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Review

From the "old NEC" to the "new NECs"

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The role of the clinical pathological dialogue in problem solving
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Abstract

Necrotizing enterocolitis (NEC) is an acute inflammatory disease of the neonatal intestine that strikes in 1 of 1,000 live births. Its etiology is unknown. This review describes in detail the new NECs especially those which affect preterm infants: contagion or lymphocytosis associated, transfusion associated and cow's milk allergy associated. A wide repertory of images are presented, together with algorithms for differential diagnosis.

Keywords

Necrotizing enterocolitis, intestinal mucosa barrier, NEC sub-classes, prevention, gastrointestinal neonatal problems.

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Definition and incidence

Necrotizing enterocolitis (NEC) is an acute inflammatory disease of the neonatal intestine. Its etiology is uncertain and it strikes in 1 of 1,000 live births. It is characterized by necrosis of the intestinal mucosa that extends to the deepest layers, mostly involving the proximal ileum and the colon. The most affected are premature infants with an incidence of 7% to 10% in very low birth weight (VLBW) infants. Incidence is inversely related to weight and gestational age (GA). Age at onset varies inversely with GA: those born at term develop the disease within a few days of birth while those of < 30 wks GA develop it after some weeks. The disease has a mortality from 20% to 50% depending on case histories [1-3].

Classification: from the "Old NEC" to the "New NECs"

At the end of the 1970s, when NEC was described prevalently in term- or late preterm infants, Bell's classification [4] provided useful grounds for grouping under a single denomination different forms that had in common a single characteristic: progression towards intestinal perforation. But with progress in neonatology and the ever-increasing onset of the disease in VLBW infants, given their constant increase in survival, clinicians realized that the use of Bell's classification, which is actually a stadiation of the disease, led to the definition of NEC ("Old NEC" in **Fig. 1**) a series of acquired neonatal intestinal diseases (ANIDs) with a different etiology that did not necessarily lead to intestinal perforation. For example, Bell's Stage I included non-specific abdominal symptoms (such as the broad range of symptoms of Feed Intolerance of Prematurity [FIP]) and systemic signs of infection, while Bell's stage IIIb could include Spontaneous Intestinal Perforation (SIP), a condition quite different from the etiological, clinical and pathological standpoints [5].

This confusion was caused by the lack of a distinction between NEC in the premature and NEC in term infants and, within these two large categories, by a line of identification of possible causes and effects, also in consideration of recent studies that show a connection with the ischemic, infective and allergic disorders, with nutrition and drugs.

In recent years a new approach [6] (NEC reductionism) has allowed to overcome the issue, at least in part ("New NEC" in **Fig. 2**), by recognizing that NEC is the point of arrival of different affections

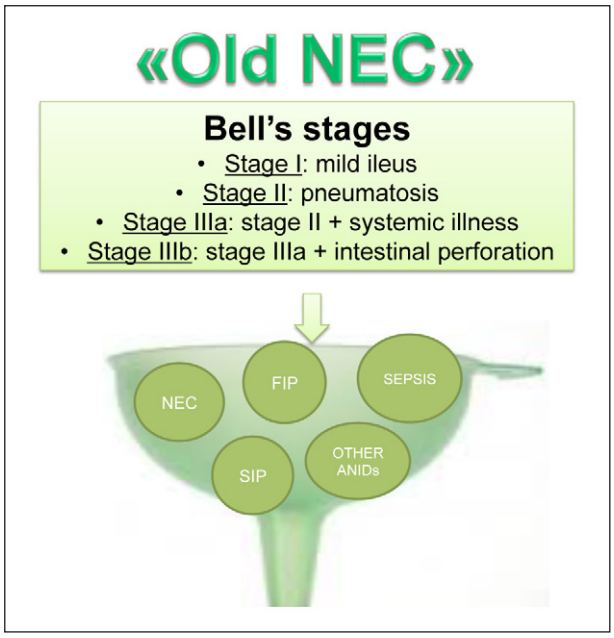

Figure 1. Bell's classification, extensively used to define NEC ("Old NEC"), may be compared with a funnel where very different neonatal conditions caracterized by mild or severe gastrointestinal symptoms are collected. NEC: necrotizing enterocolitis; FIP (Feed Intolerance of Prematurity); SIP (Spontaneus Intestinal Perforation); ANIDs (Acquired Neonatal Intestinal Diseases).

with different etiologies. This new interpretation has several advantages:

- it introduces a separation between the NEC of term and late preterm neonates and that of very preterms;
- within the NEC of preterms it provides a division into four subclasses with different etiologies according to risk factors, clinical and laboratory data:
    1. classic NEC;
    2. NEC associated with epidemics of viral gastroenteritis and/or lymphocytosis;
    3. NEC associated with cow's milk proteins intolerance;
    4. NEC associated with red blood cell (RBC) transfusions;
- it allows to establish incidence and epidemiology of different subsets in the single NICU and to implement tailored preventive and therapeutic measures.

Pathophysiology

The events leading up to NEC are multifactorial and complex, including a history of a hostile intrauterine environment, a difficult perinatal

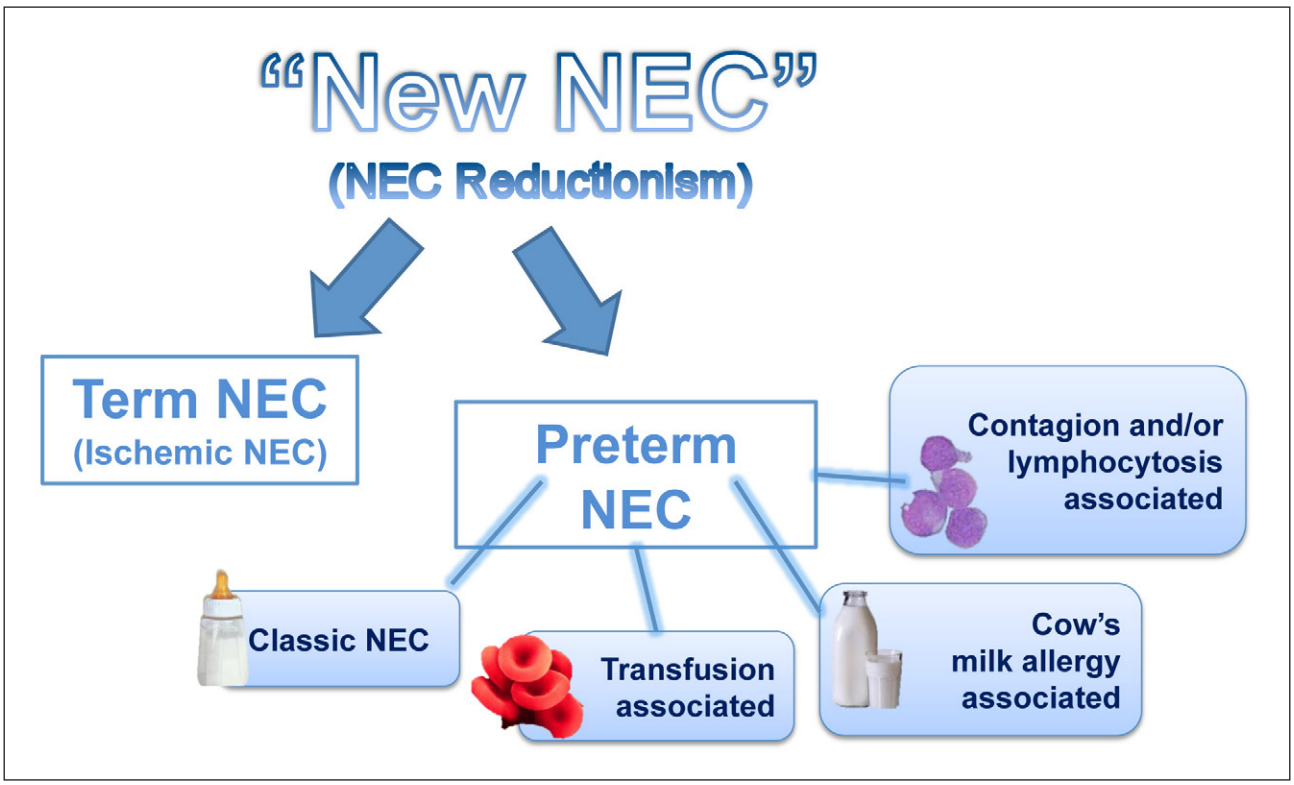

Figure 2. Necrotizing enterocolitis (NEC) reductionism [6] ("New NEC" in the figure) introduces a separation between Term-NEC and Preterm-NEC and provides a division into four subclasses with different etiologies within the latter.

transition and a complicated neonatal period. Three conditions are required for the onset of necrosis of the mucosa: a recently colonized intestine, as is that of the neonate, the presence of food in it, a triggering event that damages the mucosa barrier [6].

In the "New NEC" the first distinction between NEC in the term neonate and NEC in the preterm neonate allows us to divide them into two large groups on a pathophysiological basis: indeed, only in term newborns NEC (ischemic NEC) does the ischemia appear to be the initial event while in preterm newborns it is almost always the result of the effect of different etiological factors. Some of these act earlier (prematurity, delayed feeding, altered intestinal colonization by antibiotics, mother's milk lack, too-rapid progression in enteral feeding), while others have a later influence (viral epidemics, allergy to cow's milk proteins, transfusions) [7].

Whatever the initial trigger, ischemic or of other origin, the pathological process begins with the breaking down of the intestinal protective barrier, the invasion of the mucosa by bacteria and the triggering of an overreaction of the intestinal cells with consequent damage to the mucosa and final necrosis. Ischemia is an integral part of the process since it is caused by the endothelial dysfunction inherent in the phlogosis and being itself the cause of further damage to the mucosa.

Some elements of vulnerability of the mucosa barrier may favor the bacterial invasion, especially in preterms.

Fig. 3 schematically shows the process that seems to be more involved in the evolution of the damage and subsequent necrosis: different underlying factors are discussed in the following paragraphs.

Microbiota

The flora that normally populates the intestine (microbiota) plays an important role in maintaining the intestinal barrier: its microorganisms are recognized as commensals and perform their precious duties in the development of the mucosa and the intestinal immune system. Alteration of the microbiota (through exposure to nosocomial bacteria, antibiotics, lack of mother's milk) or its poverty due to factors that delay colonization (i.e. delay in the beginning of enteral feeding) may thus play an important role in the genesis of NEC, selecting species that may direct the immune response towards inflammation [8].

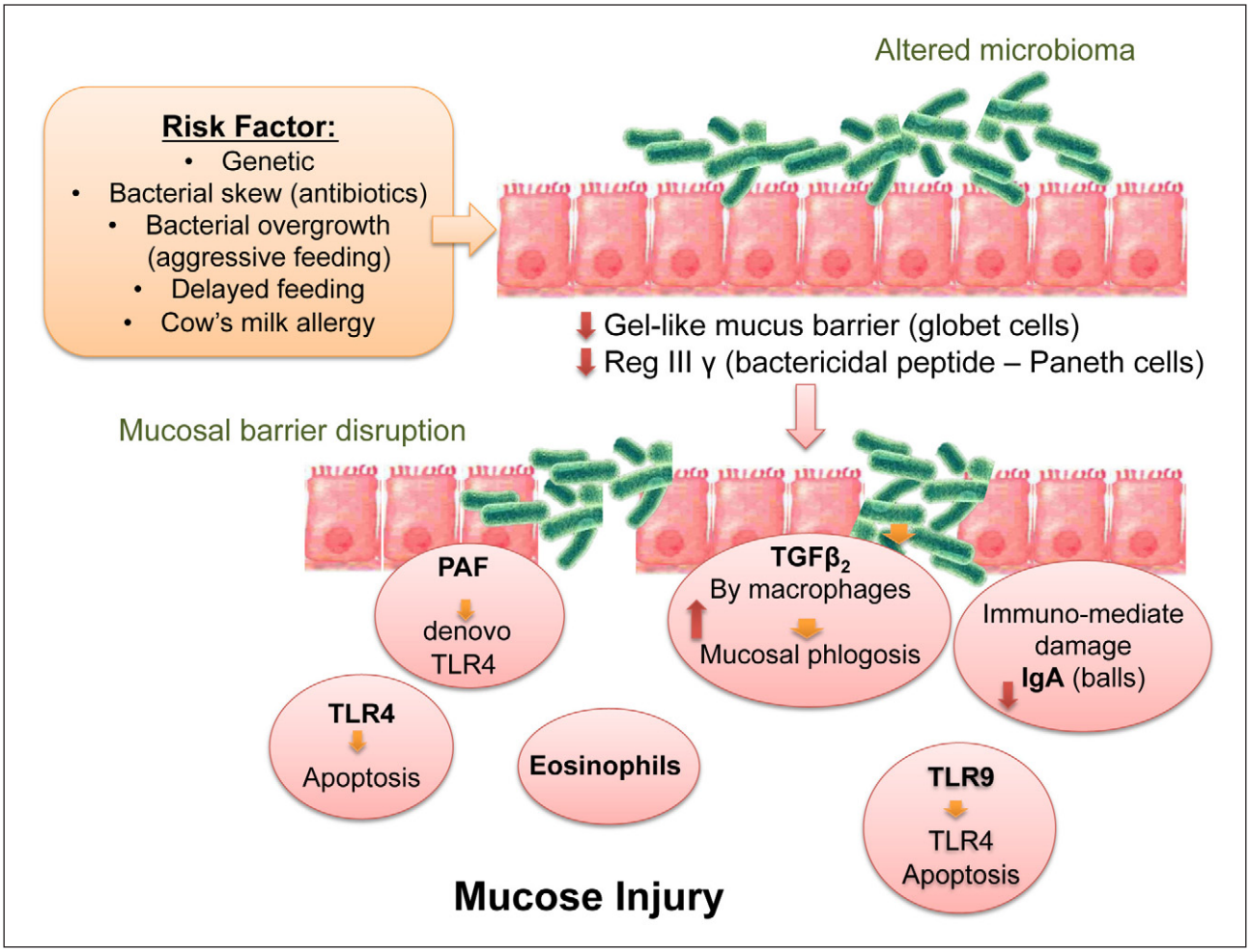

Figure 3. Regardless of the risk factor involved, the pathological process begins with the breaking down of the intestinal protective barrier, the invasion of the mucosa by bacteria and the triggering of an overreaction of the intestinal cells with consequent damage to the mucosa and final necrosis. The process is carried out by different mediators and worsened by altered microbioma and by fragile points in the mucosa barrier (gel-like mucus barrier lack and low production of bactericidal peptides by Paneth cells). PAF: Platelet Activation Factor; TLR:Toll-Like Receptors; IGFβ2:Transforming Growth Factor β2; TREG: T regulatory cells.

Fragile points in the mucosa barrier

The weakness of the tight junctions, the scarcity of the gel-like mucus layer that acts as an obstacle to the entrance of bacteria towards the surface and the reduced secretion of bactericidal substances, in particular the bactericidal C-peptide lectin RegIIIγ produced by the Paneth cells, represent further elements of fragility of the intestinal mucosa [9].

The TLR and PAF pathways

In the triggering of inflammatory mechanisms leading to necrosis of the intestinal mucosa, great importance is attributed to activation by the intestinal epithelial cells of specific receptors for the recognition of the bactericidal ligands (PPRs – pattern recognition receptors) and in particular of the TLRs (Toll-like receptors): the response, which initially is cytoprotective, may be destructive [10].

The TLR4s induce apoptosis to trap the pathogen contained within the agonizing enterocyte; in particular, they note the presence of the bacterial lipopolysaccaride (LPS) and its expression (which increases with gestational age and is drastically reduced at term), appears to have an important responsibility in determining the severity of NEC. The TLR4s appear to be up-regulated in NEC to the detriment of TLR9s, which have an antagonistic effect on apoptosis and are activated by commensals such as lactobacilli and bifidobacteria, the presence of which appears to reduce the risk of NEC by increasing the expression of TLR9 [10, 11].

Moreover, a powerful lipid mediator, PAF (platelet activation factor), is produced in large amounts by the epithelial cells in the NEC-affected

intestine: it stimulates the new production of TLR4 and in turn is stimulated by the LPS, the receptor of TLR4, which facilitates the release of the neutrophils. There thus appears to be a correlation between TLR4 and PAF, which may represent the two main pathways in NEC pathogenesis [12].

The timing of NEC may actually be determined by the TLR4s since their expression begins to be quite present (29-32 weeks) just when NEC arises a few weeks after birth [6].

In synthesis, the incapacity of the intestine to down-regulate the TLR4s hinders it from becoming tolerant of the luminal bacteria and the exaggerated TLR4 signal of the bacterial colonization results in a damage to the mucosa by apoptosis and a reduced capacity to repair itself if damaged.

The macrophages

Even the macrophages presence, starting from the 11th or 12th week of gestation, appears to participate in the onset of this intolerance: in the mature intestine they are "tolerant" to the bacteria of the nearby intestinal lumen and consequently do not have an inflammatory response to them. This tolerance, attributed to the down-regulation of TGF-β2 (transforming growth factor β2), is seemingly lacking in preterms and this facilitates the onset of the inflammatory process [13].

The eosinophils

Finally, in some forms of NEC, such as that associated with allergy to cow's milk proteins and perhaps also that associated with transfusions, the eosinophils apparently participate in the destructive process of the intestinal mucosa through recognition of a specific bacterial metabolite: fMLP (N-formyl-methionyl-leucyl-phenylalamine) [14].

The advent of adaptive immunity

With the increase in gestational age and the beginning of proper enteral feeding, the innate immunity, which includes all the processes described above, gradually gives way to the adaptive immunity mediated by the B and T cells: so the production of superficial IgA increases, the expression of TLR4 decreases, that of TGF-β2 increases with a later reduction of activation of the macrophages, the thickness of the gel-like mucus layer increases and, in absence of exaggerated bacterial growth, the risk of NEC is reduced [10, 11, 13].

Clinic

The symptomatology is similar in the different forms: it may begin with aspecific signs that progress insidiously for several days or have a fulminant onset leading in a short time to multiorgan system dysfunction and shock.

The most frequent symptoms when the terminal ileum (the typical seat in the VLBW infants) is involved are abdominal distension, gastric residuals, bilious vomiting and absence of stools. If the colon is involved the first symptom may be only blood in the stool.

As the disease progresses abdominal distension increases, the abdomen becomes firm and tender and may assume an erythematose or bluish color when intestinal perforation takes place (**Fig. 4**). In such a case, in male neonates even the scrotum may turn bluish due to the passage of peritoneal liquid from the perforated loop. Signs of overall distress, such as lethargy, apnea, bradycardia, temperature instability, impairment of peripheral perfusion and the need for artificial ventilation are quite frequent.

Laboratory tests may show anemia, neutropenia, left shift of neutrophils, metabolic acidosis and hyponatremia. In 40% to 60% of cases there may be positivity for blood cultures, commonly gram negatives.

NEC may recur in 5% of cases, especially in neonates with congenital heart disease and in the forms with viral pathogenesis or caused by allergy to cow's milk proteins [2, 15].

Diagnostics

The radiographic signs are those most useful in diagnosing the disease.

Pneumatosis (**Fig. 5**) and the presence of gas in the portal vein are pathognomonic: gas, produced by the bacteria, may generate a linear image if it is in the intestinal wall, circular if subserous and in the shape of small bubbles if submucosal. Pneumatosis is found more frequently in the lower quadrants but may involve the entire intestine.

Other non-specific radiographic aspects are the increase in thickness of the loops walls, the dilation of some of them and the presence of "fixed loops" (bowel without peristalsis) (**Fig. 6**). The series of loops struck by the pathological process may form a mass distinct from the rest of the intestine due to the considerable thickening of the wall and loss of normal shape. The persisting radiographic finding of a fixed loop is an indication of a necrotic bowel loop. The localization of

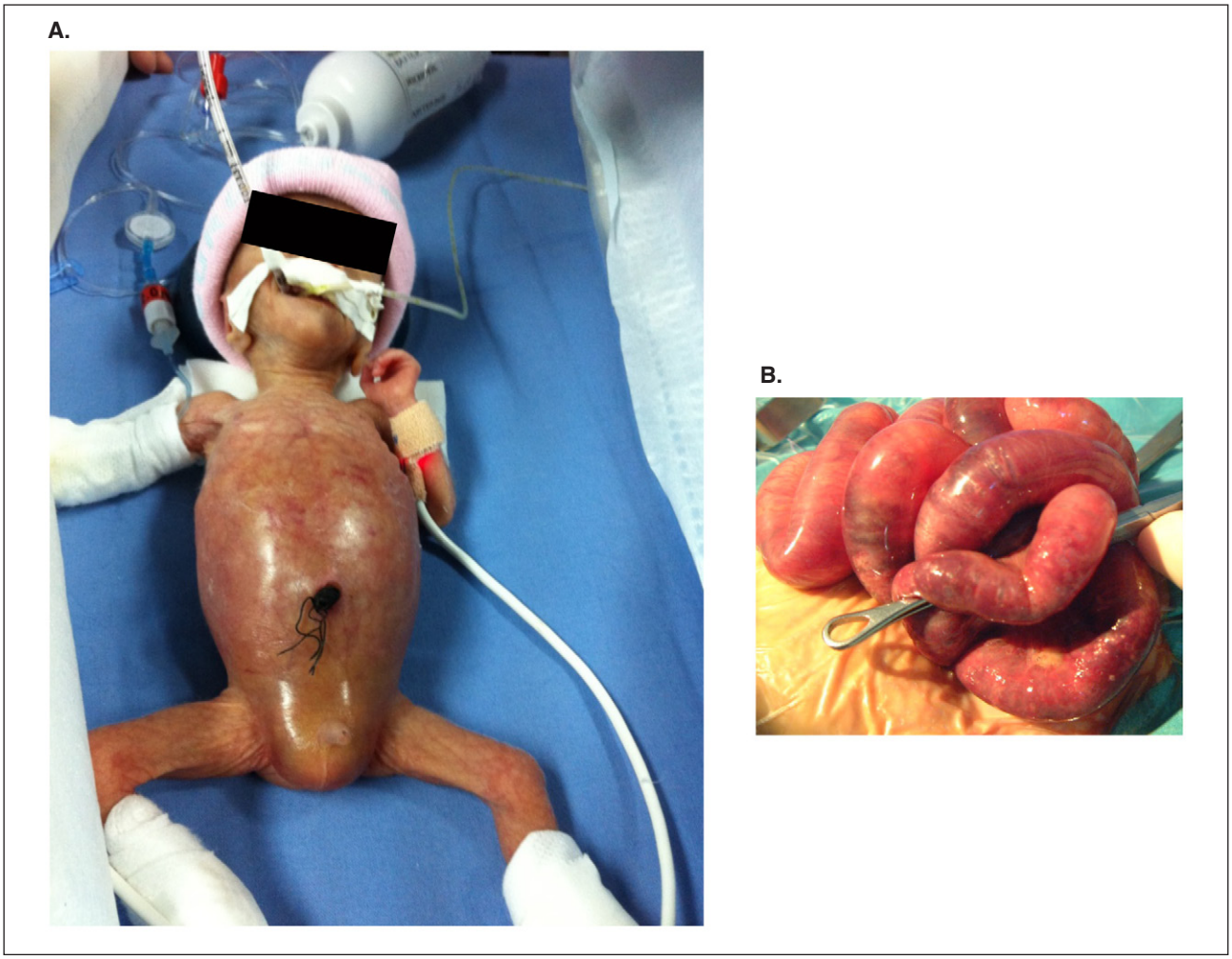

Figure 4. A. 26 wks infant with shiny, distended, erythematous abdomen (advanced necrotizing enterocolitis [NEC]) treated with ileal resection at 15 days of life. **B.** Necrotic patches on the wall of the ileum.

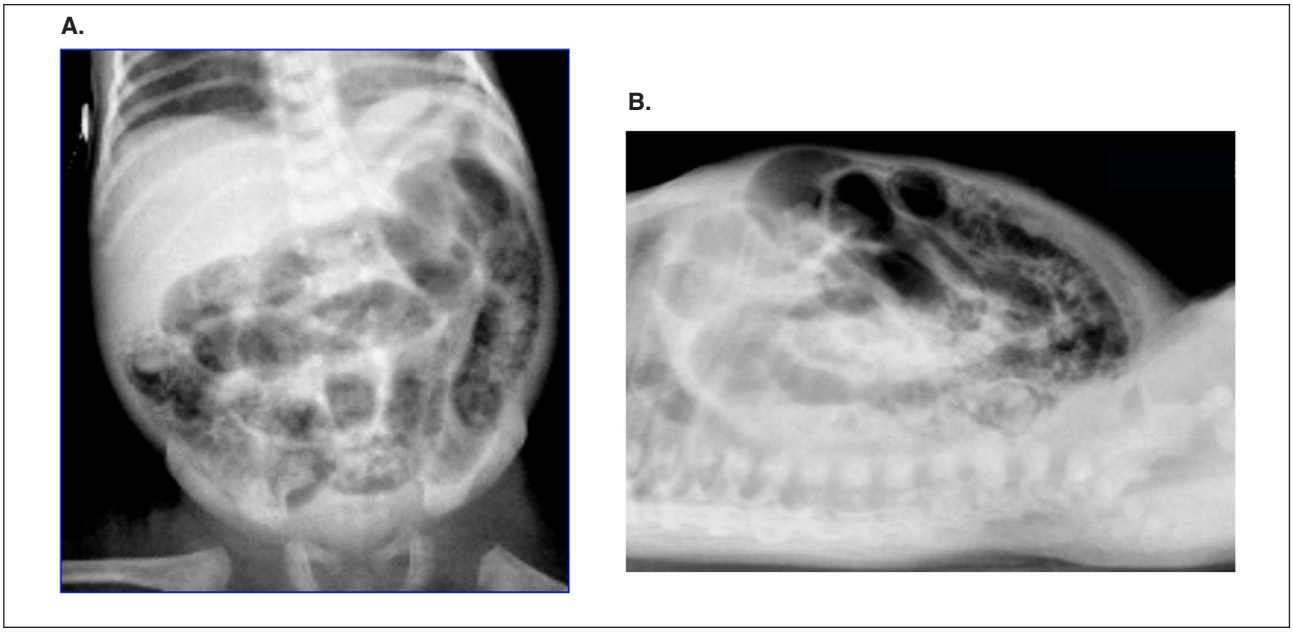

Figure 5. Supine (**A**) and lateral (**B**) radiographs of the abdomen obtained in a neonate with necrotizing enterocolitis (NEC) showing bowel wall thickening, dilatation of the loops with gas and extensive intramural gas.

dilated loops at the center of the abdomen may indicate the presence of a peritoneal effusion that causes them to "float". In cases in which main clinical features are minor abdominal distension and ileus, we see loops with no or quite limited amounts of gas inside them.

Pneumoperitoneum (**Fig. 7**) is a sign of perforation and in its initial phase it is easier to see with a left lateral decubitus radiography [2, 15].

Auxiliary to the x-ray diagnosis, abdominal ultrasound has come into wide use when NEC is suspected.

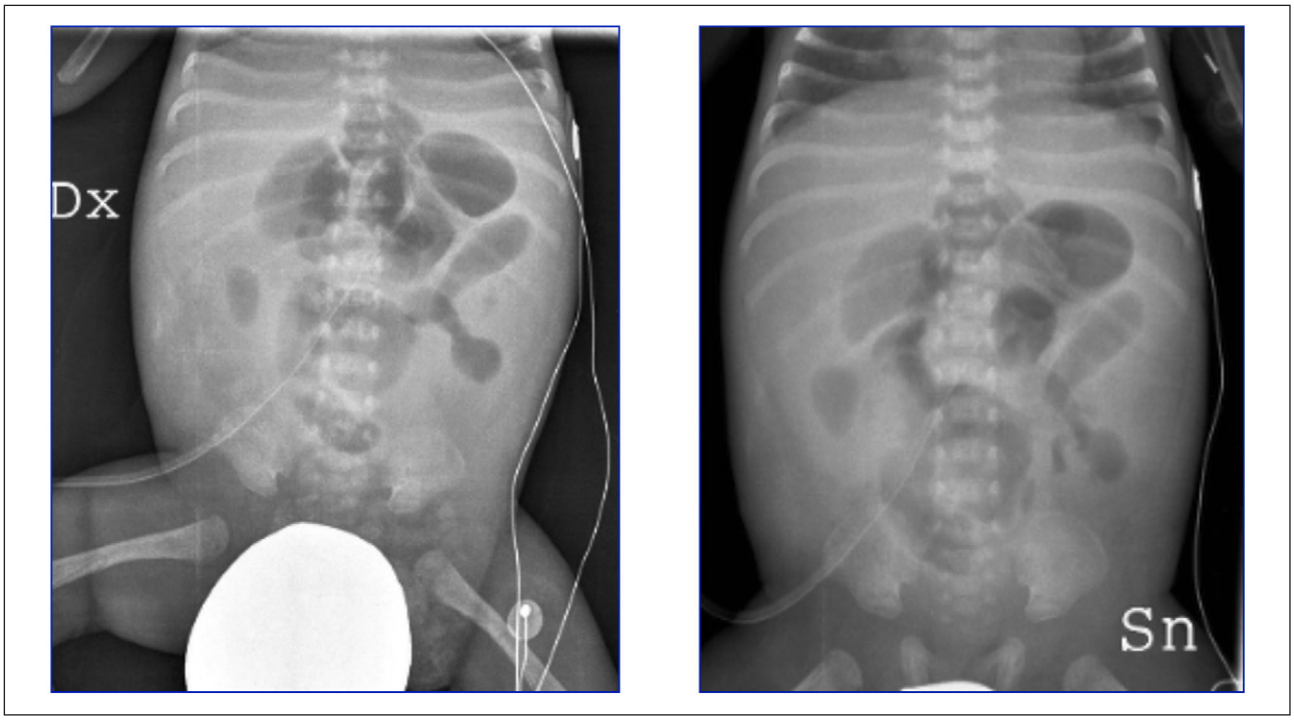

Figure 6. Images obtained at 12-hour intervals show dilated and "fixed" loops.

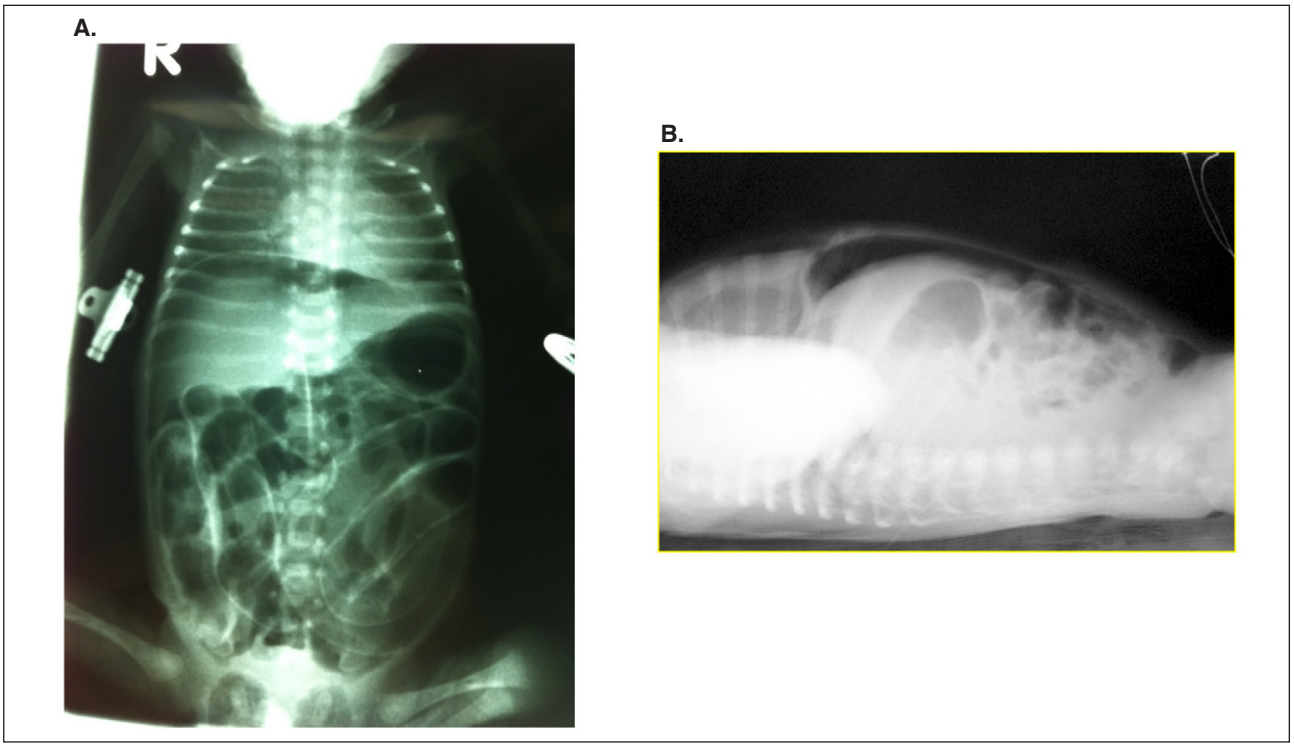

Figure 7. Supine (**A**) and lateral (**B**) radiographs of the abdomen showing dilatation of the loops with gas and free intraperitoneal gas (pneumoperitoneum).

It allows the revealing of the presence of slight effusions as well as small amounts of free gas in the abdomen. It is also possible to assess peristalsis, the thickness of the intestinal loops and the presence of pneumatosis and portal gas (**Fig. 8**). The Doppler ultrasound examination allows assessment of perfusion of the loops [16].

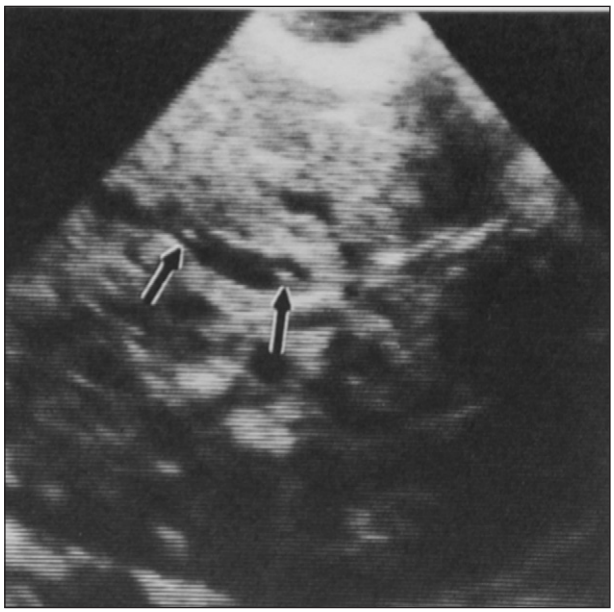

Figure 8. Supine abdominal radiograph shows bubbles of portal venous gas projected over the liver (arrow).

Inside different NECs

In the ambit of NEC subclasses, below are examined the physiopathological and clinical characteristics of each of them (**Tab. 1**), together with possible specific preventive and therapeutic measures that can be applied.

Term and late preterm NEC

This appears in approximately 1 out of 20,000 term neonates with a mortality of 25% to 30%. Its onset is generally in the first week of life in neonates in NICUs for other reasons. Certain diagnoses (congenital cyanogen heart diseases, in particular the single ventricle, perinatal asphyxia, hypotension, especially when associated with late-onset sepsis, polycythemia) have been found to be more common in these neonates as is the association with the use of formula milk and the administration of food volumes far above those that would be ingested with breastfeeding.

Relatively recently another condition associated with term NEC has been described: neonatal abstinence from oppioids. The food factor is important also in this case: breastfeeding is usually not recommended in such neonates and their extreme irritability, often interpreted as hunger, leads to a rapid increase in the amount of food administered.

Table 1. Pathophysiological and main clinical characteristics of different NECs.

	Classic NEC	NEC with contagion	NEC with cow's milk allergy	NEC with RBC transfusion	Term NEC
Where	Terminal ileum	Descending colon	Not specific	Terminal ileum	Ileocecal junction, ascending colon
When	3-4 wks	Variable	> 6 wks	5-6 wks	1 wk
Why	Prematurity, preeclampsia, PROM, feeding malpractice, antibiotics, formula feeding	Viral epidemics	Formula milk and fortifiers made with cow's milk proteins	ELBW, exposure to at least 1 previous transfusion donor, severe anemia	IUGR, congenital cardiopathy, policythemia, sepsis, hypoxia, ischemia, formula feeding
How	Coagulative necrosis	Ischemic and/ or hemorrhagic necrosis, edema and phogosis, colic pneumatosis, ascitis	Eosinofilic phlogosis, edema and necrosis, ascitis, pneumatosis	Abnormal response of mesenteric blood flow velocity, coagulative necrosis	Mesenteric ischemia, coagulative necrosis
Clinics					
Ileus	Yes	Delayed	Yes	Yes	Variable
Rectal bleeding	Rare	Yes	Yes	Yes	Variable
Pneumatosis	Yes	Yes	Yes	Yes	Yes

NEC: necrotizing enterocolitis; PROM: premature rupture of membranes; ELBW: extremely low birth weight; IUGR: intrauterine growth restriction.

In all conditions described the impairment of mesenteric perfusion is supposedly to be the first hit while the food factor would act in triggering the symptoms [3, 17, 18].

Even in the term neonate activation of the two TLR4-PAF pathways appears to play a preponderant role: the expression of TLR4s, which is running out at the end of gestation, may be re-activated by the PAF released by the enterocytes in response to hypoxia [6].

Since in term baby there is no slowing of progression along the ileum as in VLBW infant, the site of the mucosa damage in most cases is distal (ileocecal valve and ascending colon) and the symptoms of intestinal obstruction may be lacking while the emission of bloody stools may be the initial peculiar symptom (**Fig. 9**).

The use of mother's milk is, as in all forms of NEC, the gold standard for prevention. The activation of a feeding regimen that includes a slow increase in food intake is presumably to be taken into consideration even in babies born near term with a positive anamnesis for hypoxic-ischemic events or in the syndrome of abstinence from oppioids [3, 17, 18].

"Classic" NEC in the VLBW preterm

This affects approximatly 7% of neonates in this weight category, with a mortality of about 20% to 40% [2].

Symptoms usually appear after the first two weeks of life. Risk factors, other than preterm birth, are represented by pre-eclampsia, PROM (premature rupture of membranes), chorionamnionitis, poorly managed feeding regimen, antibiotics and the use of formula milk [19].

Since it is mostly the distal ileum that is affected, the main symptoms are those related to obstruction, while the presence of bloody stools is infrequent [2].

The onset generally does not take place until food volumes reach about 80 ml/kg or if enteral feeding is well under way and tolerated with volumes of > 120 ml/kg [5].

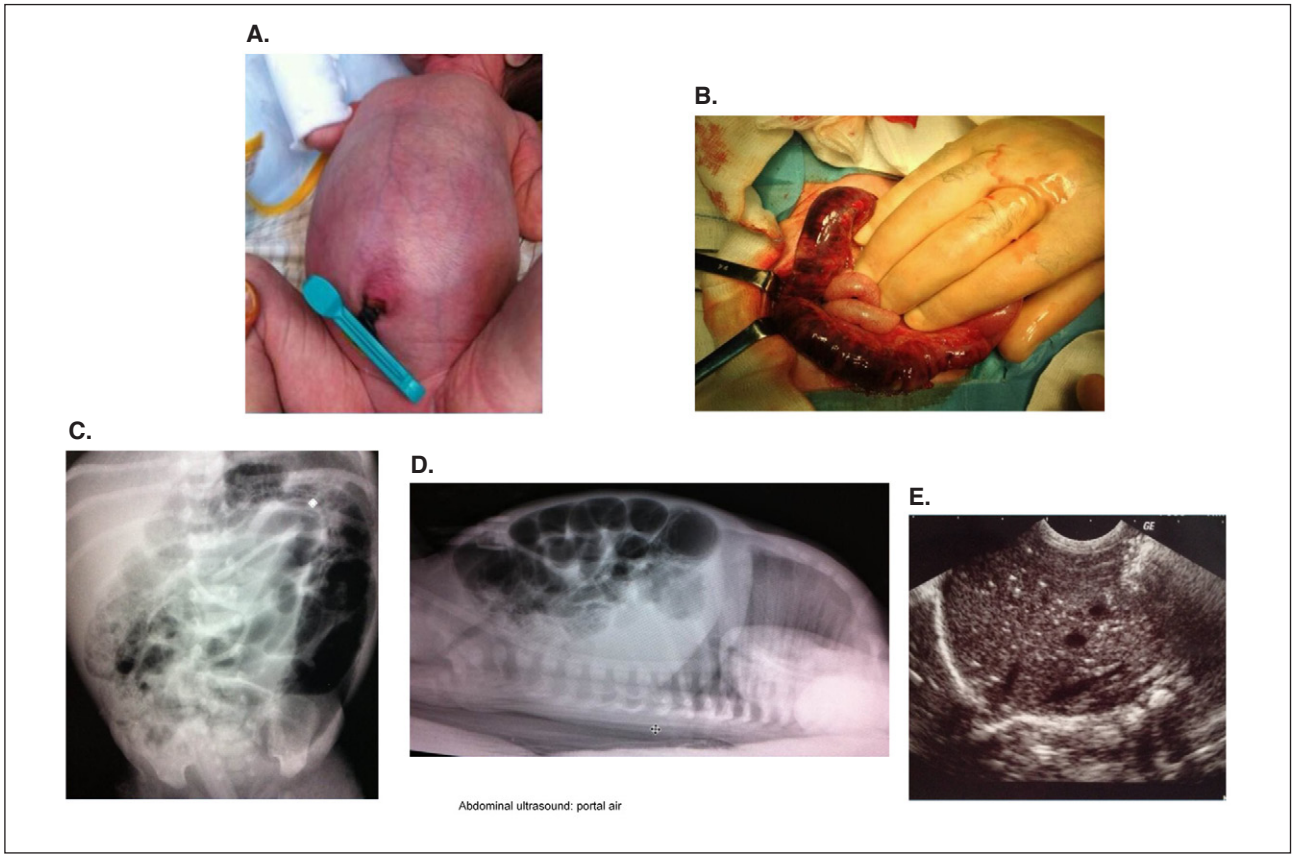

Figure 9. Necrotizing enterocolitis (NEC) in a term baby formula milk fed began with emission of bloody stools: at 4 days severe abdominal distension and tenderness. **A.** The newborn underwent surgery for total colectomy and terminal ileostomy. **B.** On exploration there were multiple gangrenous and necrotic patches on the wall of the colon. **C.** Widespread colonic pneumatosis. **D.** Radiographic evidence of ileus. **E.** Abdominal ultrasound evidence of portal gas (arrows).

In a certain number of neonates the progression is particularly serious and death takes place rapidly in a context of systemic sepsis. This most serious NEC, also known as NEC *totalis* since necrosis involves the entire intestine, has not yet received a pathogenetic explanation, although the hypothesis of a genetic predisposition is plausible [20].

NEC and FIP

NEC is frequently preceded by symptoms of FIP, a common problem in NICUs when the first attempts at enteral feeding are made. It is caused by an intestinal dysmotility (responsibility for which is probably immaturity of the peristalsis in the distal ileum) and by retarded gastric voiding [5, 21, 22]. The result is gastric retention, abdominal distension, absence of stools, sometimes bilious vomiting, apnea occurrence. FIP is a paraphysiological situation that is overcome in most cases but, given the greater risk of bacterial invasion caused by food retention, it may develop into a true NEC if not treated properly: enemas for failure to stool and appropriate diet protocol with a cautious increase in food volumes are useful measures for overcoming it.

NEC associated with delayed feeding

In the lowest gestational ages, NEC may appear in neonates in whom, following a prolonged FIP or for other causes, progression in enteral feeding is quite slow. In such cases, at the time of onset not even pertinent volumes have been reached with minimal enteral feeding [6]. Atrophy of the mucosa appears to be the preponderant pathogenetic factor: it is known that prolonged parenteral feeding is associated with this condition which, when trophism is not re-established, leads to failure of the barrier function and the onset of the necrotic process [23-25].

Prevention

Attention of clinicians and researchers concerning the prevention of preterm NEC focuses prevalently on three strategies: the use of mother's or donated breast milk, the early beginning of enteral feeding, minimal (or trophic) enteral feeding, the use of probiotics.

The efficacy of mother's milk has now been widely acknowledged [26]. Protection against NEC depends on the amount of mother's milk assumed in relation to the volume of total milk (mother's milk + formula milk). The incidence of NEC decreases with the increase in the amount of mother's milk [27].

There is strong evidence in favor of donated human milk compared to artificial milk in the prevention of NEC, but there are no randomized studies comparing mother's milk and donated milk. The administration of donated milk is thus strongly recommended when mother's milk is unavailable, keeping in mind that pasteurization and origin from mothers with term deliveries can reduce its content of T and B cells, macrophages, neutrophils, IgA, IgG and lactoferrin, thus making it less effective than the milk of one's own mother [26-28].

The early beginning of enteral feeding, owing to its positive effect on the trophism of the intestinal mucosa and the development of the microbiota, has been shown to be effective in reducing the incidence of NEC (and its mortality) [29]. Instead, there is no evidence in this sense in favor of minimal enteral feeding, but since this practice, now applied in many NICUs, has no negative effects, it can be considered a sure alternative to the rapid increase in enteral feeding [26].

As concerns probiotics, their use has been shown to be promising in preventing NEC [30, 31] but the several studies performed have not provided suitable information on what probiotic or probiotics to use, their dosage, which neonates to administer them to and when and for how long the treatment should begin and last. The possibility that their use may expose the premature intestine, already lacking adequate defenses and with a tendency to inflammation leading to sepsis, has hindered their widespread use in NICUs: although almost no studies have detected the onset of sepsis in connection with probiotics, clinical practice requires caution in this sense, especially in cases of ELBW (extremely low birth weight) infants [26].

There is universal agreement on the need to take into account the epidemiology of the disease in their own NICU as the greater the incidence of NEC the greater would be the effectiveness of probiotics [30].

In a recent editorial in response to an article by Shlomai et al. [32], in which the authors recommend the administration of probiotics to all ELBW infants regardless of the kind of milk used (mother's milk, formula milk or both together), Moodi [33] expresses the following perplexity: is it logical to administer a supplement of probiotics, (so long as we know which ones to administer) to neonates who assume colostrum from the beginning

and immediately afterwards fresh mother's milk which freely provide immuno-modulating effects that are above all tailored for that specific mother and child? And what evidence do we have that besides excluding the risk of probiotic-related sepsis it also excludes long-term repercussions on immune functions deriving from the replacement of a maternal microbiome with an exogenous one?

As emerges from the foregoing observations, there is a need for further randomized controlled studies on a large number of cases, with clear and standardized methods and objectives, before adopting the widespread use of probiotics [34-36].

NEC associated with viral epidemics

In the literature we find different mentions of cases of NEC that begin as epidemics in different seasons of the year depending on case histories, but mostly in winter and spring. Their frequency is estimated at 10% to 15% of all NECs [37, 38]. Although they prevalently involve the VLBW infants, this form is also described in term or late preterm babies. Symptoms normally appear after the first two weeks of life, when enteral feeding is well under way and tolerated with volumes of > 120ml/kg/die [5]. This form is not usually associated with ileum in the early stages, but with repeated emetic diarrhea with (secondary to enteritis) and abdominal distension.

Abdominal distension, rarely caused by the ileum, is more frequently secondary to the third space and the pneumatosis is located in the left colon.

There is frequently positivity in blood and urine cultures caused by a secondary invasion of the intestinal bacterial flora with associated symptoms of sepsis, urosepsis and meningitis. Rotaviruses, noroviruses and astroviruses have been found in stools with advanced viral enteritis in NEC. Although the clinical picture may be similar to that of classic NEC, the prognosis is more favorable with less recourse to surgery and lower mortality [5, 39].

Some recent studies have underlined the importance of the finding in this subcategory of lymphocytes at the time of diagnosing NEC: this fact, besides leading the clinician in the direction of a viral form, appears to be associated with high mortality [40, 41].

The feeding factor may be important in this case as well: Hair et al., in a study of 97 NECs diagnosed at their center between 1996 and 2007, found that neonates with lymphocytosis had at the time of diagnosis significantly higher food volumes than those without lymphocytosis and assumed a larger amount of formula milk [40, 41].

In the prevention of this subcategory of NEC, it is clearly important to adopt hygienic measures to avoid contagion. The prophylactic administration of antibiotics may be useful in inhibiting intestinal flora [6].

NEC associated with red cell transfusions

This is one of the most serious forms of NEC and is associated with a high mortality rate and frequent recourse to surgery. It appears to represent 25% to 35% of all NECs and has the following main characteristics:

- its onset is within 48 hours of the transfusion with neonates of GA and weight below those affected by classic NEC;
- symptoms appear at a chronological age of approximately 3 to 5 weeks (thus above that of NEC not associated with transfusions), at a distance from events potentially correlated to it (hypotension, hypoxia, arterial catheters);
- it occurs prevalently in neonates previously transfused one or more times with RBCs from the same donor [42-45].

TRAGI vs TRALI

NEC associated with transfusion of RBCs thus results as separate from other types of NECs in connection with a different vulnerability of the neonatal intestine. A proposed pathogenesis places it beside TRALI (transfusion-related acute lung injury) [46] in the adult and for this reason it is known as TRAGI (transfusion-related acute gut injury).

To manifest itself, a first hit, represented by immaturity of the intestinal barrier and its pro-inflammatory characteristics, is supposedly necessary: TLR4 upregulation and eosinophil action [47], often increased in this condition, supposedly favors damage of the mucosa caused by the transfusion, which represents the second hit. In the pathogenesis of this NEC subcategory, much importance appears to be assumed by the action of angiogenetic factors: the insufficient availability of O_2 present in the anemic condition requiring the transfusion increases the production of new blood vessels, a process that is already quite active at this age in the rapid development of the intestine. New blood vessels are particularly sensitive to damage

that transfused RBCs can cause: their minor deformability is known and this makes the passage into the capillaries difficult, with a consequent slowing down of flow, a tendency to hemolysis and liberation of free hemoglobin, peroxides and free radicals, all factors implicated in damage of the mucosa [44]. As infants previously transfused with RBCs from the same donor and blood type AB subjects are more affected, immunologic mechanisms cannot be excluded [6, 45].

Age of donated RBCs

There appears to be no relationship between the age of RBCs and the risk of TRAGI as recently confirmed by Christensen who, in the cases he examined, found no difference in the incidence of NEC in babies transfused with RBCs of age >10 days compared to those in whom the age of the RBCs was < 7 days [42, 48].

Role of anemia

There is an inverse relationship between the severity of the anemia and the increased risk of TRAGI. Anemia represents an independent risk factor that is accentuated by the transfusion. The risk does not change if a partial correction of the anemia is performed or if 5-20 ml/kg of RBCs are administered several times at different moments. Administration of RBCs in the first week of life to replace the blood taken for tests (≈ 15 ml/kg) is not associated with the risk of TRAGI [45, 49].

In serious anemia (Ht < 25%), vasodilatation is at a maximum to favor the use of the small amount of O_2 remaining in the intestinal cells, which are strongly O_2-dependent. Further interference in the flow of transfused RBCs would cause a worsening of the situation and further traumatize the mucosa, thus favoring the bacterial invasion. In this mechanism, nitric oxide (NO) appears to play an important role with its vasodilatating action which prevails in prematures over the vasoconstricting action of endothelin: the conserved RBCs not only contain less NO, but also cause greater consumption of it, given the major affinity of NO for the hemoglobin that is released following partial hemolysis by the transfused RBCs [45, 49].

Feeding and TRAGI

The relationship between the frequency of TRAGI and suspension of feeding before, during and/or after transfusion is quite controversial. Some authors report a significant reduction in the frequency of TRAGI if feeding is suspended during the transfusion [50], while others don't find any benefit in prevention [44]. In Christensen's study the neonates who developed TRAGI had assumed higher amounts of milk compared to controls without TRAGI in the 24 hours before and during the transfusion and were more frequently fed with formula milk: he concludes, however, that the statement of the benefit deriving from the practice of suspending feeding is speculative and that there are no data that recommend it or establish the right time to apply it [42, 47].

Still to be defined is also the role of feeding modalities (bolus vs continued nasogastric feeding) [44]: some reports consider bolus fed ELBW infants at risk since they may not be able to increase the

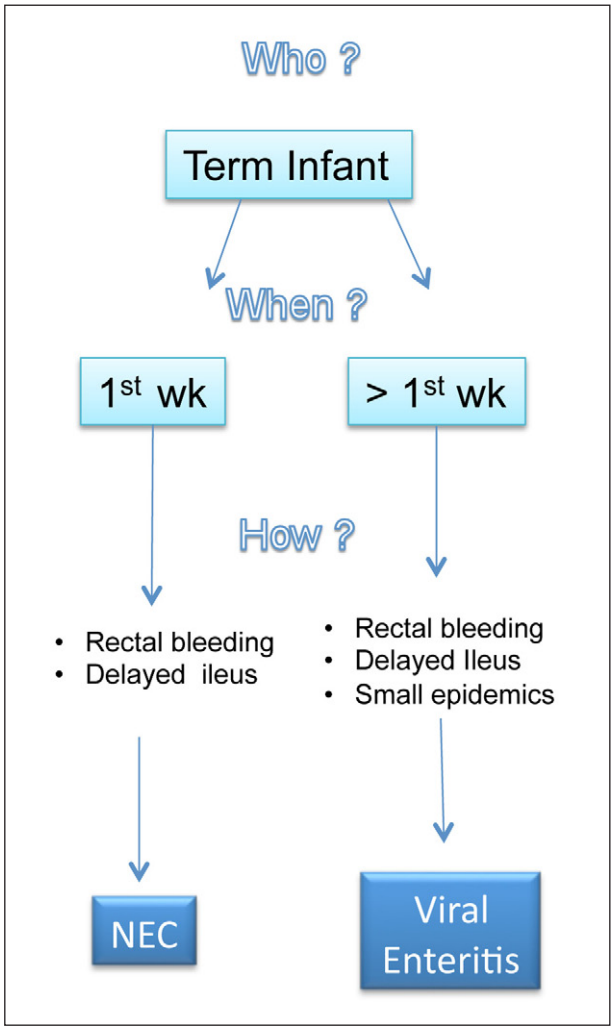

Figure 10. Differential diagnosis (DD) of severe gastrointestinal (GI) disorders in term infant (GI malformations excluded).

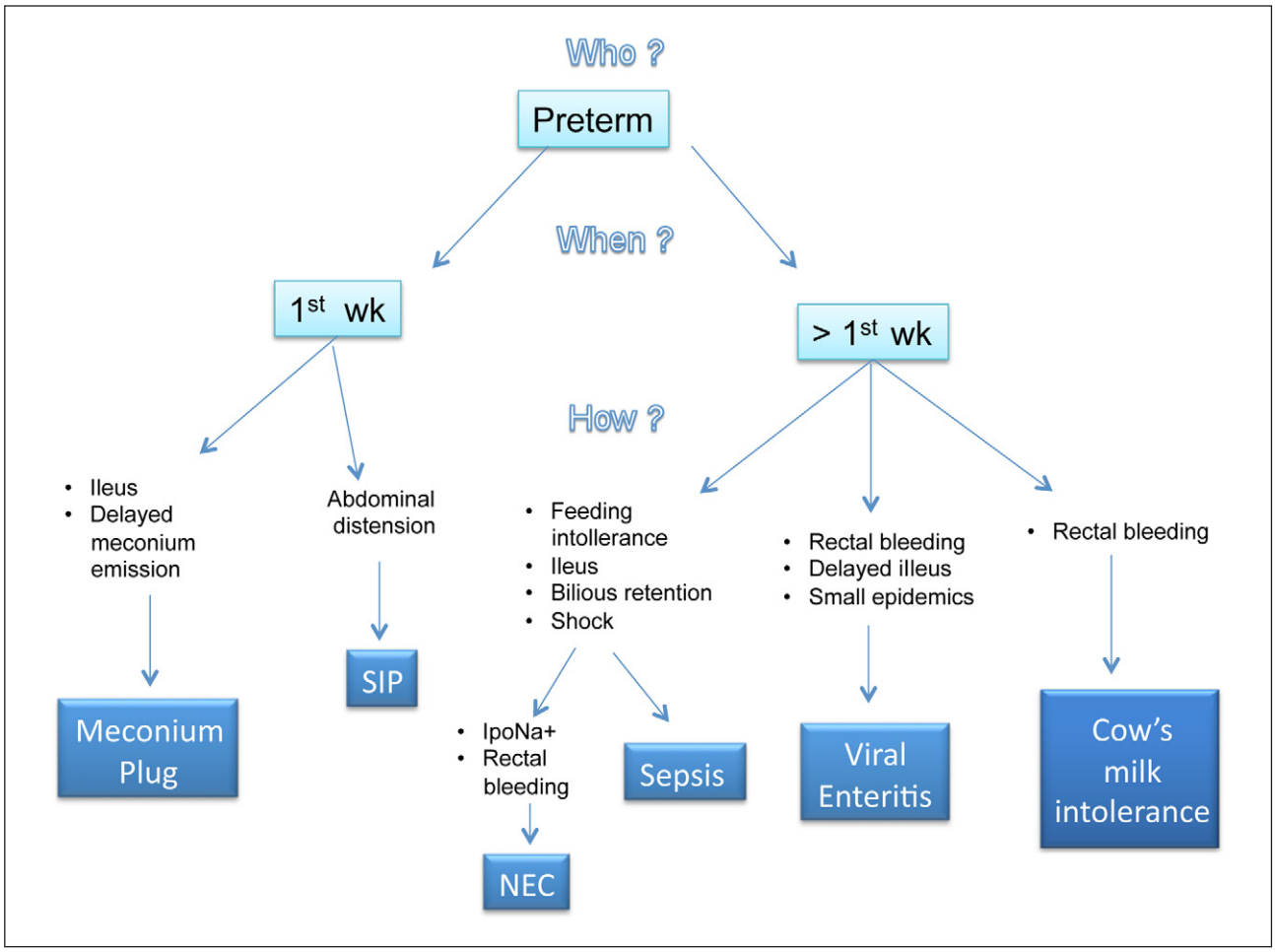

Figure 11. Differential diagnosis (DD) of severe gastrointestinal (GI) disorders in preterm infant (GI malformations excluded).

intestinal blood flow when the bolus is administered during the transfusion [51].

Prevention

Reduction of late transfusions as far as possible, preventing anemia through minimizing blood draws and use erythropoietin for preventing anemia are the main devices to prevent TRAGI. The implementation of standardized transfusion protocols and the use of washed RBCs are also important while there aren't sufficient evidences to recommend specific feeding protocols near transfusions. More studies about preventing of mesenteric flow velocity trough near-infrared spectroscopy (NIRS) and Doppler ultrasound are desirable [6, 45].

NEC associated with cow's milk protein intolerance

This presents at approximately the sixth week in VLBW infants, with abdominal distension and diarrhea, which may become bloody. The prognosis is normally favorable, but surgery may be necessary in neonates with a very low GA. Abdominal distension from the third space and pneumatosis are accompanied by the laboratory detection of eosinophilia. Essential in treating the symptomatology is the suspension of formula milks and fortifiers of mother's milk made with cow's milk proteins and the administration of an elementary formula [18, 51, 52].

Differential diagnosis

The flow-charts of **Figures 10** and **11** may help to solve the main problems of differential diagnosis (DD) of NEC when one is faced with neonates with aspecific gastrointestinal (GI) disorders when GI malformations are excluded.

Term NEC and viral enteritis

If we exclude intestinal malformations, the symptoms of NEC in term neonates should not

present many problems in DD (onset in the first week of life, a positive anamnesis for factors that may have caused mesenteric ischemia, emission of bloody stools without ileum in the first phase). It is rather the rarity of this pathology that may lead to a late diagnosis or suggest other causes. The appearance of rectal bleeding beyond the first week must instead suggest a viral origin, especially if it appears in the form of a small epidemic. Differently from VLBW infants, this form rarely evolves into NEC, although in reports on the subject cases of NEC associated with viral enteritis have been described even in late preterms [51].

FIP and NEC

FIP is typical of the first week of life, but it may appear even later if the beginning of feeding is delayed. It is not usually associated with a clinical worsening: the abdomen is distended but not tender; vomiting is not bilious as in NEC but only bile traces may be present as well as in gastric residuals. Absence of stool may be observed as in NEC (but in general it is possible to obtain the emission of stools with small enemas), and sometimes also bloody stool. There is no alteration of laboratory tests. The abdominal x-ray, if performed, often shows intestinal dilated bowel (but not thickened) loops with no sign of obstruction. Since FIP is a paraphysiological condition of VLBW infants, it is defined by Gordon as the grounds for a "perfect storm" because its progression towards NEC may be insidious or sudden [5]. For this reason its many and deceptive symptoms are often a source of concern for the neonatologist [21, 22].

NEC and meconium disease

The meconium disease includes a range of idiopathic intestinal obstructions with different severity, uncorrelated with cystic fibrosis or Hirschprung's disease: from the meconium plug syndrome (benign), in which the meconium is located in the terminal colon, to the meconium disease in which the plug forms at the level of the ileum and may cause perforation complicated by peritonitis [5, 54, 55]. Although the symptoms normally appear in the first days of life, in VLBW infants they may show up around the second week (as in NEC) preceded by spontaneous emissions of small amounts of meconium. The symptoms are bilious vomiting and ingravescent abdominal distension with palpable dilated intestinal loops with no signs of peritonitis. The x-ray of the abdomen shows dilated loops above the meconium plug, usually at the level of the terminal ileum in ELBW infants, without water or gas levels and without pneumatosis. Perforation may occur in 30% of cases.

NEC and SIP

The acronym SIP indicates an intestinal perforation, usually single and of millimetric size, prevalently located in the terminal ileum [5, 55, 56].

SIP strikes between 2% and 5% of neonates below 1,000 g [5, 57], with a gestational age at birth between 23 and 27 weeks. In the neonatal period, it represents the second cause of intestinal perforation after NEC [15]. It is characterized by:

- usual localization at the level of the terminal ileum (more rarely jejunal and colonic);
- necrotic perforation of the internal muscle with integrity of the mucosa varying in size, generally few millimeters, constantly on the antimesenteric side;
- absence of coagulative necrosis.

The first studies were fundamental in identifying a close correlation between SIP, early administration of cortisone for the prevention of bronchopulmonary displasya (BPD) [56, 58] and indometacin within 12 hours of birth for the prevention of cerebral hemorrhage [59].

Risk factors are represented by chorionamnionitis, hypoxia and ischemia, with onset in the uterus, patent ductus arteriosus (PDA) [56, 60].

In several reports, SIP is associated with infection caused by coagulase-negative staphylococcal (CoNS) sepsis and candidiasis. Infections certainly play a role in worsening the pathology and are often lethal [61]. Onset is normally in the first two to three weeks of life but cases with later onsets have been described [62]. The clinical picture is characterized by abdominal distension with a sudden onset, bluish color of the abdomen as in NEC. Bloody stools are infrequent [58].

At the beginning, the overall conditions are not impaired: thermal instability, apnea and bradycardia, emesis, gastric residuals, tender abdomen are lacking. Instead, sometimes the signs of sepsis may precede perforation. Pneumoperitoneum is the prevalent radiological sign [58] while dilated intestinal loops, pneumatosis and portal vein gas are absent [5, 58].

Most descriptions of cases of SIP report a favorable outcome but may become lethal in 25%

of cases [56] when it is associated with candidiasis, sepsis caused by CoNS [60, 61], peritonitis [24] and intraventricular hemorrhage [62, 63].

The therapy is surgical.

Sepsis and NEC

This is the medical condition more difficult to differentiate when radiological signs of NEC have not yet appeared. The onset of the two conditions is often identical: the abdominal and systemic signs of sepsis are the same as in NEC. Ileus, hypotension, respiratory distress, thrombocytopenia, neutropenia and left shift of neutrophils are present in both.

Indicating a diagnosis of sepsis is the presence of hyponatremia (> 130 mEq/l) and the emission of bloody feces, which may appear soon after resolution of the ileus.

In sepsis, the x-ray of the abdomen may show diffuse dilation of the loops or scarce presence of gas within them. The finding of "fixed" and/or thickened loops and of abdominal effusion is common to the two pathologies, while pneumatosis is characteristic of NEC [2, 15].

Therapy and outcomes

Medical therapy must begin as soon as NEC is suspected and coincides with therapy for the suspected sepsis: administration of antibiotics, parenteral feeding, maintaining of the electrolyte balance, infusion of RBCs and platelets if necessary, hemodynamic support.

Identification of pneumoperitoneum and the presence of necrotic loops are indications that surgery is absolutely required. As pneumoperitoneum cannot be present in about 20% of cases of bowel perforation a 7 point score can be helpful in making decision on surgical intervention: it involves the presence of severe metabolic acidosis, severe thrombocytopenia, hypotension, hyponatremia, neutropenia, left shift of neutrophils, and positive blood culture [2, 15].

Infants with surgical NEC die in up to 50% and have the worst long term outcomes. Mortality is inversely related to GA and birth weight. Complications include intestinal strictures and short-bowel syndrome. Strictures typically occur 3 to 8 weeks after the acute episode but can become syntomatic several months later. Short bowel syndrome is the most common long term gastrointestinal complication, affecting about 25% of survivors.

Patients with surgical NEC develop neuro-developmental problems about twice than those medical treated. Neonates with NEC who do not require surgery have long term outcomes similar to age-matched premature neonates without NEC [64, 65].

Declaration of interest

The Authors declare that there is no conflict of interest.

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