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BIOMARKERS OF NECROTIZING ENTEROCOLITIS THROUGH THE PRISM OF ETIOPATHOGENESIS

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БИОМАРКЕРЫ НЕКРОТИЗИРУЮЩЕГО ЭНТЕРОКОЛИТА С ПОЗИЦИЙ ЭТИОПАТОГЕНЕЗА

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The review analyzes the pathogenetic aspects of using diagnostic markers of necrotizing enterocolitis (NEC). The substantiation of the leading and common tags for various pathological factors depending on the period of the disease in newborns is carried out. Peculiarities of diagnostics, characteristic of different stages of the disease, are considered. Thus, using biomarkers that consider the staging and severity of the course of NEC in newborns will allow targeted interventions on various etiopathogenetic links to prevent disease progression and reduce mortality.

Keywords: necrotizing enterocolitis, biomarkers, children

В обзоре представлен анализ патогенетических аспектов использования диагностических маркеров некротизирующего энтероколита (НЭК), предложенных на сегодняшний день. Проводится обоснование ведущих и общих маркеров для разных патологических факторов в зависимости от периода заболевания у новорожденных. Рассмотрены особенности диагностики, характерные для различных этапов заболевания. Таким образом, использование биомаркеров, учитывающих стадийность и тяжесть течения НЭК среди новорождённых, позволит проводить таргетное воздействие на различные этиопатогенетические звенья для предупреждения прогрессирования заболевания и снижения летальности.

Ключевые слова: некротизирующий энтероколит, биомаркеры, дети

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AOPP – advanced oxidation protein products
CCHD – critical congenital heart defects
CRP – C-reactive protein
ELBW – extremely low birth weight
ET–1 – endothelin–1
I–FABP – intestinal fatty acid binding protein
IMA – Ischemia-Modified Albumin
L–FABP – liver-type fatty acid binding protein
LP – lipid peroxidation markers
NEC – necrotizing enterocolitis
NPBI – nonprotein-bound iron
OMP – oxidative modification of proteins
OS – oxidative stress
PAF – platelet-activating factor
SAA – serum amyloid A
SMA – smooth muscle actin
TFF3 – trefoil factor 3
TLR4 – toll-like receptor 4
VLBW – very low birth weight

Advances in neonatology in recent years have led to an increase in the survival rate of premature babies and, consequently, to a rise in child morbidity and mortality. Necrotizing enterocolitis (NEC) is one of the most frequent emergency conditions of prematurity, often requiring resuscitation and emergency surgery and leading to disability or death of this category of children. Mortality from NEC remains high despite new treatment strategies, so early diagnosis and prevention are major health priorities [1].

In recent decades, significant changes have been observed in the number of children suffering from NEC, and risk factors affecting their development are being reviewed. Whereas previously, NEC was predominantly a full-term disease of children with pronounced hemodynamic disorders, in today's world, NEC predominates in premature children with extremely low birth weight (ELBW) and very low birth weight (VLBW). They are most often associated with the most unfavorable outcome [2]. In the early 2000s, an idea of the pronounced heterogeneity of the disease began to form, the concept of phenotypes of NEC, radically different from each other in pathogenesis, with the predominance of a pathogenetic factor, on the systemic effects of NEC on the organs and tissues of the newborn [3].

Modern systematics NEC with etiopathogenesis positions

Today, the notion that NEC is a common endpoint is increasingly being developed, reducing the nosology to subsets of variants with incomparable etiology. Despite this, it is possible to distinguish common early risk factors (immaturity (including related immune system features), hypoxia, disruption of commercial microbiome of the intestine), which are the pathogenetic basis of the disease in the vast majority of cases, and later specific risk factors that allow researchers to speak of the presence of different phenotypes NEC [3].

Gordon P. et al. propose to distinguish 5 phenotypes: hemodynamically significant, transfusion-associated, lymphocytosis-associated, associated with cow's milk intolerance, and NEC associated with feeding [3].

Hemodynamically significant phenotype NEC is observed in infants with critical congenital heart defects (CCHD). CCHDs result in circulatory hypoxia of organs and tissues, including intestinal tissues. Hypoxia induces activation of free-radical metabolism and the synthesis of proinflammatory cytokines, which in constricted blood vessels, due to activation of endothelin-1 synthesis, cause vasoconstriction even more pronounced. In addition, activating the coagulating blood system results in platelet aggregation and an increased risk of clotting. All of the above mechanisms lead to ischemia of the intestinal wall with further occurrence of NEC [4, 5].

Premature newborns often require anemia replacement transfusion correction for transfusion-associated NEC [6]. In this form of correction, a persistent spasm of portal vessels develops, leading to disruption of the mesenteric blood circulation, selective circulatory ischemia of the intestine, and further necrosis of the intestine wall. In this case, the leading cause of this phenotype NEC is not the fact of blood transfusion but severe anemia and systemic hypoxic condition preceding it [3].

NEC is associated with lymphocytosis and often occurs in specific clusters, sporadic cases, united by a common space-time framework based primarily on viral etiology. It should be noted that the clinical differentiation of NEC associated with the virus and NEC exclusively bacterial etiology is currently difficult [7].

NEC associated with cow's milk intolerance is understood to mean the emergence of necrosis of the intestine as a result of IgE-associated allergic inflammation in the intestine wall. This phenotype NEC can also be called a severe manifestation of a gastrointestinal allergy to cow's milk protein [8].

As is known, premature children with ELBW are at risk for NECs. In such newborns, the beginning of enteral nutrition is often delayed, as the severity of the condition does not allow sometimes to start a full meal. Inadequate clinical tactics regarding enteral nutrition in newborns lead to developing NEC associated with enteral feeding [2, 3].

Several researchers distinguish two variants of the NEC flow based on the timing of the disease – early (first-second week of life) and late NEC [9]. Risk factors prevalent in early NEC are hemodynamic disorders due to critical heart defects in the child, severe anemia, RBC transfusions, etc. Late NEC develops in children with ELBW and VLBW disorders of microbial colonization of the intestine. In this case, the lack of compensation for functional immaturity of organs and systems plays a predominant role (slow maturation of enzymatic systems) [2].

Phenotypes and disease variants form the basis of the modern systematization of NEC. This should be considered when searching for predictors and diagnostic markers NEC, which should reflect all aspects of the development of the disease – systemic, multi-factor, heterogeneity, adjusted for the individual characteristics of each patient.

These circumstances largely determine the low efficiency of most of the methods of diagnostics and forecasting NEC, the logic of which is based on the search for highly specific and universal «monomarkers» of the disease [10].

NEC biochemical markers

To date, the full range of proposed potential biomarkers NEC can be roughly divided by tissue specificity: specific, non-specific; by the leading mechanism of pathogenesis: inflammatory markers, hypoxia-related markers, immature-related markers; by the time of detection: early, late; clinical significance: the following groups of NEC markers are advisable: prognostic, early and differential diagnosis, markers of dynamics and prediction of disease outcome [10].

Given the fact that the disease can develop in a sufficiently long period, both in the early and late neonatal period, predictive markers allow the identification of children at risk with a high probability of developing NECs, must be determined and have a high sensitivity and specificity in the first days of the child's life, until any traits of NEC are formed [9]. These indicators include markers of systemic immaturity, immaturity of the gastrointestinal

tract and immune system, hypoxia, and no factors directly detecting the development of inflammatory reactions and/or damage to the intestine [11, 12].

Markers for early and differential diagnosis describe the main etiopathogenetic mechanisms of disease development. Markers characterizing intestinal tissue damage, specific inflammatory markers [13–15] come to the fore.

Biomarkers for dynamic disease assessment, reflecting the probability of development of surgical stages and death, should also take into account the severity of the overall condition of the child, be simple and minimally invasive in the definition, reflect the severity of systemic inflammatory process (nonspecific markers of inflammation) and necrotic process in intestinal tissues [16, 17].

Markers that predict the rate of recovery of the gastrointestinal tract reflect the normalization of metabolic parameters and characterize the bowel function most often after surgery, helping in making decisions regarding the possibility of closing the stoma and transitioning to complete enteral nutrition [18, 19].

This review attempts to analyze the pathogenetic aspects of the use of potential diagnostic biochemical markers of NEC, justifying the separation of leading, typical phenotype pathogenetic factors at different periods of disease development, the use of indicators reflecting them to achieve the tasks of diagnosis, relevant at this stage of development of the disease.

Biochemical factors involved in etiopathogenesis of NEC

At present, it is essential to understand not only the choice of the correct marker but also the period at which the application of a biological indicator is appropriate. Without taking into account the later risk factors that form specific phenotypes of NEC, we can identify the top links of etiopathogenesis – immaturity, hypoxia-ischemia, and inflammation/lesion of the intestine, which in our view, are critical common non-specific vectors, creating a framework for the development of NEC (Fig.).

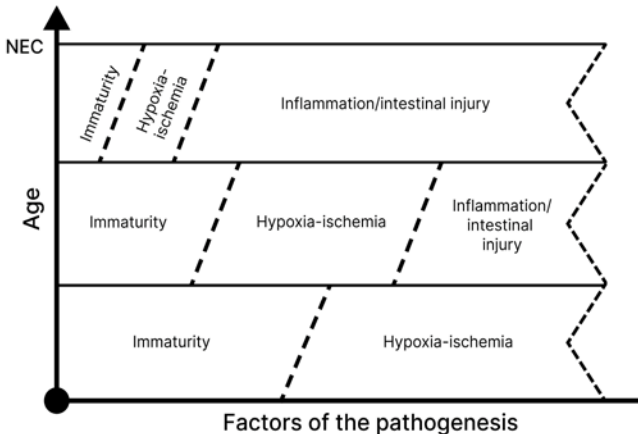

Fig. The time frame of the predominance etiopathogenetic mechanisms underlying the development of NEC

Based on the presented logic, by analyzing articles from the databases available, Pubmed, Web of Science, and ScienceDirect, a table of metabolites currently or potentially become potential biomarkers with correct and timely definitions in clinical practice (Table).

Immaturity

Features of the immature congenital immune system of premature newborns and, in particular, toll-like receptor-4 (TLR4), a key component of vulnerability to NEC, are now described in detail in the literature [20].

But the general immaturity of premature newborns, which comes to the fore when discussing risk factors for the development of NEC [2], is characterized by two levels of immaturity: anatomical (morphological) and physiological (functional). In this case, functional immaturity, which plays a crucial role in the development of NEC, may not correspond to the pace of morphological maturation and the formation of tissue structures but lie primarily in the biochemical plane. In the first place, the activity of enzymatic systems is reduced, which makes it much more difficult to assess.

Functional immaturity of enzyme systems can be determined by direct determination of enzyme activity and/or quantity [14], by detection of gene expression level [21], and indirectly by the level of metabolites being substrates/products of enzyme response [11, 22, 23].

Specific metabolites, reflecting the maturation rate and metabolic pathway formation in the tissues of the gastrointestinal tract, include some amino acids, the synthesis of which in newborns is limited to intestinal tissues. Researchers focus on the metabolism of arginine and citrulline, the concentration of which in blood or urine is most often studied in children with the first signs of the disease [24–26]. However, there are few and mixed studies of the NEC prediction, probably because the concentration of these compounds at a particular time is influenced by many factors, which is very difficult to consider in the context of a limited group of examined unborn [22, 25]. Thus, Zamora S. A. et al. has been shown to have significantly lower serum arginine levels on the third day of life in children who subsequently developed necrotizing enterocolitis than in children of the control group [25]. Arginine is an essential amino acid, and the synthesis of arginine de novo in newborns occurs almost only in the intestine. The body's arginine homeostasis is determined by intake, tissue consumption, and endogenous production. Given the specific pathogenesis of NEC, it is suggested that a decrease in plasma arginine levels in the early days of life may be due to an increased metabolic need for arginine for NO synthesis, which plays a role in gastro-plasma maintenance of intestinal blood flow and/or the consequence of limited endogenous synthesis as a result of low activity of critical enzymes of arginine synthesis in the intestine [21].

Despite numerous studies in animals that indicate low levels of arginine in premature, it is difficult to be sure, based on a bit of research, that levels of arginine at birth will differ significantly in children with a high risk of developing NECs, as shown in a large multicenter study of Sinclair T. J. et al. (2020), where levels of arginine, as well as alanine and phenylalanine, are defined in capillary blood (dry blood spots) in the first days of life were, on the contrary, higher in children, who have developed NEC [22].

The difficulty of identifying cause-effect relationships and using metabolites as specific markers of immaturity directly in the intestine is the practical impossibility of testing the hypothesis about mechanisms of reducing or increasing levels like arginine, and other amino acids, lipids, intermediates of the Krebs cycle under clinical trial conditions. These problems are partially solved by using experimental models on animals, but their translational validity in most cases is also ambiguous.

Therefore, early prediction of the development of NEC is the optimal use not only specific to the gastrointestinal tract but also system indicators of immaturity, which are highly likely to reflect the possibility of disease development.

Such biomarkers may include indicators of the severity of oxidative stress at birth or in the first days of life, the development of which, first of all, will play a role in the

Table

Possible biochemical markers of necrotizing enterocolitis

Factors of the pathogenesis	Biomarkers	Biomaterial	References	Type of diagnostics
Morpho-functional immaturity – systemic	Phospholipids (phosphatidylcholine, lysophosphatidylcholine) ↓	vein blood	[11]	Early and differential diagnostics
	Acylcarnitines	dry blood spots	[12], [22]	Prediction biomarkers
	Lipid peroxidation products↑	cord blood	[23]	Prediction biomarkers
	Thiol/disulfide homeostasis ↑	cord blood	[27]	Prediction biomarkers
Morpho-functional immaturity – intestine	Arginine ↓	vein blood	[25]	Early and differential diagnostics Prediction biomarkers
	Alanine, phenylalanine, arginine ↑	dry blood spots	[22]	Early and differential diagnostics
Hypoxia-ischemia	Lipid peroxidation products, NPBI ↑	cord blood	[22]	Prediction biomarkers
	Ischemia-modified albumin ↑	vein blood	[34], [35]	Early diagnostics Disease dynamics
	L-lactate↑	cord blood	[31]	Prediction biomarkers
	Endothelin-1↑	cord blood	[31]	Prediction biomarkers
Systemic inflammation	C-reactive protein↑	vein blood	[47], [17]	Disease dynamics
	Procalcitonin ↑	vein blood	[47], [17]	Early diagnostics Disease dynamics
	Serum amyloid A↑	vein blood urine	[18]	Early diagnostics Disease dynamics
	Advanced oxidation protein products, lipid peroxidation products ↑	cord blood	[23]	Prediction biomarkers
	Superoxide dismutase ↓, Malonic dialdehyde ↑	vein blood	[47]	Undiscussed
	Galectin-3 ↑	vein blood	[48]	Early diagnostics Disease dynamics
Inflammation/ intestinal injury	Arginine ↓	vein blood	[24], [25]	Early and differential diagnostics
	Citrulline↓	vein blood	[19], [44], [45], [46]	Disease dynamics Early and differential diagnostics
	Tyrosine ↓, arginine ↑	urine	[11]	Early and differential diagnostics
	I-FABP↑	vein blood	[16], [31], [36], [37]	Early diagnostics Disease dynamics
	I-FABP↑	urine	[18], [30], [37], [38], [43]	Early and differential diagnostics Disease dynamics
	L-FABP↑	vein blood urine	[16], [18]	Early and differential diagnostics Disease dynamics
	Platelet-activating factor ↑	vein blood	[31]	Early diagnostics Disease dynamics
	Claudin-2 ↑	urine	[15]	Differential diagnostics Disease dynamics
	Claudin-3 ↑	urine	[30]	Differential diagnostics Disease dynamics
	Faecal calprotectin ↑	stool	[30], [38],	Early and differential diagnostics
	Trefoil Factor 3 ↑	vein blood urine	[16], [18]	Differential diagnostics Disease dynamics
	Smooth muscle alpha actin ↑	vein blood	[43]	Disease dynamics
	Serum amyloid A ↑	urine	[18]	Disease dynamics
	D-lactate ↑	urine vein blood	[40]	Disease dynamics Early diagnostics
	β-glucosidase	vein blood	[14]	Early diagnosis

development of immature enzymatic antioxidant systems of the body, forming the inability to resist the effects of prooxidant factors arising in premature in response to rapidly increasing aerobic metabolism and high energy demand or against the background of hypoxic-ischemic conditions. Further disruption of the balance of antioxidant/oxidant systems will lead to oxidative damage to the intestinal cells and an increased probability of the development of NEC [4]. This was shown by Perrone S. et al. and Gurel S. et al. on samples of umbilical cord blood, in which markers of oxidative damage of molecules and non-enzymatic antioxidants prevailed in children who subsequently developed NEC [23, 27].

Precise changes in the profile of the acylcarnitines [12, 22] and the phospholipids [11] immediately after birth were identified for premature newborns developing NEC. Such differences are understandable in terms of immaturity and late functional inclusion in the work of some and predominance in the fetus of the activity of other enzymatic systems, which in turn indicates specific features of metabolic pathway maturation of children at risk for NEC, which, if exposed to other pathogenetic factors, will contribute to intestinal wall lesions.

In the above studies, it has been shown that there is a significant difference in the blood metabolite concentrations of some metabolites at birth between children who develop or do not develop NECs, respectively. The differences are probably due to the degree of the immaturity of the enzymatic systems critical to the functioning of the digestive tract and their rate of development, which could, if studied more closely, use specific patterns of these exchange rates as early markers – predictors of the NEC. But it is unlikely that these characteristics can be used effectively for early or differential diagnosis of the disease, where other links of pathogenesis will come to the fore.

Studies aimed at the marker potential of specific intestinal wall proteins, including claudins and occludes, which have had a significant impact in recent years, are also not of interest in prediction, even though model animals showed low expression of these proteins in premature experimental animals [28]. Unfortunately, in clinical practice, their detection is possible only in case of intestinal wall damage, and high concentrations were found only on the day of diagnosis, mainly in children who developed stage III NEC [15, 29, 30]. Early identification of premature newborns with a risk of NECs using biomarkers is particularly promising, as it would allow risk assessment and timely application of preventive measures (feeding protocols, introduction of breast milk, probiotics). The detection of biomarkers with high potential diagnostic value can lead to the creation of a reliable multi-biomarker panel for early prediction of NECs. Unfortunately, such markers are not currently available.

Hypoxia / Ischemia

Hypoxia/ischemia comes to the forefront of pathogenesis in late premature or full-term newborns, where NEC usually develops in the first week of life due to hemodynamic disorders. At the same time, despite allocating a different phenotype NEC-associated with ischemia [3], the development of «classical» NEC hypoxia and reduction of intestinal wall perfusion also play a leading role, without which the development of the disease would seem unlikely.

As in the case of immaturity, it is possible to distinguish two main directions of search of biomarkers: system hypoxia-related markers and intestinal ischemia-related markers. At the same time, the former, most often with an integrative assessment with other indicators, can be early predictors of the development of NEC long before the first clinical manifestations. And the latter, although more likely and specific, identify the presence of NECs, but the time frame for this definition is shifted towards the height of the disease and, Probably, such markers can be used mainly in differential diagnostics of NEC and prediction of the severity of the disease, the development of surgical stages.

Systemic hypoxia-related markers studied in children with NEC include L-lactate, ischemia-modified albumin (IMA), and other indicators of oxidative stress (non protein bound iron (NPBI), lipid peroxidation markers (LP) and oxidative modification of proteins (OMP)), endothelin-1.

Mammalian plasma L-lactate, the final product of anaerobic glycolysis, is a non-specific marker of acute cellular hypoxia. An increase in intraperitoneal L-lactate will be a fact of intestinal ischemia; however, hyperlactatemia will not be detected in blood at local intestine ischemia due to a compensatory rise in lactate absorption to the liver. An increase in L-lactate in the cord blood of premature newborns will indicate a pronounced hypoxic state, developed by perinatal hypoxia, predisposed to the development of NEC [31]. An increase in the level of lactate in the venous blood, if diagnosed with NECs, may develop predominantly in a severe general condition of the child and will not indicate a specific lesion of the intestine [13].

Plasma NPBI indicates a form of iron free of high affinity with the transferrin. Free iron can be released in large quantities from hemoglobin when red blood cells undergo oxidative stress. Premature newborns are very susceptible to oxidative stress caused by free iron [32]. Increases in NPBI and PL in the umbilical cord blood of newborns in a study by Perrone S. et al. indicate hypoxic conditions in premature, more pronounced in children with increased risk of necrotizing enterocolitis [23].

IMA is a modified serum albumin with a reduced ability to bind to metals such as cobalt, nickel, and copper in the N-terminal amino-acid region. Ischemia, hypoxia, free radical damage, and acidosis can change the ability of circulating serum albumin to bind metals. IMA can occur in several potentially life-threatening states that can cause both local and generalized hypoxic conditions [33]. Yakut I. et al. studied the relationship between levels of IMA, IL-6, and C-reactive protein (CRP) in blood at NEC. Serum IMA, CRP, and IL-6 levels determined on the first, third, and seventh days of diagnosis in the NEC group were significantly higher than in the control group. IMA levels increased in the following days in newborns with stage II and stage III NEC, and these increases were higher, especially in children with stage III NEC [34]. Meneza S. et al. also showed a positive correlation between serum IMA level and healing time [35].

Oxidative stress (OS) indicators in the context of the definition of the hypoxic lesion are widely investigated for various conditions and premature diseases (bronchopulmonary dysplasia, retinopathy of prematurity, intraventricular hemorrhage, periventricular leukomalacia) but poorly understood for necrotizing colitis prediction. In our view, it is possible to study other metabolites of proteins, lipids, and nucleic acids to identify the most prognostically effective OS parameters specific to NEC or to use basic OS parameters combined with specific and non-specific markers, reflecting other links of pathogenesis.

The delicate balance between vasodilatory and vasodilatory factors regulates intestinal microcirculation. NO is the primary vasodilator, and endothelin-1 (ET-1) is the main vasoconstrictor. Any condition that disturbs this balance can cause ischemia and bowel damage. Onay et al. has been shown to increase endothelin-1 levels in the umbilical cord blood of premature newborns who developed early NEC [31], in addition to which most re-

searchers of the amino acid profile of the blood plasma indicated a marked decrease in arginine levels, substrate for NO synthesis in children with NEC, compared to other premature newborns [24, 25]. In addition, elevated levels of reactive oxygen or nitrogen species (ROS or RNS) can react with already synthesized NO, turning it into peroxynitrite and playing a vasoconstrictor role.

The specific D-lactate ischemia-related markers, I-FABP, described below in the section on inflammatory markers, often intersect or are simultaneously markers of inflammation and damage to the intestinal wall, which also reduces their diagnostic value for early detection of the disease but increases the probability of prediction of heavy current and allows differentiation of NEC with other septicemic states.

In summary, it can be concluded that an isolated definition of any hypoxia markers for predicting NEC, especially in cord blood and the first days of life, is not advisable due to the absence of pronounced perinatal hypoxic-ischemic lesions in the pathogenesis of NEC. Better use of a combination of markers covering different pathogenesis links.

Inflammation/intestinal injury

CRP and procalcitonin commonly used in clinical practice, and non-specific biomarkers are essential mediators for any inflammatory cascade. They cannot differentiate NECs from other inflammatory diseases. They should be used together with the general (intestinal fatty acid binding protein (I-FABP), hepatic fatty acid binding protein (L-FABP), trefoil factor-3 (TFF3)) with low molecular mass, which when intestinal damage enters the systemic bloodstream where and can be easily detected. Onay et al. noted an increase in I-FABP in venous blood in the first days of life in children with hemodynamic disorders at birth, who developed NEC in the first week of life (NEC-associated with ischemia), but not in children with late NEC, for which the increase began only on admission to the intensive care unit and corresponded to clinical manifestation of the disease [31]. Briana D. D. et al. showed no rise in I-FABP in the umbilical cord blood of premature children who subsequently developed NEC [36]. There is no evidence of severe bowel damage in children with NEC during the perinatal period and in the first days of life, which minimizes the effectiveness of using these markers in the early years of the child's life, with a predictive purpose. This aligns with the current understanding of NEC pathogenesis (Fig.). The diagnostic value of their use increases dramatically with the onset of clinical symptoms and disease dynamics. In numerous studies [37–39], it has been shown that the concentration of markers in the blood and urine increased sharply on the first day of the onset and remained stable high throughout the disease period only in children with severe surgical stages. In contrast, in children with stages I–II NEC, it gradually decreased within a few days after the demonstration.

For a significant increase in I-FABP concentration in the blood, an apparent bowel injury is required, which is likely to result in a perforation of the intestine in children who maintain this level for a long time. Ng E. W. et al., Coufal S. et al., Thuijls G. et al. noted an increase in I-FABP, L-FABP, and TFF3 biomarker concentrations in blood plasma and urine at stages II–III NEC, but not at sepsis, except for the combination of these markers in urine, and in the blood better predicted the outcome of the disease, the duration of hospitalization [16, 18, 30].

Claudines are dense contact proteins with intercellular adhesion complexes found on the apical part of the intestinal epithelial cells. In NEC research, Claudine is mainly studied as a marker reflecting intestinal integrity [28]. In a newborn study, reduced expression of Claudine-2 in the intestine epithelium and increased levels of Claudine-2 [15] and Claudine-3 [30] in urine were found to be correlated with NEC severity and independent of other conditions, including sepsis. Premature newborns with NEC III showed reduced expression of tight contact proteins in bowel preparations taken during surgery [40]. In larger-scale clinical trials, it is possible to consider Claudine's in urine as non-invasive markers for differential diagnosis of NECs and prediction of their severity.

D-Lactate is a byproduct of bacterial activity, so elevated levels indicate bacterial translocation. Intestinal ischemia and bacterial colonization increase intestinal wall permeability, allowing D-lactate to enter the portal circulation with increased plasma lactate levels in both portal and peripheral venous blood. Due to metabolism, D-lactate may be present in human blood, but usually in nanomolar concentrations. Lei G.et al. showed the effectiveness of using D-lactate for early diagnosis of NEC as a marker of inflammatory lesions of the intestine [41].

Moreno J. L. et al. and Benkoe T. et al. have been shown to increase the tissue-specific enzyme β-glucosidase activity in blood plasma in children with diagnosed NEC [14, 42]. Cytosolic β-glucosidase belongs to the widespread hydrolase family. In lower mammals, the enzyme plays a detoxification role. Its function in humans is unknown.

A more well-known non-invasive marker widely used in clinical practice for diagnosing inflammatory bowel diseases is fecal calprotectin, also proposed for differential diagnosis of NEC [43]. Thuijls G. et al. and Arisanti D. et al. showed a high diagnostic accuracy of fecal calprotectin in detecting newborns with NEC [30, 39] on par with specific intestinal wall proteins found in blood or urine. Still, the impossibility of obtaining biomaterial from most newborns suspected of NEC reduces the potential effectiveness of using this marker in routine practice.

Smooth muscle alpha-actin (SMA), a smooth muscle component released into the bloodstream by severe intestinal wall breakdown, can potentially be used as a specific marker of severe bowel damage to identify surgical stages of NEC [43, 44].

Some amino acids, the synthesis of which is premature individuals is limited primarily to intestinal tissues, may serve as an early indicator of intestinal distress in children with suspected NEC. So it was shown that a few days before the development of NEC in children, there was a decrease in levels of citrulline [19, 45–48] and arginine [24, 25] in the blood, which continued until recovery. At the same time, the increase in the levels of these amino acids correlated with the positive clinical dynamics of recovery of the overall and local status of the child.

As additional non-specific markers of inflammation at any stage of NEC, various indices of oxidative damage of molecules mediated by the immune response can be used because prolonged intestinal ischemia leads to an inflammatory state by activating inflammatory cytokines and neutrophils. Activated neutrophils migrate to the inflamed area of the intestine, causing tissue damage due to myeloperoxidase producing ROS and causing a respiratory explosion. Excessive prooxidant action, lack of antioxidant systems, and increased enteral load can lead to damage of lipids, proteins, enterocytes DNA, and activation of apoptotic pathways. These indicators are universal and, when used with more specific markers, can solve problems such as a prognosis of the development of the disease, its dynamics, and the rate of recovery of intestinal tissue [23, 37].

Galectin-3 is an organonespecific protein, a mediator of inflammation and fibrosis. Children diagnosed with NEC showed increased blood levels compared to the control group [49, 50].

Platelet-activating factor (PAF) is an endogenous phospholipid with a powerful inflammatory effect. It is synthe-

sized by many cells, including neutrophils and gut parenchyma, and can be found in most tissues and bodily fluids. PAF has a very short half-life. PAF initiates an intense inflammatory reaction in the epithelium of the intestine, leading to apoptosis, necrosis, and clinical and pathological signs of NEC. Enteral feeding in prematurity can stimulate PAF synthesis, provided bacteria and bacterial endotoxins are present in the intestine. Animal models suggest blocking PAF effects reduces the risk of developing NECs [51]. Attempts have been made to use PAF not only as a target for therapeutic action but also as a marker for NEC [31].

Conclusion

The data in the survey indicate that the diagnosis of NEC should be based on the clinician's objective, meet the time criteria for disease development, and take into account the common early and late pathogenesis factors and risks, individual for each child.

At this stage of development of ideas about biochemical diagnostic markers, NEC scientifically based and clinically proven data are available for methods of early and differential diagnosis of NEC, reflecting the fact of development of damage and inflammation of the intestinal wall. There is a problem of validation of methods of their determination and full implementation in clinical practice, perhaps, first for the most complex and heavy cohorts of patients.

NEC prediction markers, which should be used as a prospective screening of premature newborns, are at the stage of finding unique metabolites, enzymes, etc., forming immaturity and involved in developing hypoxia/ischemia. All the markers in the review require further in-depth study, both in model animals and in multi-center clinical trials.

From this point of view, it seems promising to develop methods of prediction of NEC involving the use of integral indicators, including a set of parameters, taking into account the main early and late pathogenetic links of NEC, with the possibility of constructing a mathematical model and allocating a leading risk factor, followed by a targeted action directly on this link to prevent the development of the disease, reduce the risk of surgical stages of NEC, reduce its lethality. Based on this system approach, it is possible to correctly and timely interpret and use existing and new diagnostic markers.

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