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Lactobacillus paracasei subsp. paracasei F19 in Bell's Stage 2 of necrotizing enterocolitis

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***Aim*. The aim of this trial is to evaluate the role *Lactobacillus paracasei* in Bell's stage 2 in order to prevent the clinical progression to stage 3.**

***Methods*. A prospective study was approved and started in December 2008. Patients were infants with birth weight 600 to 1500 g. One group received probiotic supplementation (*L. paracasei susp.paracasei* F-19) and the control group received only standard medical treatment. The primary outcome was the progression to stage 3 as defined by Bell's modified criteria. Inclusion and exclusion criteria were created and discussed with parents before treatment.**

***Results*. Thirty-two patients (stage 2 NEC) were considered eligible for the study. Group A: 18 patients and Group B: 14 patients. Three patients in group A and six patients in group B had a clinical history of Bell's stage 3 NEC (P<0.05); oral supplementation of *L. paracasei* reduced the clinical progression of NEC. It was considered that an improvement in intestinal motility might have contributed to this result.**

***Conclusion*. The use of *Lactobacillus paracasei subsp. paracasei* F-19 is safe; the low progression rate to stage 3 NEC suggests that the use of this probiotic in stage 2 NEC could be a valuable therapeutic option.**

KEY WORDS: Enterocolitis, necrotizing - Bacterial proteins - Infant, very low birth weight.

Necrotizing enterocolitis (NEC) remains one of the most common neonatal gastrointestinal emergencies.[1] Many factors have been suggested to be predictive of NEC but the pathogenesis is still controversial.[2-4]

The preferred treatment remains to be standardized: in the initial stage the treatment is exclusively medical and is based on suspension of nutrition in favor of total parenteral nutrition, with administration of large-spectrum antibiotics. In the following stages, and particularly if there is evidence of intestinal perforation, surgical treatment is advisable. In current literature discussion is still ongoing as to which surgical approach is the most effective to maximize patient survival: laparotomy or percutaneous drainage in case of intestinal perforation. However, currently available data do not give definite answers regarding which is to be preferred.[5]

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Although many different investigational approaches have been proposed in the study of NEC prevention,[6] (use of probiotics, early *vs*. late nutrition, etc.), the aim of the present study was to investigate the role of probiotics in the management of stage 2 NEC.

In the treatment of NEC Bell's Stage 2 is to be considered the key stage in the necrotic development of this condition. For this reason when NEC reaches this stage, clinicians should use all possible resources to prevent intestinal perforation.

The primary aim of this trial was to identify the role of *Lactobacillus paracasei subsp. paracasei* F19 in Bell's Stage 2 and to compare the efficacy and safety of *Lactobacillus* administration *versus* no treatment in the prevention of severe NEC (Stage 3).

This approach may result useful in the prevention of intestinal perforation, since it should reduce bacterial translocation.

Scientific rationale

NEC is characterized by the lack of intestinal mucosal integrity leading to acute clinical evidence of feeding intolerance, blood in the stools, and *pneumatosis intestinalis*. Moreover, it is accompanied by cardiorespiratory disorders and severe hemodynamic instability.[7]

Preterm infants are particularly vulnerable to the development of NEC due to inappropriate intestinal colonization by pathogenic bacteria with a consequent immature immune response which favors a pro-inflammatory state. Identification of the benefits of probiotics, *i.e*., maintaining mucosal barrier integrity, triggering general intestinal immune defenses, regulating appropriate bacterial colonization, modulating intestinal inflammation, may be the first step to prevent the pathogenesis of NEC. The benefits of probiotics in the prevention of NEC is supported by findings in animal experiments.[8, 9]

Lactobacillus paracasei subsp. paracasei F19 plays an important role in many diseases such as irritable bowel syndrome (IBS) [10] and others,[11-14] as it has genetic stability and it is able to suppress human t cell proliferation.[15-19]

Materials and methods

The present prospective study was approved by the Ethics Committee of the University of Verona in December 2008 (no. 1786) for a duration of 3 years (December 2008-December 2011).

This study was programmed in two phases: necrotizing enterocolitis was diagnosed or suspected according to Bell's staging criteria [20] (Table I). Phase 1: *Lactobacillus paracasei subsp. paracasei F19* for all infants. To identify any changes regarding any adverse effects (*i.e*., sepsis) (6 months). Phase 2: *Lactobacillus paracasei subsp. paracasei F19* only for Stage 2 (3 years). After selection of patients, *Lactobacillus paracasei subsp. paracasei F19* was used for 21 days.

All patients received standard therapy for suspected NEC. Two main groups were created. Group A: patients who received standard therapy plus *Lactobacillus paracasei subsp. paracasei F19* in Stage 2; Group B: standard management of Stage 2 NEC (standard therapy and "wait and see" approach).

Inclusion criteria for the trial were as follows: weight at birth <1500 g; gestational age <32 weeks; radiological evidence of Stage 2 NEC (*pneumatosis intestinalis* and/

TABLE I.—*Bell's clasification (1973)*

STAGE 1 Suspect
– Perinatal distress
– Fever,
– Gastrointestinal signs: stop feeding, gastric stasis, biliar vomiting, blood in stools
– Abdominal X-ray® abdominal distension
STAGE 2 Definite
– Perinatal distress, acidosis, thrombocytopenia
– Abdominal X-ray® abdominal distension, fixed loop, pneumatosis intestinalis, portal vein gas
STAGE 3 Advanced
– Perinatal distress
– Shock, intestinal bleeding
– Abdominal X-ray ® abdominal distension, pneumoperitoneum

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or portal vein gas). Since it is not always possible to distinguish these two categories with plain abdominal X-rays, all radiologists were invited to review and compare previous X-rays of the abdomen in NEC patients retrospectively. Prospectively, all X-rays were observed by two pediatric radiologists.

Exclusion criteria for the trial were: bilateral Grade IV intraventricular hemorrhage; multiple episodes of NEC; treatment in Neonatal Intensive Care Units with no involvement of pediatric surgeons; other malformations; previous laparotomy; parents' decision, at any time, by submission of relevant Form (Exclusion from the study).

The primary endpoint of the study was the efficacy of "early" treatment (absence of subsequent intestinal perforation). Secondary endpoints of the trial were: duration of hospitalization (hospital stay); mortality and morbidity (short-term); duration of parenteral nutrition (days); time to full enteral feeding (days); long-term intestinal complications.

Study design

When a consultant (neonatologist or pediatric surgeon) of the study decides that a neonate under his/her care is potentially eligible for the treatment, the procedure is as follows (Figure 1): 1) informed consent

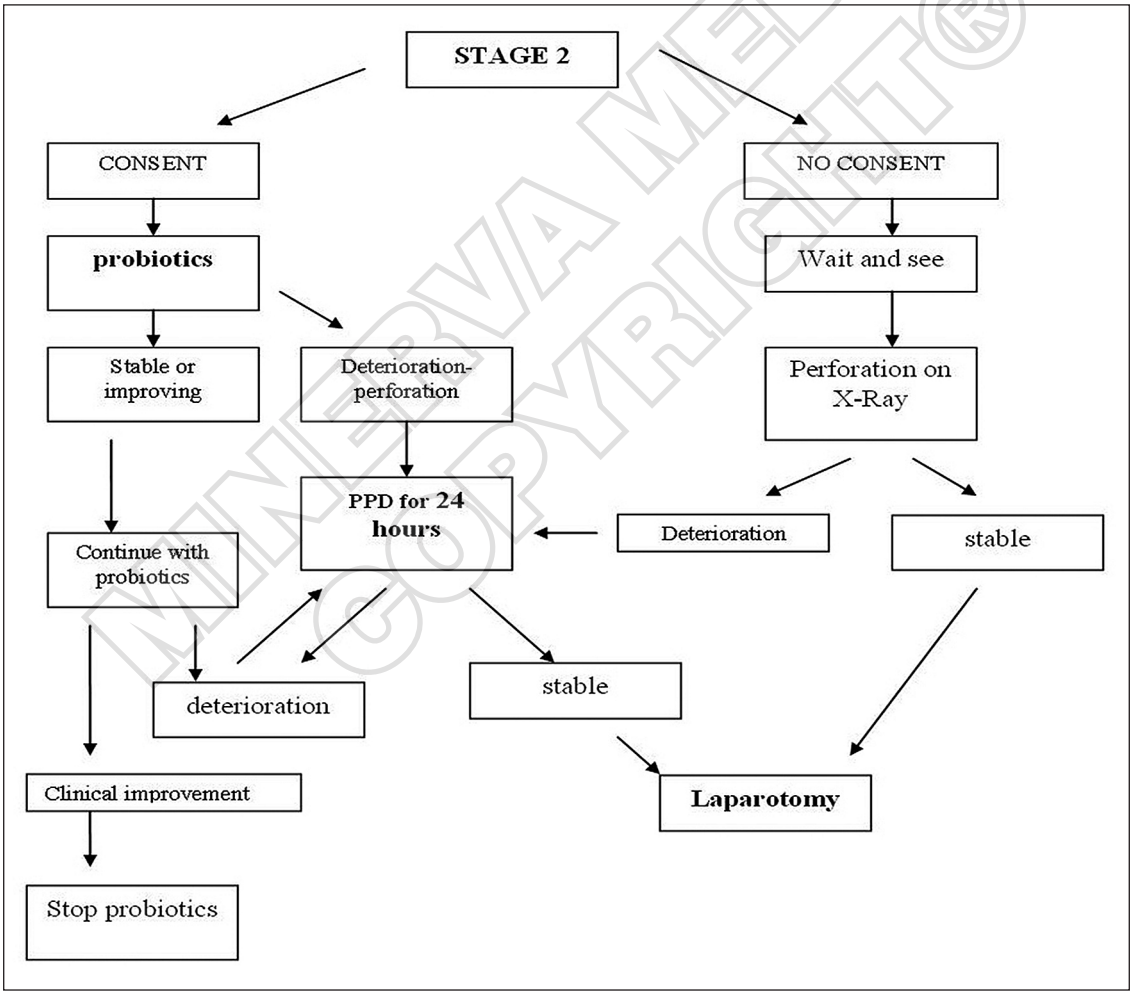

Figure 1.—Bell's modified classification. PPD: percutaneous peritoneal drainage.

from parent is obtained; 2) the clinician in charge of the patient will fax the form to the study center; 3) random allocation of patient into one of the two study groups (*Lactobacillus paracasei subsp. paracasei* F19 group or "wait-and-see"). The parents are informed of treatment allocation; 4) the principal investigator (N.Z.) is responsible for coordinating data monitoring; 5) the study coordinator will confirm the diagnosis of stage 2 by reviewing the patient's clinical data.

Randomization

All patient details for randomization were collected locally and forwarded by fax or mobile phone to the randomization service based in the Pediatric Surgical Unit. A randomization code was obtained from the randomization service within 20 minutes.

Consent for randomization was obtained from the parents at the time of diagnosis of Stage 2 NEC. The parents were given adequate explanation in order to make an informed decision as to whether or not they wanted to enroll their child in the trial. In each center an experienced surgeon or neonatologist was responsible for obtaining consent.

To randomly allocate patients into study groups, a casual list generated by a computer (controlled by a standard computer entry order set) was obtained.

Clinical monitoring of patients

Clinical charts were used to monitor the clinical status of patients upon enrolment in the study, and then at days 1, 2, 7 and 21. Neutrophils, coagulation indices and C-reactive protein were also measured at the same time points. Blood culture was performed once every week and when clinically indicated.

Clinical data and blood tests were checked daily by the study Investigators. Abdominal X-rays were performed daily and when clinically indicated: a Pediatrician and a Surgeon performed daily examinations.

Procedure

The experimental product (no profit) was supplied free of charge by SIFFRA pharmaceuticals. The product was administered by the nurses in the Departments taking part in the trial after standard preparation, *via* either nasogastric tube (with syringe) or oral administration (syringe).

Dosage (standard dose for ach patients)

Pooling data from three randomized controlled trials,[21-23] the following dosage was selected and decided: 6 x 10^9 CFU/day for 21 days L-F19 (*Lactobacillus paracasei subsp. paracasei F19*); each bottle contained: 10 mL, 6 mld *Lactobacillus paracasei subsp. paracasei F19* + 600 mg glucoligosaccharides; final dosage: half a bottle (5 mL) with *Lactobacillus* – with a 5-mL syringe.

The product was administered orally in 5 hours, 1 mL/hour; if a nasogastric tube was used, the nurse was advised to put first 2 mL into the tube and then 1 mL.

The medical charts of all infants with NEC or suspected NEC were reviewed and the perinatal data recorded, including maternal age, birth weight, 1- and 5-minute Apgar scores, gestational age, use of prenatal steroids, need of ventilatory support after birth, and post-natal clinical support.

The risk and comorbid factors of the two main groups (developing advanced NEC and not developing advanced NEC) were also analyzed.

Postnatal clinical support

The following elements were recorded: need of ventilatory support, need for open nasogastric tube (caused by gastric residuals), time of feeding, type of feeding, meconium evacuation. Standard medical treatment, especially for drugs and antibiotics, was administered following the standard neonatal protocol for NEC (triple-coverage antibiotics).

At our Institution feeding is usually started within 12 h after birth with 6 mL/kg every 3 h and then, if well tolerated, this

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is increased daily up to a maximum of 16 mL/kg. Feeding intolerance was defined as a gastric residual exceeding a single feeding dose between two consecutive doses detected after aspiration (nasogastric tube). The type of residual was also considered.

Data analysis

Presuming that principal end point of the trial is the efficacy of treatment, *i.e.*, absence of subsequent intestinal perforation, we believe that in order to obtain this result we have to reduce the percentage about 10-15% and it is necessary to enrol 30 patients (80% power, alpha 0.05)

Data were processed using the S.P.S.S. statistical program. The following statistical tests were used: Student's *t*-test, χ^2 tests, Fisher exact test when indicated (for parametric and non-parametric data): % intestinal perforation, % mortality, hospitalization (days), (chi-square) - blood tests (difference between groups), duration of parenteral nutrition, time to full enteral feeding (Student's *t*-test).

Results

During the study period 194 infants were admitted to the study Neonatal Intensive Care Units. 32 patients (stage 2 NEC) were considered eligible for the study. Group A: 18 patients and Group B: 14 patients. No patient was excluded due to parents' decision.

Maternal characteristics

Data regarding patients' mothers in both groups were similar in terms of age, body mass index, and number of previous pregnancies. There were no statistically significant differences (P>0.05).

Study population

The mean birth weight in Group A was inferior to that in Group B (mean 1100 g *versus* 1280 g, P>0.05), ranging between 640 and 1420 g for Group A and between 850 and 1480 g for Group B. The mean ± SD Apgar score in Group A was 4±2 and 6±2 at 1 and 5 minutes, and 5±2 and 7±1 at 1 and 5 minutes for Group B (P>0.05).

All infants in Group A were delivered before gestational week 31 (one at week 24, 6 at week 26±6 days; and the others at week 29 ± 6 days); 90% of these infants required intubation after birth due to respiratory distress.

All infants in Group B were delivered before gestational week 32 (8 at week 25, 2 at week 28 and the others at week 32); no difference was found between mean gestational week at delivery in the two study groups (P>0.05). Overall, 85% of the infants in Group B required intubation following respiratory distress (P>0.05). No significant differences were observed between the two groups in terms of type of feeding and use of nasogastric tube (P>0.05). The type of gastric residue was closely correlated with the development of advanced NEC (Figure 1).

Group B infants evacuated meconium later than infants in Group A (mean 5 *vs.* 3 days, P<0.05), and started feeding earlier (12 days *vs.* 18 days) (P<0.05).

The use of *Lactobacillus paracasei subsp. paracasei F19* was associated with lower progression to stage 3 (3 patient *vs.* 6 patients) (P<0.05), lower mortality rate (2 patients *vs.* 5 patients) and shorter hospital stay (38 *vs.* 54 days) (P<0.05). Mortality was also associated with lower gestational age and lower Apgar score at 1 minute (mean gestational age was 26 weeks ± 4 days and mean Apgar score was 3±1). Infants in Group A received oral feeding earlier than infants in Group B (11 *vs.* 19 days) (P<0.05).

No collateral effects were observed during the study period; none of our patients presented sepsis due to *Lactobacillus paracasei subsp. paracasei F19*. No patient required preventive exclusion from the trial. In Group A no patient presented treatment-related intestinal complications (*i.e.*, diarrhea).

Three patients developed stage 3 NEC with intestinal perforation at day 6±1 after

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beginning of treatment; 6 patients in Group B developed Stage 3 NEC 3±2 days after enrolment (P>0.05).

Discussion

The incidence of NEC in premature infants with birth weight <1500 g has increased in recent years mainly in relationship to an increased survival rate related to better post-natal support treatment and more efficient Neonatal Intensive Care Units (NICU). It has recently been reported that more than 50% of patients with surgically treated NEC have a birth weight <1500 g. These patients certainly show a higher incidence of co-morbid factors which may affect their clinical course.[24]

Recent studies have shown that surgical mortality in premature infants with NEC varies inversely to weight and gestational age; however, the authors maintain that birth weight is a risk factor since it is strictly related to lower multi-organ function with a consequent reduced tolerance for anesthesia and surgery.[25, 26]

Subspecies of *Lactobacillus* were the commonly used probiotic organisms in trials reported in the literature. The colonization rates of *Lactobacillus paracasei subsp. paracasei F19* are reported to range from 60% to 87% in preterm neonates. The intestinal microbial flora of preterm neonates differs from that of normal-term neonates. Neonates with a very low birth weight usually become infected by microbial flora mainly from the N.I.C.U. rather than from their mother.[27-32]

Stools of breastfed neonates have a predominance of *Lactobacillus paracasei subsp. paracasei F19* species which compete with species of Bacteroides, Clostridia, and Enterobacteria.[16-22]

Lactobacillus species are ubiquitous anaerobic or facultative anaerobic strains. There are no studies in the literature considering the use of *Lactobacillus paracasei subsp. paracasei F19* or probiotics in stage 2 NEC to avoid clinical progression to stage 3.

Even if the preventive role of probiotics in the prevention of NEC is still under discussion, the most important clinical key points to consider are which patients will develop a stage 3 NEC and what clinicians can do to prevent it.

The results of our study do not demonstrate new clinical aspects of NEC or new predictive data.

They demonstrate the efficacy of probiotics if used in Stage 2, the reduction in the perforation rate, the reduction in the overall mortality rate and hospital stay.

Some criticism could be raised regarding this discussion: 1) this study involved a small number of patients; 2) oral administration of 5 mL (1 mL per h) may not be correct (probably due to possible loss of dose); 3) long-term administration (21 days) could be reduced to 14 days; 4) dosage may not be useful for all infants, but it should probably be standardized according to birth weight.

Conclusions

The use of *Lactobacillus paracasei subsp. paracasei F19* is safe and effective; the low incidence rate of Stage 3, shorter hospital stay, earlier oral feeding and lower morbidity seem to justify the use of probiotics also in Bell's stage 2. Gestational age <28 week and low 1-minute Apgar score are risk factors (in both groups) related to the development of advanced NEC.

Study results support the use of the probiotic *Lactobacillus paracasei subsp. paracasei F19* for Very Low Birth Weight infants in the prevention of Stage 3 NEC. However, more detailed results are required in order to define the most suitable dose and timing of administration, as well as to detect potential adverse effects of the treatment. Long-term follow-up should also focus on other important outcomes such as cost-benefit ratio and therapeutic options, as well as feasibility and duration of therapy. In light of the results obtained in this study further analysis should be carried out in another multicenter trial in order to guarantee an adequate number of patients and to detect possible adverse events.

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Riassunto

Lactobacillus paracasei subsp. paracasei F19 *nello stadio 2 di Bell di enterocolite necrotizzante*

Obiettivo. Lo scopo di questo studio è di valutare il ruolo del *Lactobacillo paracasei subspecie paracasei F-19* nello stadio 2 di NEC per evitare la progressione verso lo stadio 3.

Metodi. Lo studio prospettico è stato approvato dal comitato etico in dicembre 2008. I pazienti erano neonati dal peso compreso tra 600 e 1500 grammi. Un gruppo è stato trattato con terapia usuale e probiotico mentre un secondo gruppo solo con terapia usuale per NEC. L'obiettivo primario era di valutare la progressione verso lo stadio 3. Criteri di inclusione ed esclusione sono stati creati prima dell'arruolamento dei pazienti.

Risultati. Trentadue pazienti sono stati considerati idonei allo studio: gruppo A 18 pazienti e gruppo B 14 pazienti. 3 pazienti del gruppo A e 6 del gruppo B hanno sviluppato lo stadio 3 (P<0,05); l'assunzione di probiotico ha aumentato la motilità intestinale favorendo quindi una ridotta traslocazione batterica.

Conclusioni. L'utilizzo del *Lactobacillo paracasei F-19* è efficace e sicuro; la ridotta progressione verso lo stadio 3 suggerisce che questa terapia potrebbe essere utilizzata in tutti i pazienti.

PAROLE CHIAVE: Enterocolite necrotizante- Proteine batteriche - Neonati con basso peso alla nascita.

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