunction have also been described in neonates in previous reports. Cases with high CRP tend to develop clinical manifestations such as fever and not doing well, making differential diagnosis from severe infection difficult.
In Japan, most neonates with suspected CMA are treated by neonologists who tend to avoid conducting an OFC due to the risk of inducing serious symptoms. However, Host et al. found that OFC-induced allergic symptoms occurred in only one-third of neonates with gastrointestinal symptoms that suggested CMA. Most neonates with food-related symptoms suggestive of CMA develop gastrointestinal symptoms and few develop shock symptoms, as revealed in the current study, which suggests that there is a low risk of inducing serious immediate symptoms in an OFC test, at least in cases with only mild gastrointestinal symptoms at the onset.

We confirmed that clinical manifestations of food-related symptoms suggestive of CMA in neonates were distinctly different from those of common immediate type food allergies and that these manifestations were largely affected by underlying complications such as prematurity and gastroenterological surgery. Further OFC-based prospective accumulation of cases of CMA in neonates will be particularly important to reveal the full clinical features of this disease.

ACKNOWLEDGEMENTS

The study was supported by a Grant-in-Aid for Scientific Research into prevention and treatment of allergic diseases from the Ministry of Health, Labour and Welfare, Japan. We acknowledge all the collaborating research institutions for providing detailed clinical data for the subjects.

REFERENCES