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Extensively-hydrolyzed formula of cow's milk proteins for refeeding preterm infants with necrotizing enterocolitis: Results of national survey

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Abstract

Objective: The aims of this study were to evaluate the frequency and reasons of use of extensively hydrolyzed formula of cow's milk proteins (EHF) in French neonatal units (NUs) and the modality of use when prescribed for refeeding infants with NEC.

Methods: Clinical practice survey by the mean of a questionnaire addressing 1) the prevalence of use and the reasons for prescribing an EHF in hospitalized neonates and 2) the duration of use for infants who have had NEC, and the protocol used for weaning these infants of EHF. The questionnaire was send to one senior physician per neonatal unit.

Results: 91% of the NUs surveyed used EHF. Out of the 1 969 infants hospitalized the day of the survey, 11% were fed an EHF with a higher frequency of use in level II (14%) than in level III units (8.5%) ($p<0.0001$). Eleven percent of the prescriptions of EHF were due to a previous NEC. The main reasons for using an EHF as the preferred milk for feeding infants post NEC were the absence of human milk (75%) and the need for surgery (17%). When given, EHF was mainly prescribed for a period varying between 15 days and 3 months. None of the units were continuing EHF after 6 months of age. More than half the units declared hospitalizing the infant for the initiation of weaning from EHF but only 21% of them performed allergic test for cow's milk allergy.

Conclusions: The prevalence of use of EHF in the French NUs is high. Refeeding infants with NEC is one the reasons of such high prevalence. The main driver for choosing an EHF is the absence of human milk, either bank human milk or mother's milk.

Key Words: hydrolyzed formula, feeding methods, low birth weight infants, milk hypersensitivity, necrotizing enterocolitis

Abbreviations: NEC – necrotizing enterocolitis; MCT - medium-chain triglycerides; GI – gastrointestinal; EHF - extensively hydrolyzed formula of cow's milk proteins; GA - gestational age

Strengths of this study:

- This nationwide survey shows for the first time that extensively-hydrolyzed formula of cow's milk proteins are frequently used in neonatal departments.
- Refeeding infants after necrotizing enterocolitis is one of the main reasons for prescribing an extensively-hydrolyzed formula to preterm infants especially when mother's milk or banked human milk is not available.
- The modalities for weaning from extensively hydrolyzed formula are extremely variable demonstrating a lack of consensus.

Limits of this study:

- This is a clinical practice survey by the mean of a questionnaire
- The benefits/risk ratio, as well as the modality for weaning of EHF should be evaluated in further studies.

Introduction

Necrotizing enterocolitis (NEC) is a major issue in preterm, especially extremely preterm (<28 weeks' gestation) neonates worldwide. Its mean prevalence among very preterm infants is about 7% with a reported mortality rate of 20 to 30% [1]. Many clinical trials have evaluated the safety and benefits from preventive strategies which include trophic feedings, standardized feeding regimens, provision of breast milk, arginine supplementation, probiotic therapy, and infection control measures [2].

The severity of intestinal involvement influences the decision for medical or surgical management. However some aspects are common to both medical NEC and surgical NEC. The initial management of an infant with NEC includes providing supportive respiratory and hemodynamic care, discontinuation of all enteral feedings and medications, placement of a gastric tube to allow gastric and intestinal decompression, start of parenteral nutrition to support energy and protein needs, and administration of broad-spectrum intravenous antibiotics [3]. Surgical intervention may be required especially if an intestinal perforation is diagnosed, but there is a lack of comparative evidence to support primary anastomosis over enterostomy after intestinal resection during laparotomy for acute NEC in infants [3].

There is a lack of consensus on when enteral feeding should be reintroduced, and on the method and the rate of reaching goal feeding volumes [4]. The choice of feedings post-NEC remains controversial. In most instances, breast milk is considered as the optimal feeding when available [5]. In the case of the absence of breast milk, some physicians use premature milks if the gastrointestinal (GI) injury is limited. Some others use banked human milk or hydrolyzed formula. In the case of larger GI injury, hydrolyzed formula with no lactose and variable amounts of medium-chain triglycerides (MCT) or elemental formula preparations are used to improve absorption [5].

The purpose of this study is to evaluate the frequency of use of extensively hydrolyzed formula of cow's milk proteins (EHF) in French neonatal units and the modality of their use when prescribed for refeeding infants with NEC.

1. Materials and methods

To perform this study, we designed a survey using a questionnaire specially designed to investigate nutrition routine practices in neonatology departments. The survey technique used a closed-answer questionnaire to limit variability of answers and to decrease the number of incomplete answers. It focused on the enteral nutrition protocol after the initial management or postoperatively of the infants with NEC. The first series of questions aimed at determining the frequency of use and the reasons for prescribing an EHF in neonates. To achieve this goal, the physicians answering the questionnaire were asked to report the total number of infants hospitalized in their unit the day of filling the questionnaire as well as the number of infants receiving an EHF because of a previous NEC. The second series of questions focused on the nutritional protocol in the unit and assessed the modality of use of EHF for refeeding infants with NEC. More specifically, questions targeted the duration of use, and the protocol used for weaning the infants from EHF.

The extensive list of neonatal departments of metropolitan France and overseas territories was established by combining the lists of national scientific societies involved with newborn care and those of all regional health care services each contacted individually. Neonatal departments that had high acuity or intensive care beds were selected for the study. Only one questionnaire was sent per unit; it was accompanied by a cover letter and a reply envelope, and sent by mail to the head of the department. The senior physician in each NICU was asked to complete the survey questionnaire or delegate the task to a colleague devoting ≥20% of their time to patient care and with >3 years of clinical experience in neonatal intensive care.

We aimed at surveying at least half of the national neonatal units to have a precise view of the nutritional care of the preterm infants with NEC. One subsequent mailing was sent one month later to the non-respondents to achieve our goal. The units were divided as level II and level III units as previously described [6]. The identity of the physicians contacted and requested to complete the survey remained blinded for the analysis.

Statistical analysis

Statistical analyses were restricted to completed questionnaires with evaluable results. Data were analyzed using Minitab® 13.3 software (Minitab Inc., State College, PA, USA). General frequency responses to all survey items were determined and then used to test for associations among the categorical variables. When needed, data were split to cross tabs with respect to various grouping variables. Results from level II and III units were compared by Pearson χ^2 test of independence. A p-value ≤ 0.05 was considered statistically significant.

2. Results

2.1 Characteristics of the units

The goal of surveying at least half of the French neonatal units was reached since we received 174 answers from the 296 units contacted (58.8%). The answer rate was similar to the level III (42/66 = 64%) and the level II units (132/230 = 57%) (p=0.364). The characteristics of the units are reported in the Table I. Out of the 174 units surveyed, 158 out of them (91%) routinely use EHF (Table I).

2.2 Prevalence of use and reasons for feeding hospitalized neonates with EHF

Out of the 1 969 infants hospitalized the day of the filling of the questionnaire, 216 received an extensively-hydrolyzed formula (12.1%) with a higher frequency of use in level II (14%) than in level III units (8.5%) (p<0.0001). The main reasons for feeding hospitalized neonates with EHF are indicated in Table II. Shortage of human milk is overall the main reasons for prescribing EHF either for the initiation of feeding in preterm infants or for complementary feeding of breastfed infants.

Among the infants receiving EHF, 10.5% of the prescriptions were made because of a previous NEC with a higher percentage of the prescriptions for NEC observed in the level III than in the level II units (p=0.025).

2.3 Nutritional protocols when using EHF for feeding infants with NEC

Out of the 174 units surveyed, 93 (53.4%) routinely took care of infants with NEC. EHF were routinely used in 88 of them (95%) (Table I).

The main reasons for using an EHF as the preferred milk for feeding infants post NEC were the absence of human milk (n= 65/93, 75%) and the need for surgery (n= 15/93, 17%). The other reasons cited were NEC in term babies for whom EHF is nutritionally adapted, other associated digestive problems, NEC severity at onset, and salvage of bank human milk.

EHF, when given, were mainly prescribed for a period which varies from 15 days to 3 months. None of the units continued giving EHF after 6 months of age (Table III).

Table III describes the mode of weaning from EHF. In the absence of breast milk, 83% of the units declared switching to cow's milk formula, the others using either bank human milk or partially hydrolyzed formula. Most of the units weaned from EHF progressively for a mean (SD) period of 6.9 (3.1) days. More than half of the units declared hospitalizing the infant for the initiation of weaning but only 21% of them performed allergic test for cow's milk allergy.

3. Discussion

To our knowledge this first study which attempts to determine the frequency of use of EHF in neonatology departments. We found that the prevalence of the prescriptions of EHF is high, reaching 12% of the prescriptions of enteral or oral nutrition. There are many reasons for prescribing such formula to neonates. They include the absence of human milk, a poor feeding tolerance [7], a severe gastrointestinal reflux [8], a family history or clinical signs of cow's milk allergy [9], or an history of gastrointestinal surgery or intestinal resection [10]. Our study clearly shows that the refeeding of infants with NEC is a frequent reason of EHF prescription in sick neonates.

We found a statistically significant difference between the prevalence for using EHF in level II units compared to that of level III units. This fits well with the observation that the main reason for using EHF is the shortage of mother's milk. Indeed the availability of pasteurized human milk is lower in the level II units since, in France, the milk banks are usually located close to the level III units, not to the level II units. In contrast, it is not surprising that level III units are more prone to use EHF for refeeding infants after NEC since only these units have high acuity beds and are able to care for such surgical, or potentially surgical, patients.

Most of the textbooks and the literature focus on the nutritional prevention, not the treatment of NEC. Furthermore, they are extremely vague with regards to the timing of refeeding and the type of milk that should be used after the initial management or postoperatively of infants who have had NEC. It is, however, recognized that the feeding should be suspended for a period that is dependent of the severity of clinical disease but no clear recommendations for when to restart feeding after the episode of NEC have been made [5 11]. The choice of formula milk for feeding NEC infants depends on many considerations such as the gestational age of the infant, the availability of human milk, the risk of small bowel syndrome and/or malabsorption, and the risk of cow's milk allergy. Our study clearly shows that the main driver for choosing an EHF is the absence of human milk, either bank human milk or mother's milk.

Prescribing EHF in infants with NEC is not in line with the few guidelines we are aware of, in which it is recommended to start feeding NEC infants with preterm formula in absence of human milk [5] [11]. There are, however, several putative reasons for choosing an EHF. Premature infants recovering from mucosal inflammation and prolonged period of bowel rest are potentially at an increased risk of antigen response to intact protein [11]. Several case reports have shown that cow's milk protein allergy is closely related to NEC [12] [13] [14] or may occur after NEC. This suggests that cow's milk protein tolerance should be evaluated when NEC occurs in the case of absence of classical risk factors [15]. In this context, extensively hydrolyzed formula may be useful for feeding infants with NEC.

Cow's milk allergy is well recognized as a significant cause of morbidity in formula-fed term infants (14) and more recently, in preterm infants [16]. More specifically, it has been shown that allergy to cow's protein milk in surgical newborns is higher than expected and may reach 4% in absence of family history of allergy [17]. There are also evidence of in vitro sensitization to cow's milk protein in peripheral blood mononuclear cells of preterm infants with NEC [18] [19]. In addition to that, there is a debate on the direct contributory role of cow's milk protein sensitization in the pathogenesis of NEC [20]. The use of EHF for feeding infants with NEC in the view of preventing cow's milk protein sensitization should however be tested by further studies since to date, no studies were able to confirm the usefulness of hydrolyzed formula in prevention of allergy, in either unselected cohorts of very low birth weight infants [21] or in preterm infants with an atopic predisposition [22].

The use of EHF may also be considered because of their nutritional values. Indeed they usually do not contain lactose and some of them do contain significant amount of medium-chain triglycerides (MCT). These characteristics may improve the absorption during the refeeding period especially in surgical patients. Lactose is poorly tolerated in neonates with small bowel loss because of the decrease in available mucosal lactase; Medium-chain triglycerides improve fat absorption which is reduced because of loss of absorptive area, rapid transit, bile acid depletion, and/or bacterial overgrowth [10]. In contrast, the theoretical

advantage for using hydrolyzed protein because of a possible better absorption than the whole protein in face of a reduced absorptive area and decreased pancreatic enzyme output, remains uncertain since it has been shown that dietary protein absorption capacity of the small intestine is normal for most neonates after intestinal surgery [23].

If there are possible nutritional benefits for using EHF for feeding infants after NEC, they should be waited against possible disadvantages [24]. Indeed these formulas have an energy density close to term formula and usually low in minerals and polyunsaturated fatty acids compared to preterm formula. Urinary nitrogen excretion is higher [25], calcium and phosphorus absorption and nitrogen retention are lower in preterm infants fed hydrolyzed formula compared to those fed whole protein formula [26] [27]. This may alter the quality of growth or decrease lean mass accretion even in absence of similar growth rate of preterm infants receiving hydrolyzed preterm formula vs non-hydrolyzed formula [28] [29].

In the absence of specific recommendations and studies aiming at assessing the risk of food allergy in infants with NEC, it is not surprising that our study shows a great heterogeneity in the protocols for weaning the infants of the EHF. In the contrary of the suggestions made by El Hassani et al [17] and more importantly to the guidelines for prevention of food allergy [30], our study shows that the duration of use is lower than the 4 to 6 months recommended. In addition to that, most of the units introduce cow's milk protein without performing any appropriate diagnostic work-up.

It should be recognized that our study has several limitations. It was performed in only one country. On the other hand, it was performed in a large number of neonatal departments (> 50 %) and thus gives a precise picture of the management of the NEC infants in France. Furthermore, since this survey was performed in a large number of neonatal departments and since the respondents were asked to identify, among the infants in their unit, those who were receiving EHF the day the questionnaire was filled, we have a precise estimate of the prevalence of use of EHF. Unfortunately we did not obtain the total number of infants with NEC precluding us to calculate the percentage of NEC infants who were fed with EHF.

Finally as the questionnaire was designed, this survey did not allow us to assess the exact routine timing for restarting feeds after an episode of NEC nor the rate of advancement of feeds.

In conclusion, this study shows that the use of EHF in the neonatal departments is frequent. Refeeding infants with NEC is one the reasons of such high prevalence. The main driver for choosing an EHF is the absence of human milk, either bank human milk or mother's milk. NEC patients are a group of infants who may benefit from these specific formula but the benefits/risk ratio, as well as the modality for weaning of EHF, should be evaluated in further studies.

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Contributors' Statement

Alexandre Lapillonne served as the coordinator for the survey. He had access to all of the primary data and performed the statistical analyses. He drafted the manuscript. He also participated in the review, revision and approval of the final manuscript.

Maroun Matar drafted the manuscript. He had access to all of the primary data and participated to the statistical analyses. He also participated in the review, revision and approval of the final manuscript.

Ariane Adleff-Genot designed and performed the survey. She had access to all of the primary data and participated to the statistical analyses. She participated in the review, revision and approval of the final manuscript.

Elsa Kermorvant-Duchemin participated in the study and critically revised the manuscript. She participated in the review, revision and approval of the final manuscript

Florence Campeotto critically revised the manuscript and provided significant scientific input. She participated in the review, revision and approval of the final manuscript.

Competing interests

The authors have no conflicts of interest relevant to this article to disclose and have no financial disclosure to declare.

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Data sharing statement

Data are not currently shared but are available on request by the journal.

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Table I.

Characteristics of the units

	Level III units	Level II units	Total
Units surveyed			
Number of units surveyed (n)	42	132	174
Number of units using EHF routinely (n)	41	117	158
Number of units routinely caring infants after NEC (n)	41	52	93
Number of admissions			
Number of admissions per year in the units surveyed (n) *	23 175	38 403	61 578
GA <37 weeks (n) *	11 476	16 553	28 029
GA <28 weeks (n) *	1 947	347	2 394

*based on the responses of 150 units; GA = gestational age

Table II.

Frequency of use of extensively-hydrolyzed formula (EHF) among 1969 neonates hospitalized the day of the survey.

	n (%)
Number of infants hospitalized the day of survey	1 969
Number of infants receiving an EHF, n (% of hospitalized infants)	238 (12.1%)
Reasons for feeding neonates with EHF	
Refeeding	
after NEC	25 (10.5%)
after perinatal asphyxia	8 (3.3%)
after surgery	4 (1.7%)
Shortage of human milk	
feeding initiation of preterm infants	63 (26.5%)
feeding hospitalized breastfed neonates	84 (35.3%)
Allergy prevention in high risk neonates	2 (0.8%)
Gastrointestinal symptoms (compatible or not with cow's milk protein allergy)	31 (13.0%)
Others (research protocol, hypoglycemia, cholestasis, metabolic disease, etc., no reasons indicated)	21 (8.8%)

* Significantly different from level III units; EHF = Extensively-hydrolyzed formula of cow's milk proteins;

Table III.

Nutritional protocols of units using extensively-hydrolyzed formula (EHF) for feeding preterm infants after NEC

Duration of EHF use	Percent of units
< 15 days	8%
15 days – 1 month	30%
1-3 months	50%
4-6 months	12%
≥ 7 months	0%
Weaning of EHF in hospital	
Yes	52%
No	48%
Weaning of EHF progressively over several days	
Yes	96%
No	4%
Weaning of EHF after performing cow's milk allergy test	
Yes	21%
No	79%
Type of milk for weaning of EHF in absence of mother's milk	
Human milk bank	13%
Regular cow's milk formula	83%
Partially hydrolyzed formula or other	7%

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Use of extensively-hydrolyzed formula for refeeding neonates with necrotizing enterocolitis: A national survey-based, cross sectional study

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Abstract

Objective: The aims of this study were to evaluate the prevalence and reasons of use of extensively hydrolyzed formula of cow's milk proteins (EHF) in French neonatal units and the modality of use when prescribed for refeeding infants with necrotizing enterocolitis (NEC).

Methods: Multicentric national cross sectional study using a questionnaire addressing 1) the prevalence of use and the reasons for prescribing an EHF in hospitalized neonates and 2) to examine the protocols and reasons of use when prescribed for refeeding infants with NEC . The questionnaire was sent to one senior physician per neonatal unit.

Results: More than half of the French neonatal departments were surveyed. Ninety-one percent of the units used EHF. Out of the 1 969 infants hospitalized the day of the survey, 12% were fed with an EHF. Eleven percent of the prescriptions of EHF were due to a previous NEC. The main reasons for using an EHF to feed infants post NEC were the absence of human milk (75%) and when surgical management of the episode of NEC was required (17%). When given, EHF was mainly prescribed for a period varying between 15 days and 3 months. None of the units were continuing EHF after 6 months of age. More than half the units declared hospitalizing the infant for the initiation of weaning from EHF but only 21% of them performed allergic test for cow's milk allergy.

Conclusions: The prevalence of use of EHF in the French NUs is high. Refeeding infants post NEC is one of the reasons of such high prevalence. The main drive for choosing an EHF is the absence of human milk, either banked human milk or mother's milk.

Key Words: hydrolyzed formula, feeding methods, low birth weight infants, necrotizing enterocolitis, refeeding

Abbreviations: NEC – necrotizing enterocolitis; EHF - extensively hydrolyzed formula(s) of cow's milk proteins; GA - gestational age

Strengths of this study:

- This nationwide survey shows for the first time that the prevalence of use of extensively-hydrolyzed formula of cow's milk proteins is high in neonatal departments.
- Refeeding infants after necrotizing enterocolitis is one of the main reasons for prescribing an extensively-hydrolyzed formula to preterm infants especially when mother's milk or banked human milk is not available.
- The modalities for weaning from extensively hydrolyzed formula are extremely variable demonstrating a lack of consensus.

Limits of this study:

- This is a clinical practice survey by the means of a questionnaire
- The benefits-risk ratio, as well as the modality for weaning from EHF should be evaluated in further studies.

Introduction

Necrotizing enterocolitis (NEC) is a major issue in preterm, especially extremely preterm (<28 weeks' gestation) neonates worldwide. Its mean prevalence among very preterm infants is about 7% with a reported mortality rate of 20 to 30% [1]. Many clinical trials have evaluated the safety and benefits from preventive strategies while others have attempted to determine the best possible medical or surgical management [2,3].

In contrast there is a lack of consensus on when enteral feeding should be reintroduced, and on the method and the rate of reaching target feeding volumes [4]. The choice of feeding post-NEC remains controversial. In most instances, breast milk is considered as the optimal feeding when available [5]. In the case of the absence of breast milk, some physicians use premature milks if the gastrointestinal injury is limited. Some others use banked human milk or hydrolyzed formula. In the case of larger gastrointestinal injury, hydrolyzed formula with no lactose and variable amounts of medium-chain triglycerides or elemental formula preparations are used to get over the problem of malabsorption [5].

The purpose of this study is to evaluate the prevalence of use of extensively hydrolyzed formula of cow's milk proteins (EHF) in French neonatal units and to examine the protocols and reasons of use when prescribed for refeeding infants with NEC.

Materials and methods

To perform this nationwide study, we ran a survey using a questionnaire specially designed to investigate routine feeding practices in neonatology departments. The survey technique used a closed-answer questionnaire to limit variability of answers and to decrease the number of incomplete answers (questionnaire available on demand). It focused on the enteral feeding practices post-medical or surgical NEC.

The first series of questions aimed at determining the prevalence of use and the reasons for prescribing an EHF in neonates. To achieve this goal, we performed a multicentric national cross sectional study and asked the physicians answering the questionnaire to report the

total number of infants hospitalized in their unit the day of filling the questionnaire as well as the main reasons for prescribing such formula. Only infants who have had NEC of grade II or III were classing as infants with NEC, those with grade I NEC being classified as "gastrointestinal symptoms".

The second series of questions focused on the feeding protocols and reasons for EHF use when prescribed for refeeding infants recovering from NEC of stage II or III. More specifically, questions targeted the duration of use, and the protocol used for weaning the infants from EHF to regular cow's milk formula or human milk. It should be noted that the medical decision on choosing an EHF in France, particularly during hospitalization, is not accounted by any financial issues because every legal resident of France has access under the law of universal coverage to full coverage of the cost of the hospitalization of a preterm infant.

The extensive list of neonatal departments of metropolitan France and overseas territories was established by combining the lists of national scientific societies involved with newborn care and those of all regional health care services each contacted individually. Neonatal departments that had high acuity or intensive care beds were selected for the study. Only one questionnaire was sent per unit; it was accompanied by a cover letter and a reply envelope, and sent by mail to the head of the department. The senior physician in each neonatal unit was asked to complete the survey questionnaire or delegate the task to a colleague devoting ≥20% of their time to patient care and with >3 years of clinical experience in neonatal intensive care.

In order to reduce the risk of selection bias, we aimed at surveying at least half of the national neonatal units to have a precise view of the nutritional care of the preterm infants post NEC. One subsequent mailing was sent one month later to the non-respondents to achieve our goal. The identity of the physicians contacted and requested to complete the survey remained blinded for the analysis.

Statistical analysis

Statistical analyses were restricted to completed questionnaires with evaluable results. Data were analyzed using Minitab® 13.3 software (Minitab Inc., State College, PA, USA). General frequency responses to all survey items were determined and then used to test for associations among the categorical variables. When needed, data were split to cross tabs with respect to various grouping variables. Comparison were made by Pearson χ^2 test of independence. A p-value ≤ 0.05 was considered statistically significant.

Results

Characteristics of the units

The goal of surveying at least half of the French neonatal units was reached since we received 174 responses from the 296 units contacted (58.8%). The characteristics of the responding units are reported in the Table I. Out of the 174 units surveyed, 158 (91%) routinely use EHF (Table I).

Prevalence of use and reasons for feeding hospitalized neonates with EHF

Out of the 1 969 infants hospitalized the day of the filling of the questionnaire, 238 received an extensively-hydrolyzed formula (238/1969; 12.1%). The reasons for feeding hospitalized neonates with EHF were indicated in all cases and are reported in Table II. Shortage of human milk is overall the main reason for prescribing EHF either for the initiation of feeding in preterm infants or for complementary feeding of breastfed infants. Among the infants receiving EHF, 10.5% of the prescriptions were made because of a previous NEC.

Nutritional protocols when using EHF for feeding infants recovering from NEC

Out of the 174 units surveyed, 93 (53.4%) routinely took care of infants post NEC. EHF were routinely used in 88 of them (95%) (Table I).

The main reasons for using an EHF as the preferred milk for feeding infants post NEC were the absence of human milk (n= 65/93, 75%) and when surgical management of the episode of NEC was required (n= 15/93, 17%). The other reasons cited were NEC in term babies for

whom EHF is nutritionally adapted, other associated digestive problems, NEC severity at onset, and shortage of banked human milk.

EHF, when given, was mainly prescribed for a period which varies from 15 days to 3 months. None of the units continued giving EHF after 6 months of age (Table III). The mode of weaning from EHF to regular cow's milk formula or banked human milk is described in Table III. In the absence of breast milk, 83% of the units declared switching to cow's milk formula, the others using either banked human milk or partially hydrolyzed formula. Most of the units weaned from EHF progressively for a mean (SD) period of 6.9 (3.1) days. More than half of the units declared hospitalizing the infant for the initiation of weaning from EHF but only 21% of them performed allergic test for cow's milk allergy.

Discussion

To our knowledge this is the first study which attempts to determine the frequency of use of EHF in neonatology departments. We found that the prevalence of the prescriptions of EHF is high, reaching 12% of the prescriptions of enteral feeding. There are many reasons for prescribing such formula to neonates. They include absence of human milk, poor feeding tolerance [6], severe gastrointestinal reflux [7 8], family history or clinical signs of cow's milk allergy [9], or history of gastrointestinal surgery or intestinal resection [10]. Our study clearly shows that the refeeding of infants recovering from NEC is a frequent reason of EHF prescription in hospitalized neonates.

Most literature focuses on the nutritional prevention, not the treatment of NEC. Furthermore, they are extremely vague regarding the timing of refeeding and the type of milk that should be used after the initial management or postoperative of infants who have had NEC. It is, however, recognized that the feeding should be suspended for a period that is dependent on the severity of clinical disease but no clear recommendations for when to restart feeding after the episode of NEC have been made [5 11]. The choice of formula milk for feeding NEC infants depends on many considerations such as the gestational age of the infant, the availability of human milk, the risk of short bowel syndrome and/or malabsorption, and the risk of cow's

milk allergy. Our study clearly shows that the main drive for choosing an EHF for refeeding NEC infants is the absence of human milk, either banked human milk or mother's milk.

EHF is not a mode of feeding that is usually cited in the few textbooks describing the choice of feeding post-NEC [5 11]. There are, however, several putative reasons for choosing an EHF. Firstly, there is a debate on the direct contributory role of cow's milk protein sensitization in the pathogenesis of NEC [12]. Cow's milk allergy is well recognized as a significant cause of morbidity in formula-fed term infants and more recently, in preterm infants [13]. Several case reports have shown that cow's milk protein allergy may be closely related to NEC [14 15 16] and there are also evidence of *in vitro* sensitization to cow's milk protein in peripheral blood mononuclear cells of preterm infants with NEC [17 18]. This suggests that cow's milk protein tolerance should be evaluated when NEC occurs in the case of absence of classical risk factors [19].

Secondly, premature infants recovering from mucosal inflammation and prolonged period of bowel rest are potentially at an increased risk of antigen response to intact protein [11]. It has been shown that allergy to cow's protein milk in newborns who underwent gastrointestinal surgery is higher than expected in absence of family history of allergy [20]. In this context, EHF may be useful for feeding infants post NEC despite no study to date have assessed the usefulness of this strategy for feeding infants recovering from NEC in the view of preventing cow's milk protein sensitization.

Finally, the use of EHF may also be considered because of their nutritional values. Indeed they usually do not contain lactose and some of them do contain significant amount of medium-chain triglycerides. These characteristics may improve the absorption during the refeeding period especially in surgical patients. Lactose is poorly tolerated in neonates with small bowel loss because of the decrease in available mucosal lactase; Medium-chain triglycerides improve fat absorption which is reduced because of loss of absorptive area, rapid transit, bile acid depletion, and/or bacterial overgrowth [10]. In contrast, the theoretical advantage for using hydrolyzed protein because of a possible better absorption than the

whole protein in face of a reduced absorptive area and decreased pancreatic enzyme output, remains uncertain since it has been shown that dietary protein absorption capacity of the small intestine is normal for most neonates after intestinal surgery [21].

If there are possible nutritional benefits for using EHF for feeding infants after NEC, they should be weighed against possible disadvantages [22]. Indeed these formulas have an energy density close to term formula and usually low in minerals and polyunsaturated fatty acids compared to preterm formula. Urinary nitrogen excretion is higher [23], calcium and phosphorus absorption and nitrogen retention are lower in preterm infants fed with hydrolyzed formula compared to those fed with whole protein formula [24 25]. This may alter the quality of growth or decrease lean mass accretion even in absence of similar growth rate of preterm infants receiving hydrolyzed preterm formula vs non-hydrolyzed formula [26 27].

In the absence of specific recommendations and studies aiming at assessing the risk of food allergy in infants post NEC, it is not surprising that our study shows a great heterogeneity in the protocols for weaning the infants from the EHF. On the contrary of the suggestions made by El Hassani et al [20] and more importantly of the guidelines for prevention of food allergy [28], our study shows that the duration of use is lower than the 4 to 6 months recommended. In addition to that, cow's milk proteins are frequently introduced without performing any appropriate diagnostic work-up.

It should be recognized that our study has several limitations. This study was performed in one country only and results may not be applicable in other countries. It may be argued that this study cross sectional was performed at a single point in time only, not over a long period of time. However, it is recognized that such study design is particularly suitable for assessing the prevalence of a disease or treatment in a population [29]. Although we aimed and succeeded at assessing more than half of the French units, such study design is prone to selection bias[30]. We therefore cannot exclude that the non-respondent units were those in which EHF was used the least or the most frequently. Finally, this survey did not allow us to

assess any longitudinal follow-up nor the incidence of NEC [29]. However, we were able to assess the intent-to-treat modalities of feeding infants post NEC.

In conclusion, this study shows that the use of EHF in the neonatal departments is frequent. Refeeding infants post NEC is one of the reasons of such high prevalence. The main drive for choosing an EHF is the absence of human milk, either banked human milk or mother's milk. NEC patients are a group of infants who may benefit from these specific formula but the benefits/risk ratio, as well as the modality for weaning from EHF, should be evaluated in further studies.

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Contributors' Statement

Alexandre Lapillonne served as the coordinator for the survey. He had access to all of the primary data and performed the statistical analyses. He drafted the manuscript. He also participated in the review, revision and approval of the final manuscript.

Maroun Matar drafted the manuscript. He had access to all of the primary data and participated to the statistical analyses. He also participated in the review, revision and approval of the final manuscript.

Ariane Adleff-Genot designed and performed the survey. She had access to all of the primary data and participated to the statistical analyses. She participated in the review, revision and approval of the final manuscript.

Marwa Chbihi provided significant scientific input and critically revised the manuscript. She participated in the review, revision and approval of the final manuscript

Elsa Kermorvant-Duchemin participated in the study and critically revised the manuscript. She participated in the review, revision and approval of the final manuscript

Florence Campeotto critically revised the manuscript and provided significant scientific input. She participated in the review, revision and approval of the final manuscript.

Competing interests

The authors have no conflicts of interest relevant to this article to disclose and have no financial disclosure to declare.

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Data sharing statement

Data are not currently shared but are available on request by the journal.

There are no additional data available.

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Table I.

Characteristics of the units

	Total
Units surveyed	
Number of units surveyed (n)	174
Number of units using EHF routinely (n)	158
Number of units routinely caring infants after NEC (n)	93
Number of admissions	
Number of admissions per year in the units surveyed (n) *	61 578
GA <37 weeks (n) *	28 029
GA <28 weeks (n) *	2 394

*based on the responses of 150 units; GA = gestational age

Table II.

Frequency of use of extensively-hydrolyzed formula (EHF) among 1969 neonates hospitalized the day of the survey.

	n (%)
Number of infants hospitalized the day of survey	1 969
Number of infants receiving an EHF, n (% of hospitalized infants)	238 (12.1%)
Reasons for feeding neonates with EHF	
Initiation of feeds	
after NEC stage II or III	25 (10.5%)
after perinatal asphyxia	8 (3.3%)
after any kind of surgery	4 (1.7%)
Shortage of human milk	
feeding initiation of preterm infants in absence of human milk	63 (26.5%)
complementary feeding of breastfed neonates	84 (35.3%)
Allergy prevention in high risk neonates	2 (0.8%)
Gastrointestinal symptoms (compatible or not with cow's milk protein allergy)	31 (13.0%)
Others (research protocol, hypoglycemia, cholestasis, metabolic disease, etc., no reasons indicated)	21 (8.8%)

EHF = Extensively-hydrolyzed formula of cow's milk proteins;

Table III.

Nutritional protocols of units using extensively-hydrolyzed formula (EHF) in preterm infants recovering from NEC

Duration of EHF use	Percent of units
< 15 days	8%
15-30 days	30%
1-3 months	50%
4-6 months	12%
≥ 7 months	0%
Weaning from EHF in hospital	
Yes	52%
No	48%
Weaning from EHF progressively over several days	
Yes	96%
No	4%
Weaning from EHF after performing cow's milk allergy test	
Yes	21%
No	79%
Type of milk for weaning from EHF in absence of mother's milk	
Banked human milk	13%
Regular cow's milk formula	83%
Partially hydrolyzed formula or other	7%

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	NA
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	6
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA

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Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(*b*) Report category boundaries when continuous variables were categorized	8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Use of Extensively-hydrolyzed Formula for Refeeding Neonates Post-necrotizing enterocolitis: A Nationwide Survey-based, Cross sectional study

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Use of Extensively-hydrolyzed Formula for Refeeding Neonates Post-necrotizing enterocolitis: A Nationwide Survey-based, Cross sectional study

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Abstract

Objective: To evaluate the prevalence and reasons for using extensively hydrolyzed formulas (EHF) of cow's milk proteins in the French neonatal units as well as the modality of their prescription for refeeding infants recovering from necrotizing enterocolitis (NEC).

Methods: Multicenter nationwide cross sectional study using a questionnaire to address the prevalence of use and the reasons for prescribing EHF in hospitalized neonates and to examine the protocols and the actual reasons of their use for refeeding infants in recovery from NEC. The questionnaire was sent to only one senior neonatologist in each neonatal unit included in the study.

Results: More than half of the French neonatal units participated I the survey. Ninety-one percent of the surveyed units used EHF. Of 1 969 infants hospitalized the day the survey was run, 12% were fed on an EHF. Eleven percent of the EHF prescriptions were due to previous NEC. The main reasons for using an EHF to feed infants post NEC were the absence of human milk (75%) and when surgical management of NEC was performed (17%). When given, EHF was mainly prescribed for a period varying between 15 days and 3 months. None of the involved units continued using the EHF after 6 months of age. More than half of the surveyed units acknowledged hospitalizing infants for the initiation of weaning EHF but only 21% of them tested these infants for cow's milk allergy.

Conclusions: The prevalence of EHF use in the French neonatal units is high. Refeeding infants post NEC is one of the main reasons for such a high prevalence. The main incentive for using an EHF is the absence of human breast milk, either maternal or donor milk.

Key Words: hydrolyzed formula, feeding methods, low birth weight infants, necrotizing enterocolitis, refeeding

Abbreviations: NEC – necrotizing enterocolitis; EHF - extensively hydrolyzed formula(s) of cow's milk proteins; GA - gestational age

Strengths of this study:

- This nationwide survey shows for the first time that the prevalence of use of extensively-hydrolyzed formula (EHF) of cow's milk proteins is high in the French neonatal units.
- Refeeding infants after necrotizing enterocolitis is one of the main reasons for prescribing EHF for preterm infants especially when maternal or donor breast milk is not available.
- The weaning modalities of EHF varied between the units surveyed signifying a considerable lack of consensus.

Limits of this study:

- This is a questionnaire-based clinical practice survey.
- The benefits-risk ratio of the EHF use, as well as the modality for their weaning need to be evaluated by more studies.

Introduction

Necrotizing enterocolitis (NEC) is a major concern in preterm, especially extremely preterm (<28 weeks' gestation) neonates worldwide. Its mean prevalence among very preterm infants is about 7% with a reported mortality rate of 20 to 30% [1]. Many clinical trials have evaluated the safety and benefits of preventive strategies while others have attempted to determine the best possible medical or surgical management [2,3].

In contrast there is a perceived lack of consensus on when, and how enteral feeding should be reintroduced, and advanced till achieving the target volumes [4]. The choice of post-NEC feeding remains controversial. In most instances, when available, maternal breast milk is considered the optimal feeding [5]. In case of non-availability, some neonatologists use preterm milk formula, provided if the gastrointestinal injury is limited. Some others would use either donor breast milk or hydrolyzed formulas. When the gastrointestinal injury is substantial, elemental or lactose-free hydrolyzed formulas with variable content of medium-chain triglycerides, are used to get over the problem of malabsorption [5].

The purpose of this study was to evaluate the prevalence and indications of use of extensively hydrolyzed formula (s) (EHF) of cow's milk proteins in the French neonatal units and to examine the protocols guiding their use for refeeding infants post NEC.

Materials and methods

To conduct this study on a nationwide level, we ran a survey using a questionnaire especially designed to investigate routine feeding practices in the involved neonatal units. The survey used the technique of a closed-answer questionnaire to limit the variability of answers and decrease the number of incomplete answers focusing on enteral feeding practices post-medical or surgical NEC (Questionnaire available on demand).

The first series of questions aimed at determining the prevalence and indications of EHF use in neonates. To achieve this, we ran a multicenter nationwide cross-sectional study, and requested the neonatologists responding to the questionnaire to report the total number of

infants actually hospitalized in their units the day the questionnaire was filled in, together with the main reported indications of EHF. Only infants who had NEC of grade II or III were considered for the study, grade I NEC can be confused with other causes of feeding intolerance and were classified as “gastrointestinal symptoms”.

The second series of questions focused on the feeding protocols and reasons for EHF use when prescribed for refeeding infants recovering from NEC of stage II or III. More specifically, questions targeted the duration of use, and the protocol used for weaning infants of EHF to regular cow’s milk formula or human milk. It should be noted that the medical decision to use an EHF in France, particularly during hospitalization, was not made under any financial pressure, conflict of interest, or mitigation as every legal resident of France, including preterm infants, has, by law, a full universal coverage of healthcare.

The exhaustive list of neonatal units of metropolitan France and overseas territories was established by combining the lists of the national scientific societies involved in neonatal care and those of all the regional health care services. Each unit was individually contacted. Neonatal units having a high-acuity or intensive care beds were selected for the study. Only one questionnaire per unit accompanied by a cover letter and a reply envelope, was posted by mail to the head of the unit. He was asked to complete the survey questionnaire or to delegate the task to a colleague with more than 3 years of clinical experience in neonatal medicine, and more than 20% of time devoted to direct patient care.

In order to reduce the risk of selection bias, we aimed at surveying at least half of the nationwide neonatal units to have a picture of the nutritional care of the preterm infants post NEC as clear, and as accurate as possible. A reminder letter was subsequently posted one month later to the non-responders to achieve our goal. The identity of the neonatologists contacted and requested to complete the survey remained blinded for the analysis.

Statistical analysis

Statistical analyses were restricted to completed questionnaires with evaluable results. Data were analyzed using Minitab® 13.3 software (Minitab Inc., State College, PA, USA). General frequency responses to all survey items were determined and then used to test for associations among the categorical variables. When needed, data were split to cross tabs with respect to various grouping variables. Comparisons were made by Pearson χ^2 test of independence. A p-value ≤ 0.05 was considered statistically significant.

Results

Characteristics of the units

The goal of surveying at least half of the French neonatal units was reached since we received 174 responses from the 296 units contacted (58.8%). The characteristics of the responding units are reported in the Table I. Of the 174 units surveyed, 158 (91%) routinely used EHF (Table I).

Prevalence of use and reasons for feeding hospitalized neonates with EHF

Of the 1 969 infants hospitalized the day of the filling of the questionnaire, 238 (12.1%) received an extensively-hydrolyzed formula. The reasons for feeding hospitalized neonates with EHF were indicated in all cases as reported in Table II. Shortage of human milk is overall the main reason for prescribing EHF either for the initiation of feeding in preterm infants or for complementary feeding of breastfed infants. Among all the infants receiving EHF, 10.5% of the prescriptions were made because of a previous NEC.

Nutritional protocols when using EHF for feeding infants recovering from NEC

Of the 174 units surveyed, 93 (53.4%) routinely took care of infants post NEC. EHF were routinely used in 88 (95%) of them (Table I).

The main reasons for using EHF as the preferred milk for feeding infants post NEC were the absence of human breast milk (n= 65/93, 75%) and when surgical management of NEC was required (n= 15/93, 17%). The other reasons cited were NEC in term babies for whom EHF is

nutritionally adapted, other associated digestive problems, NEC severity at onset, and shortage of donor breast milk.

EHF, when given, were mainly prescribed for a period which varies from 15 days to 3 months. None of the units continued giving EHF after 6 months of age (Table III). The mode of weaning from EHF to regular cow's milk formula or donor breast milk is described in Table III. In absence of breast milk, 83% of the units switched to a cow's milk formula, while others shifted to using either donor breast milk or partially a hydrolyzed formula. Most of the surveyed units progressively weaned the EHF over a mean (SD) period of 6.9 (3.1) days. More than half of the units reported having the infants hospitalized for initiating the weaning process. However, only 21% of these units tested the infants for cow's milk allergy.

Discussion

To our knowledge this is the first study which attempts to determine the frequency of use of EHF in the neonatal units. We found that the prevalence of EHF use is high, approaching 12.1% of the enteral feeding prescriptions. The indications of EHF in neonates reported in literature include absence of human milk, poor feeding tolerance [6], severe gastrointestinal reflux [7 8], family history or clinical signs of cow's milk allergy [9], or history of gastrointestinal surgery or intestinal resection [10]. Our study clearly shows that refeeding infants recovering from NEC is a frequent indication of EHF use in hospitalized neonates.

Most available literature focuses on the nutritional prevention, not the treatment of NEC. Furthermore, it is extremely vague regarding the timing of refeeding and the type of milk to be used after initial management or postoperatively for infants who have had NEC. Although it is well established that feeding should be suspended for a period of time that depends on the disease severity, there are no clear recommendations on when to restart feeding after the subsidence of the acute-stage NEC [5 11]. The choice of formula milk for refeeding infants post NEC depends on many of such factors as gestational age, the availability of human breast milk, risk of short gut syndrome and/or malabsorption, as well as risk of cow's milk

allergy. Our study clearly shows that the main drive for choosing an EHF for refeeding NEC infants is the absence of human breast milk, either maternal or donor.

EHF is not the feeding formula usually cited in the few textbooks defining the feeding choices post-NEC [5 11]. There are, however, several putative reasons for choosing an EHF.

Firstly, there is a debate on the direct contributory role of cow's milk protein sensitization in the pathogenesis of NEC [12]. Cow's milk allergy is well recognized as a significant cause of morbidity in formula-fed term and more recently, in preterm infants [13]. Several case reports have shown that cow's milk protein allergy may be closely related to NEC [14 15 16] and there is also evidence of *in vitro* sensitization to cow's milk protein in peripheral blood mononuclear cells of preterm infants with NEC [17 18]. This suggests that cow's milk intolerance should be evaluated when NEC occurs in case of absence of classical risk factors [19].

Secondly, premature infants recovering from mucosal inflammation and prolonged periods of bowel rest are potentially at increased risk of antigenic response to intact proteins [11]. It has been shown that allergy to cow's milk proteins in newborns who underwent gastrointestinal surgery is higher than expected in absence of family history of allergy [20]. In this context, EHF may be useful for refeeding infants post NEC. Nevertheless, no study to date has assessed the usefulness of such a strategy for possibly preventing cow's milk protein sensitization.

Finally, the use of EHF may also be considered for their nutritional value. Indeed they do not usually contain lactose and some of them do contain significant amount of medium-chain triglycerides. These characteristics may improve absorption during refeeding especially in surgical patients. Lactose is poorly tolerated in neonates with a small bowel disease or resection because of the decrease in available mucosal lactase. Medium-chain triglycerides also improve fat absorption, known to be also reduced in cases of loss of absorptive area, rapid transit, bile acid depletion, and/or bacterial overgrowth [10]. In contrast, the theoretical advantage of hydrolyzed over whole protein formulas of better absorption in case of a reduced absorptive area and decreased pancreatic enzyme output, remains uncertain since

it has been shown that dietary protein absorption capacity of the small intestine is normal for most neonates after intestinal surgery [21].

If there are possible nutritional benefits for using EHF for feeding infants after NEC, they should be weighed against possible disadvantages [22]. Indeed these formulas have an energy density close to that of term formulas, in addition to usually low mineral and polyunsaturated fatty acid contents as compared to the preterm formulas. Urinary nitrogen excretion is higher [23], calcium and phosphorus absorption and nitrogen retention are lower in preterm infants fed with hydrolyzed formula compared to those fed with whole protein formula [24] [25]. These drawbacks may alter the quality of growth or decrease the lean body mass accretion in preterm infants receiving hydrolyzed formula when compared to those receiving non-hydrolyzed formula whether the growth rate was similar or not [26] [27].

In absence of specific recommendations or studies guiding or helping to assess the risk of food allergy in infants post NEC, it is not surprising that our study shows a great heterogeneity in the weaning protocols of EHF. In contrast to the recommendations of El Hassani et al [20], and more importantly to the guidelines for food allergy prevention [28], our study shows that the duration of use is less than the recommended duration of 4 to 6 months. In addition to that, cow's milk proteins are frequently introduced without performing any appropriate diagnostic work-up.

It should be recognized that our study has several limitations. This study was performed in one country only and results may not be valid to other countries. It may be argued that this is a cross sectional study that was performed at a single point of time. However, it is well known that such study design is particularly suitable for assessing the prevalence of a disease or a treatment in a specific population [29]. Although we aimed and succeeded at assessing more than half of the French units, such study design is prone to selection bias [30]. We, therefore, cannot state that the non-responding units were those who used EHF less or more than the responding ones. Finally, this survey did not allow us to assess any

longitudinal follow-up or incidence data of NEC [29]. However, we were able to assess the intent-to-treat modalities of feeding infants post NEC.

In conclusion, this study shows that the use of EHF in the neonatal units is frequent. Refeeding infants post NEC is one of the reasons of such high prevalence. The main drive for using EHF is the absence of human breast milk, either maternal or donor. NEC patients represent a group of infants who may benefit from these EHF. However, benefits/risk ratio of their use, as well as the modality of their weaning need to be further evaluated by more studies.

Acknowledgments

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Contributors' Statement

Alexandre Lapillonne served as the coordinator for the survey. He had access to all of the primary data and performed the statistical analyses. He drafted the manuscript. He also participated in the review, revision and approval of the final manuscript.

Maroun Matar drafted the manuscript. He had access to all of the primary data and participated to the statistical analyses. He also participated in the review, revision and approval of the final manuscript.

Ariane Adleff-Genot designed and performed the survey. She had access to all of the primary data and participated to the statistical analyses. She participated in the review, revision and approval of the final manuscript.

Marwa Chbihi provided significant scientific input and critically revised the manuscript. She participated in the review, revision and approval of the final manuscript

Elsa Kermorvant-Duchemin participated in the study and critically revised the manuscript. She participated in the review, revision and approval of the final manuscript

Florence Campeotto critically revised the manuscript and provided significant scientific input. She participated in the review, revision and approval of the final manuscript.

Competing interests

The authors have no conflicts of interest relevant to this article to disclose and have no financial disclosure to declare.

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Data sharing statement

Data are not currently shared but are available on request by the journal.

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Table I.

Characteristics of the responding neonatal units

	Total
Units surveyed	
Number of units (n)	174
Number of units using EHF routinely (n)	158
Number of units routinely caring infants post NEC (n)	93
Number of admissions	
Number of admissions per year (n) *	61 578
GA <37 weeks (n) *	28 029
GA <28 weeks (n) *	2 394

*based on the responses of 150 units; GA = gestational age

Table II.

Prevalence of use and reasons for feeding hospitalized neonates with extensively-hydrolyzed formula (EHF).

	n (%)
Infants hospitalized the day of the filling of the questionnaire (n)	1 969
Infants receiving an EHF, n (% of hospitalized infants)	238 (12.1%)
Reasons for feeding neonates with EHF	
Initiation of feeds	
after NEC stage II or III	25 (10.5%)
after perinatal asphyxia	8 (3.3%)
after any kind of surgery	4 (1.7%)
Shortage of human milk	
feeding initiation of preterm infants in absence of human milk	63 (26.5%)
complementary feeding of breastfed neonates	84 (35.3%)
Allergy prevention in high risk neonates	2 (0.8%)
Gastrointestinal symptoms (compatible or not with cow's milk protein allergy)	31 (13.0%)
Others (research protocol, hypoglycemia, cholestasis, metabolic disease, etc., no reasons indicated)	21 (8.8%)

EHF = Extensively-hydrolyzed formula of cow's milk proteins;

Table III.

Nutritional protocols of neonatal units using extensively-hydrolyzed formula (EHF) in preterm infants post necrotizing enterocolitis.

Duration of EHF use	Percent of units
< 15 days	8%
15-30 days	30%
1-3 months	50%
4-6 months	12%
≥ 7 months	0%
Weaning EHF in hospital	
Yes	52%
No	48%
Weaning EHF progressively over several days	
Yes	96%
No	4%
Weaning EHF after testing for cow's milk allergy	
Yes	21%
No	79%
Type of milk for weaning of EHF in absence of mother's breast milk	
Donor breast milk	13%
Regular cow's milk formula	83%
Partially hydrolyzed formula or other	7%

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	NA
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	6
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA

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Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(*b*) Report category boundaries when continuous variables were categorized	8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Use of Extensively-hydrolyzed Formula for Refeeding Neonates Post-necrotizing Enterocolitis: A Nationwide Survey-based, Cross Sectional Study

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Use of Extensively-hydrolyzed Formula for Refeeding Neonates Post-necrotizing Enterocolitis: A Nationwide Survey-based, Cross Sectional Study

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Abstract

Objective: To evaluate the prevalence of and reasons for using extensively hydrolyzed formulas (EHF) of cow's milk proteins in the French neonatal units as well as the modality of their prescription for refeeding infants recovering from necrotizing enterocolitis (NEC).

Methods: Multicenter nationwide cross sectional study using a questionnaire to address the prevalence of use and the reasons for prescribing EHF in hospitalized neonates and to examine the protocols and the actual reasons of their use for refeeding infants in recovery from NEC. The questionnaire was sent to only one senior neonatologist in each neonatal unit included in the study.

Results: More than half of the French neonatal units participated in the survey. Ninety-one percent of the surveyed units used EHF. Of 1 969 infants hospitalized the day the survey was run, 12% were fed on an EHF. Eleven percent of the EHF prescriptions were due to previous NEC. The main reasons for using an EHF to feed infants post NEC were the absence of human milk (75%) and surgical management of NEC (17%). When given, EHF was mainly prescribed for a period varying between 15 days and 3 months. None of the involved units continued using the EHF after 6 months of age. More than half of the surveyed units acknowledged hospitalizing infants for the initiation of weaning EHF but only 21% of them tested these infants for cow's milk allergy.

Conclusions: The prevalence of EHF use in the French neonatal units is high. Refeeding infants post NEC is one of the main reasons for such a high prevalence. The main incentive for using an EHF is the absence of human breast milk, either maternal or donor.

Key Words: hydrolyzed formula, feeding methods, low birth weight infants, necrotizing enterocolitis, refeeding

Abbreviations: NEC – necrotizing enterocolitis; EHF - extensively hydrolyzed formula (s) of cow's milk proteins; GA - gestational age

Strengths of this study:

- This nationwide survey shows for the first time that the prevalence of use of extensively-hydrolyzed formula of cow's milk proteins is high in the French neonatal units.
- Refeeding infants after necrotizing enterocolitis is one of the main reasons for prescribing EHF for preterm infants especially when maternal or donor breast milk is not available.
- The weaning modalities of EHF varied between the units surveyed signifying a considerable lack of consensus.

Limitations of this study:

- This is a questionnaire-based clinical practice survey.
- The benefit-risk ratio of the EHF use, as well as the modality for their weaning need to be evaluated by more studies.

Introduction

Necrotizing enterocolitis (NEC) is a major concern in preterm, especially extremely preterm (<28 weeks' gestation) neonates worldwide. Its mean prevalence among very preterm infants is about 7% with a reported mortality rate of 20 to 30% [1]. Many clinical trials have evaluated the safety and benefits of preventive strategies, while others have attempted to determine the best possible medical or surgical management [2,3].

In contrast, there is a perceived lack of consensus on when, and how enteral feeding should be reintroduced, and advanced till achieving the target volumes [4]. The choice of post-NEC feeding remains controversial. In most instances, when available, maternal breast milk is considered the optimal feeding [5]. In case of non-availability, some neonatologists use preterm milk formula, provided that the gastrointestinal injury is limited. Some others would use either donor breast milk or hydrolyzed formulas. When the gastrointestinal injury is substantial, elemental or lactose-free hydrolyzed formulas with variable content of medium-chain triglycerides, are used to get over the problem of malabsorption [5].

The purpose of this study was to evaluate the prevalence and indications of use of extensively hydrolyzed formula (s) (EHF) of cow's milk proteins in the French neonatal units and to examine the protocols guiding their use for refeeding infants post NEC.

Materials and methods

To conduct this study on a nationwide level, we ran a survey using a questionnaire especially designed to investigate routine feeding practices in the involved neonatal units. The survey used the technique of a closed-answer questionnaire to limit the variability of answers and decrease the number of incomplete answers, focusing on enteral feeding practices post-medical or surgical NEC (Questionnaire available on demand).

The first series of questions aimed at determining the prevalence and indications of EHF use in neonates. To achieve this, we ran a multicenter nationwide cross-sectional study, and requested the neonatologists responding to the questionnaire to report the total number of

infants actually hospitalized in their units the day the questionnaire was filled in, together with the main reported indications of EHF. Only infants who had NEC of grade II or III were considered for the study, grade I NEC can be confused with other causes of feeding intolerance.

The second series of questions focused on the feeding protocols and reasons for EHF use when prescribed for refeeding infants recovering from NEC of stage II or III. More specifically, questions targeted the duration of use, and the protocol used for weaning infants of EHF to regular cow's milk formula or human milk. It should be noted that the medical decision to use an EHF in France, particularly during hospitalization, was not made under any financial pressure, conflict of interest, or mitigation as every legal resident of France, including preterm infants, has, by law, a full universal coverage of healthcare.

The exhaustive list of neonatal units of metropolitan France and overseas territories was established by combining the lists of the national scientific societies involved in neonatal care and those of all the regional health care services. Each unit was individually contacted. Neonatal units having a high-acuity or intensive care beds were selected for the study. Only one questionnaire per unit accompanied by a cover letter and a reply envelope, was posted by mail to the head of the unit. He was asked to complete the survey questionnaire or to delegate the task to a colleague with more than 3 years of clinical experience in neonatal medicine, and more than 20% of time devoted to direct patient care.

In order to reduce the risk of selection bias, we aimed at surveying at least half of the nationwide neonatal units to have a picture of the nutritional care of the preterm infants post NEC as clear, and as accurate as possible. A reminder letter was subsequently posted one month later to the non-responders to achieve our goal. The identity of the neonatologists contacted and requested to complete the survey remained blinded for the analysis.

Statistical analysis

Statistical analyses were restricted to completed questionnaires with evaluable results. Data were analyzed using Minitab® 13.3 software (Minitab Inc., State College, PA, USA). General frequency responses to all survey items were determined and then used to test for associations among the categorical variables. When needed, data were split to cross tabs with respect to various grouping variables. Comparisons were made by Pearson χ^2 test of independence. A p-value ≤ 0.05 was considered statistically significant.

Results

Characteristics of the units

The goal of surveying at least half of the French neonatal units was reached since we received 174 responses from the 296 units contacted (58.8%). The characteristics of the responding units are reported in the Table I. Of the 174 units surveyed, 158 (91%) routinely used EHF (Table I).

Prevalence of use and reasons for feeding hospitalized neonates with EHF

Of the 1 969 infants hospitalized the day of the filling of the questionnaire, 238 (12.1%) received an extensively-hydrolyzed formula. The reasons for feeding hospitalized neonates with EHF were indicated in all cases as reported in Table II. Shortage of human milk is overall the main reason for prescribing EHF either for the initiation of feeding in preterm infants or for complementary feeding of breastfed infants. Among all the infants receiving EHF, 10.5% of the prescriptions were made because of a previous NEC.

Nutritional protocols when using EHF for feeding infants recovering from NEC

Of the 174 units surveyed, 93 (53.4%) routinely took care of infants post NEC. EHF were routinely used in 88 (95%) of them (Table I).

The main reasons for using EHF as the preferred milk for feeding infants post NEC were the absence of human breast milk (n= 65/93, 75%) and when surgical management of NEC was required (n= 15/93, 17%). The other reasons cited were NEC in term babies for whom EHF is

nutritionally adapted, other associated digestive problems, NEC severity at onset, and shortage of donor breast milk.

EHF, when given, were mainly prescribed for a period which varies from 15 days to 3 months. None of the units continued giving EHF after 6 months of age (Table III). The mode of weaning from EHF to regular cow's milk formula or donor breast milk is described in Table III. In absence of breast milk, 83% of the units switched to a cow's milk formula, while others shifted to using either donor breast milk or a partially hydrolyzed formula. Most of the surveyed units progressively weaned the EHF over a mean (±SD) period of 6.9 (±3.1) days. More than half of the units reported having the infants hospitalized for initiating the weaning process. However, only 21% of these units tested the infants for cow's milk allergy.

Discussion

To our knowledge this is the first study which attempts to determine the frequency of use of EHF in the neonatal units. We found that the prevalence of EHF use is high, approaching 12.1% of the enteral feeding prescriptions. The indications of EHF in neonates reported in literature include absence of human milk, poor feeding tolerance [6], severe gastro-esophageal reflux [7 8], family history or clinical signs of cow's milk allergy [9], or history of gastrointestinal surgery or intestinal resection [10]. Our study clearly shows that refeeding infants recovering from NEC is a frequent indication of EHF use in hospitalized neonates.

Most available literature focuses on the nutritional prevention, not the treatment of NEC. Furthermore, it is extremely vague regarding the timing of refeeding and the type of milk to be used after initial management or postoperatively for infants who have had NEC. Although it is well established that feeding should be suspended for a period of time that depends on the disease severity, there are no clear recommendations on when to restart feeding after the subsidence of the acute-stage NEC [5 11]. The choice of formula milk for refeeding infants post NEC depends on many of such factors as gestational age, the availability of human breast milk, risk of short gut syndrome and/or malabsorption, as well as risk of cow's milk

allergy. Our study clearly shows that the main drive for choosing an EHF for refeeding NEC infants is the absence of human breast milk, either maternal or donor.

EHF is not the feeding formula usually cited in the few textbooks defining the feeding choices post-NEC [5 11]. There are, however, several putative reasons for choosing an EHF.

Firstly, there is a debate on the direct contributory role of cow's milk protein sensitization in the pathogenesis of NEC [12]. Cow's milk allergy is well recognized as a significant cause of morbidity in formula-fed term and more recently, in preterm infants [13]. Several case reports have shown that cow's milk protein allergy may be closely related to NEC [14-15] and there is also evidence of *in vitro* sensitization to cow's milk protein in peripheral blood mononuclear cells of preterm infants with NEC [16 17]. This suggests that cow's milk intolerance should be evaluated when NEC occurs in case of absence of classical risk factors [18].

Secondly, premature infants recovering from mucosal inflammation and prolonged periods of bowel rest are potentially at increased risk of antigenic response to intact proteins [11]. It has been shown that allergy to cow's milk proteins in newborns who underwent gastrointestinal surgery is higher than expected in absence of family history of allergy [19]. In this context, EHF may be useful for refeeding infants post NEC. Nevertheless, no study to date has assessed the usefulness of such a strategy for possibly preventing cow's milk protein sensitization.

Finally, the use of EHF may also be considered for their nutritional value. Indeed they do not usually contain lactose and some of them do contain significant amount of medium-chain triglycerides. These characteristics may improve absorption during refeeding especially in surgical patients. Lactose is poorly tolerated in neonates with a small bowel disease or resection because of the decrease in available mucosal lactase. Medium-chain triglycerides also improve fat absorption, known to be also reduced in cases of loss of absorptive area, rapid transit, bile acid depletion, and/or bacterial overgrowth [10]. In contrast, the theoretical advantage of hydrolyzed over whole protein formulas of better absorption in case of a reduced absorptive area and decreased pancreatic enzyme output, remains uncertain since

it has been shown that dietary protein absorption capacity of the small intestine is normal for most neonates after intestinal surgery [20].

If there are possible nutritional benefits for using EHF for feeding infants after NEC, they should be weighed against possible disadvantages [21]. Indeed these formulas have an energy density close to that of term formulas, in addition to usually low mineral and polyunsaturated fatty acid contents as compared to the preterm formulas. Urinary nitrogen excretion is higher [22], calcium and phosphorus absorption and nitrogen retention are lower in preterm infants fed with hydrolyzed formula compared to those fed with whole protein formula [23, 24]. These drawbacks may alter the quality of growth or decrease the lean body mass accretion in preterm infants receiving hydrolyzed formula when compared to those receiving non-hydrolyzed formula whether the growth rate was similar or not [25, 26].

In absence of specific recommendations or studies guiding or helping to assess the risk of food allergy in infants post NEC, it is not surprising that our study shows a great heterogeneity in the weaning protocols of EHF. In contrast to the recommendations of El Hassani et al [19], and more importantly to the guidelines for food allergy prevention [27], our study shows that the duration of use is less than the recommended duration of 4 to 6 months. In addition to that, cow's milk proteins are frequently introduced without performing any appropriate diagnostic work-up.

It should be recognized that our study has several limitations. This study was performed in one country only and results may not be valid to other countries. It may be argued that this is a cross sectional study that was performed at a single point of time. However, it is well known that such study design is particularly suitable for assessing the prevalence of a disease or a treatment in a specific population [28]. Although we aimed and succeeded at assessing more than half of the French units, such study design is prone to selection bias [29]. We, therefore, cannot state that the non-responding units were those who used EHF less or more than the responding ones. Finally, this survey did not allow us to assess any

longitudinal follow-up or incidence data of NEC [28]. However, we were able to assess the intent-to-treat modalities of feeding infants post NEC.

In conclusion, this study shows that the use of EHF in the neonatal units is frequent. Refeeding infants post NEC is one of the reasons of such high prevalence. The main drive for using EHF is the absence of human breast milk, either maternal or donor. NEC patients represent a group of infants who may benefit from these EHF. However, benefit/risk ratio of their use, as well as the modality of their weaning need to be further evaluated by more studies.

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Contributors' Statement

Alexandre Lapillonne served as the coordinator for the survey. He had access to all of the primary data and performed the statistical analyses. He drafted the manuscript. He also participated in the review, revision and approval of the final manuscript.

Maroun Matar drafted the manuscript. He had access to all of the primary data and participated to the statistical analyses. He also participated in the review, revision and approval of the final manuscript.

Ariane Adleff-Genot designed and performed the survey. She had access to all of the primary data and participated to the statistical analyses. She participated in the review, revision and approval of the final manuscript.

Marwa Chbihi provided significant scientific input and critically revised the manuscript. She participated in the review, revision and approval of the final manuscript

Elsa Kermorvant-Duchemin participated in the study and critically revised the manuscript. She participated in the review, revision and approval of the final manuscript

Florence Campeotto critically revised the manuscript and provided significant scientific input. She participated in the review, revision and approval of the final manuscript.

Competing interests

The authors have no conflicts of interest relevant to this article to disclose and have no financial disclosure to declare.

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Data sharing statement

Data are not currently shared but are available on request by the journal.

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Table I.

Characteristics of the responding neonatal units

	Total
Units surveyed	
Number of units (n)	174
Number of units using EHF routinely (n)	158
Number of units routinely caring for infants post NEC (n)	93
Number of admissions	
Number of admissions per year (n) *	61 578
GA <37 weeks (n) *	28 029
GA <28 weeks (n) *	2 394

*based on the responses of 150 units; GA = gestational age

Table II.

Prevalence of use and reasons for feeding hospitalized neonates with extensively-hydrolyzed formula (EHF).

	n (%)
Infants hospitalized the day of the questionnaire filling (n)	1 969
Infants receiving an EHF, n (% of hospitalized infants)	238 (12.1%)
Reasons for feeding neonates with EHF	
Initiation of feeds	
after NEC stage II or III	25 (10.5%)
after perinatal asphyxia	8 (3.3%)
after any kind of surgery	4 (1.7%)
Shortage of human milk	
feeding initiation of preterm infants in absence of human milk	63 (26.5%)
complementary feeding of breastfed neonates	84 (35.3%)
Allergy prevention in high risk neonates	2 (0.8%)
Gastrointestinal symptoms (compatible or not with cow's milk protein allergy)	31 (13.0%)
Others (research protocol, hypoglycemia, cholestasis, metabolic disease, etc., no reasons indicated)	21 (8.8%)

EHF = Extensively-hydrolyzed formula of cow's milk proteins;

Table III.

Nutritional protocols of neonatal units using extensively-hydrolyzed formula (EHF) in preterm infants post necrotizing enterocolitis.

Duration of EHF use	Percent of units
< 15 days	8%
15-30 days	30%
1-3 months	50%
4-6 months	12%
≥ 7 months	0%
Weaning EHF in hospital	
Yes	52%
No	48%
Weaning EHF progressively over several days	
Yes	96%
No	4%
Weaning EHF after testing for cow's milk allergy	
Yes	21%
No	79%
Type of milk used for weaning EHF in absence of mother's breast milk	
Donor breast milk	13%
Regular cow's milk formula	83%
Partially hydrolyzed formula or other	7%

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	NA
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	6
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA

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Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(*b*) Report category boundaries when continuous variables were categorized	8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.