The purpose of this work is to clarify the latest opinions of clinicians about the pathogenesis, diagnosis and treatment of necrotizing enterocolitis, as well as the role of microflora in the pathogenesis of this pathology. Search Strategy: A literature search was conducted in the following electronic databases: PubMed, Web of Science (Thomson Reuters), Scopus, Cochrane Library, SpringerLink, and EBSCOhost ScienceDirect. The search spanned 20 years, from 2000 to 2020. In this review, we included 39 articles on this topic, which were available in full text and analyzed through critical evaluation.

Results. According to the literature, necrotizing enterocolitis is primarily attributed to prematurity, compounded by the disruption of intestinal microflora composition due to artificial feeding, leading to an inflammatory reaction in the immature intestine. Delayed detection of the initial signs of necrotizing enterocolitis exacerbates treatment outcomes. In clinical practice, two main dilemmas in necrotizing enterocolitis diagnosis arise. Firstly, distinguishing early necrotizing enterocolitis from sepsis can be challenging, resulting in delayed diagnosis and treatment. Secondly, the optimal timing for surgical intervention remains unclear. Conclusion. There is a need to elucidate the latest opinions of clinicians regarding the pathogenesis, diagnosis, and treatment of necrotizing enterocolitis, including the role of microflora in its pathogenesis. Treatment strategies should integrate various models of clinical prognosis and biomarkers to accurately diagnose and tailor therapy for necrotizing enterocolitis.

Key words: premature infants, necrotizing enterocolitis, biomarkers, intestinal microflora.
child's life. From this age, anaerobic bacteria begin to colonize the baby's intestines, including pathogens that cause inflammatory processes. Bacteria played a crucial role in the pathogenesis of NEC [7]. Therefore, the effectiveness of antibiotic therapy in the treatment and reduction of the frequency of NEC is associated with the eradication of pathogenic microbes in the intestine [8].

Dysbiosis, defined as a violation of the precarious balance between beneficial protective species and harmful intestinal bacteria, has already been described as a provocative factor in the pathogenesis of NEC. More recent studies have shown that the main Clostridium species such as Clostridium perfringens, C. butyricum and C. neonatale play an important role in the etiology of NEC [9].

In 2004, de la Cochetiere et al. [10] reported a link between early colonization of the intestine of C. perfringens and the development of NEC. However, this supposed role of C. perfringens has not been confirmed in later studies [11].

The predominance of proteobacteria was observed in fecal samples of patients with NEC 2 weeks before the clinical development of NEC, which suggests that dysbiosis precedes the onset of NEC [12].

Reports on the overall diversity and abundance of enterococcal populations are contradictory, some studies describe a decrease in diversity and a decrease in the number of enterococci in some patients with NEC; however, these data have not been confirmed in other studies [13].

So far, no pathogen has been identified as a specific cause of NEC.

Diagnostics

NEC usually manifests itself in the second week of life, when enteral feeding is introduced (especially a mixture based on cow's milk). The age of NEC manifestation is inversely proportional to gestational age; in extremely premature infants, NEC may develop at a later date [14].

Clinically, NEC can be manifested by both gastrointestinal and non-specific systemic signs, such as delayed gastric emptying, bloating or soreness (or both), bloody stools, lethargy, apnea, respiratory distress or poor perfusion. The progression of NEC usually leads to tenderness and erythema of the abdominal wall, decreased peristalsis, and swollen intestinal loops are often palpated. Abdominal wall erythema is one of the main predictors of NEC, but is detected in no more than 10% of affected patients. Cyanosis or discoloration of the skin of the abdomen may be a sign of intestinal perforation [15].

Due to the fact that the early clinical signs of NEC usually do not have specificity, it is difficult to distinguish it from sepsis. The course of NEC also varies greatly, non-specific signs can progress slowly over several days, but within just a few hours NEC can also manifest itself with the lightning appearance of gastrointestinal symptoms and shock with multiple organ failure Laboratory data in patients with NEC may include an increased or decreased number of leukocytes (with a shift of neutrophils to the left), thrombocytopenia (rapid a drop in platelet count is a poor prognostic sign), metabolic acidosis, hypoglycemia or hyperglycemia, and electrolyte imbalance, but all of these have low specificity [16]. Infection of the bloodstream, mainly by gram-negative bacteria, is present in 43% of cases of NEC, which proves a complete violation of the barrier of the intestinal mucosa.

Non-specific radiological signs of NEC include thickened intestinal walls, enlarged and fixed intestinal loops, and an insignificant amount of gas volume in the intestinal lumen. The expansion and fixation of the intestinal loop may indicate necrosis of the intestinal wall [17].

Pneumatosis in NEC is usually detected in the lower right quadrant of the abdomen. The extent of pneumatosis is not always associated with the severity of NEC, and its disappearance does not necessarily mean clinical improvement [18].

When the present gas is absorbed into through the mesenteric vessels, on a simple X-ray it can be recognized as a linear shadow in the portal vein. Ultrasound can also detect gas in the portal vein [19]. Gas in the portal vein is associated with the severity of the disease, but its presence does not always correlate with mortality or the need for surgical intervention [20].

The most important radiological sign of NEC is the presence of pneumoperitoneum caused by perforation of the intestinal wall.

Ultrasound examination of the abdominal cavity can be useful in the process of NEC diagnosis. It can detect pneumatosis, portal vein gas, pneumoperitoneum and decreased intestinal perfusion. Moreover, ultrasound can rule out other gastrointestinal abnormalities, such as intestinal malformation and intestinal inversion, the clinical symptoms of which may resemble NEC. However, the results of an ultrasound examination strongly depend on the doctor who conducts it, which in practice limits the use of his data in interpretation. Other imaging studies, such as contrast studies, computed tomography and magnetic resonance imaging, currently have no additional value in the diagnosis and monitoring of NEC [21]. With simple radiography, premature infants are less likely to develop intestinal pneumatosis and gases in the portal vein, but they have an increased risk of developing pneumoperitoneum [22].

The modified Bells criteria of Walsh and Kligman are used to build an assessment system that includes clinical symptoms, laboratory parameters and radiological data. This can be useful for deter-
mining the severity of the disease and choosing appropriate treatment in daily clinical practice [23].

**Treatment**

The therapeutic tactics of newborns with NEC includes two methods: conservative and surgical. **Conservative treatment**. The basis of NEC treatment is medical stabilization and preservation of general homeostasis. This regimen usually includes abdominal decompression, intestinal rest, parenteral nutrition with sufficient protein intake and intravenous administration of antibiotics. Decompression of the abdomen can be achieved using a two-light gastric tube with continuous or periodic suction. Significant volumes of aspiration should be filled [24].

Antibiotic therapy usually consists of ampicillin (or cephalosporin) in combination with aminoglycosides. In the case of peritonitis or intestinal perforation, the addition of metronidazole or clindamycin is justified to protect against anaerobic bacteria. Antibiotic treatment and intestinal rest should be continued for 5-7 days in the case of stage II NEC and 10 days in the case of stage III NEC.

Further supportive treatment consists of intubation for respiratory failure, inotropic support according to indications, adequate liquid infusion and correction of anemia, thrombocytopenia and electrolyte imbalance. Since NEC causes pain, adequate anesthesia of the infant is an important aspect of conservative treatment [25]. With conservative NEC therapy, it is necessary to periodically re-examine the newborn in order to timely identify clinical deterioration and the presence of pneumoperitoneum, which requires surgical intervention. It should be borne in mind that the appearance of soreness and palpation "neoplasm in the abdominal cavity" indicate the development of a severe stage of NEC. Therefore, in order to timely identify complications requiring surgical placement, it is necessary to conduct serial radiographs of the abdominal cavity every 6-24 hours and in case of clinical deterioration or enlargement of the abdominal cavity; periodically determining the level of C-reactive protein (CRP) can also be useful for solving treatment tactics [26].

**Surgical treatment**. Approximately 20% to 40% of newborns with NEC eventually need surgery. However, many aspects of surgical intervention are still debatable. Setting indications and choosing the time of surgical intervention cause difficulties. Intestinal perforation and intestinal necrosis are absolute indications for urgent surgery. On radiographs, intestinal perforation can be diagnosed when pneumoperitoneum is detected. Intestinal necrosis can be thought of in the absence of improvement against the background of drug treatment with persistent metabolic acidosis and thrombocytopenia. On the other hand, palpable fixed formation in the abdominal cavity and erythema of the abdominal wall are highly specific signs of intestinal necrosis. The presence of gas in the portal vein (if it is the only sign present) does not provide a definitive basis for surgical intervention. In extremely premature infants, in 20% of cases, it is not possible to detect pneumoperitoneum and changes indicating necrosis of the intestinal wall [27].

Currently, there are 2 methods of surgical treatment of NEC: primary peritoneal drainage (PPD) and laparotomy. Two recent randomized controlled trials compared the results of PPD and laparotomy. A meta-analysis of these two studies showed that there were no significant differences between the two groups in terms of mortality and the total duration of parental nutrition [28].

The classic surgical approach during laparotomy is to remove all areas of the necrotic intestine and extract it [29]. However, stomas, especially skinny stomas, are poorly tolerated by premature infants, as they predispose to nutritional and metabolic disorders, as well as poor growth as a result of impaired water-electrolyte balance. Therefore, some surgeons recommend primary anastomosis [30].

**Discussion**

**Ways to improve the diagnosis and treatment of NEC: prognostic models and biomarkers.** In everyday clinical practice, there are 2 main dilemmas in the diagnosis of NEC. Firstly, the differentiation of early NEC and sepsis can be difficult or even impossible, which can lead to a delay in adequate diagnosis and treatment. Secondly, the optimal timing of surgical intervention is unclear.

In their research, various authors have tried to determine which (early) parameters are predictive for severe NEC. Christensen et al. [31] identified bloody stools (32%), increased abdominal volume (66%) and increased gastric residues before feeding or vomiting (48%) as the most frequent specific precursors of severe NEC (grade III). In another study of the same group, an increase in CRP, immature neutrophils to the total number and average volume of platelets, a low pH value, previous blood transfusion and the first feeding, which is not colostrum, were identified as factors associated with the severity of NEC [32].

Moss et al found that 12 factors are associated with progressive NEC. However, it was not possible to develop a proper clinical model to predict the progression of NEC.

To optimize the time of surgical intervention, Tepas et al. developed a score system that includes 7 points: metabolic acidosis (pH <7.25), severe thrombocytopenia (<50 × 10^9 / l), hypotension requiring volume increase or drug therapy, hypotension <130 mmol/L, neutropenia, neutrophil shift to the left (ratio L / T <0.2) and positive blood culture. In a retrospective study, a medical center with surgical cases...
intervention based on a score of ≥3 was compared with a control center that did not use this system. Using this point system showed the best results. However, this scoring system should always be used in conjunction with clinical assessment and serial radiography.

Clinicians typically used clinical and laboratory parameters during diagnosis to develop a data-based algorithm that can automatically classify the NEC stage by Bell and predict the risk of NEC progression to the need for surgery.

Although the low- and high-risk groups of disease progression could be identified with a high degree of confidence, researchers still had problems predicting the intermediate-risk group.

It has been suggested that new biomarkers may help in predicting the prognosis of NEC. Indeed, in a previously published study, a similar algorithm placed 40% of infants with NEC in an intermediate risk group in which it is impossible to accurately predict the progression of the disease. The introduction of peptide biomarkers in urine dramatically improved the algorithm's ability to predict disease progression [33].

The results of X-ray examination of the abdominal cavity in NEC. Recently, a 10-point radiographic scale for assessing the severity of NEC (Dukes scale) has been developed, which is used in practice. Zero points mean "normal gas pattern", and 5 points mean "fixed or persistent loops of the enlarged intestine". A score of 10 is indicative of the "pneumoperitoneum". A recent assessment of this indicator showed that an increase in the Duke score is associated with an increase in the severity of the disease and necessitating surgical intervention [34].

The role of biomarkers in the diagnosis of the clinical course of NEC. Early diagnosis of NEC and especially differentiation from neonatal sepsis is a serious problem. Despite extensive research, there are currently no reliable (early) biomarkers. The biomarker must acquire such properties so that it can be accepted as specifically significant for the detection of NEC. At the same time, this biomarker should not respond to sepsis and at the beginning of NEC should be in sufficient concentration in blood, urine or stool. The magnitude of its increase should be proportional to the severity of the damage to the intestinal tissue.

Biomarkers can be divided into different categories. Non-specific biomarkers, which are mainly biomarkers of inflammation and specific biomarkers, are biomarkers of damage to the mucous membrane of the gastrointestinal tract or NEC.

Non-specific biomarkers. The most commonly used non-specific biomarker is C-reactive protein (CRP). CRP levels may increase 12-24 hours after the onset of inflammation, specificity is low, and it is impossible to use NEC in differentiation with sepsis. Another non-specific biomarker is serum amyloid A (SAA). The levels of SAA in an infant with NEC in the blood serum can increase up to 1000 times within 8-24 hours after the onset of inflammation. It can be used to diagnose and follow-up NEC with the same accuracy as CRP, since there is also a small correlation with the height of the SAA and the severity of the NEC. Other non-specific biomarkers are procalcitonin [35, 36]. All of these biomarkers have been studied in small quantities. research, and their role in the diagnosis of NEC has yet to be confirmed by larger multicenter studies. In addition to proteins, cytokines such as interleukin-6 and interleukin-8 are considered early biomarkers for the diagnosis of neonatal sepsis and NEC [37]. Consequently, both pro-inflammatory and anti-inflammatory cytokines are elevated in the context of sepsis and NEC. The combination of anti-inflammatory cytokines in the ratio of pro-inflammatory and anti-inflammatory cytokines can reflect the severity of the disease and accurately predict the complications of these diseases [38].

Specific biomarkers. With NEC, the permeability of the intestinal mucosa increases; consequently, proteins are secreted into the fecal stream [39]. One of the secreted proteins present in stool is calprotectin, which binds calcium and zinc. It consists of 60% soluble cytosol proteins of human neutrophil granulocytes. It is resistant to bacterial decomposition and has been proposed as a useful biomarker in adults and children with inflammatory bowel diseases. In the studies on NEC, many calprotectin values went beyond the normal reference values for adults. However, there are also large differences in calprotectin levels in healthy newborns (1.0–625.1 mcg/g), as well as for newborns with NEC (107.6–7847.6 mcg/g). In addition, differences in their indicators were found depending on gestational and postnatal age. Wide differences and overlap between healthy newborns and NEC, as well as differences between studies, limit the use of this biomarker in daily practice.

Conclusions and prospects for further investigation. The diagnosis and progression of necrotizing enterocolitis (NEC) remain largely unpredictable with current clinical, biological, and radiological markers. Existing diagnostic tools rely on signs of established or progressive disease. Early diagnosis is critical for treatment and outcomes, underscoring the need for more accurate predictive biomarkers of NEC. Further research should focus on understanding the biological and microbiotic processes that drive NEC development. There is a compelling rationale for investigating biomarkers based on altered microbiotic profiles in NEC. Developing a prognostic model incorporating various markers could enhance diagnostic capabilities, guide customized probiotic-based feeding strategies, and inform therapeutic interventions. Given
the persistently high mortality and morbidity rates associated with NEC, it is imperative to continue the challenging quest for optimal diagnostic and therapeutic strategies.

References


Актуальні проблеми сучасної медицини

Реферат

КЛІНІЧНИЙ ОГЛЯД ДІАГНОСТИЧНИХ БІОМАРКЕРІВ ТА РОЛІ КИШКОВОЇ МІКРОБІОТИ У НЕДОНОШЕНИХ НЕМОВЛЯТ З НЕКРОТИЧНИМ ЕНТЕРОКОЛІТОМ

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Ключові слова: недоношені діти, некротичний ентероколіт, біомаркери, кишкова мікрофлора.

Метою даної роботи було дослідження останніх думок і поглядів клініцистів про патогенез, діагностику та лікування некротичного ентероколіту, а також роль мікрофлори в патогенезі даної патології.


Результати. За даними літератури причиною некротичного ентероколіту здебільшого є недоношеність; при цьому через незавершення повного розвитку кишківника штучне годування сприяє порушенню складу мікрофлори, що викликає запальну реакцію в стінці незрілого кишківника. Упущення своєчасного виявлення первинного прояви некротичного процесу в стінці кишківника призводить до різкого погіршення результатів лікування некротичного ентероколіту. У повсякденній клінічній практиці при діагностиці некротичного ентероколіту зустрічаються дві основні дилеми. По-перше, диференціація раннього некротичного ентероколіту та сепсису може бути важкою або навіть неможливою, що може призвести до затримки адекватного діагнозу та лікування. По-друге, незрозумілі оптимальні терміни хірургічного втручання. Зазвичай клініцисти використовують клінічні та лабораторні параметри під час постановки діагнозу для розробки алгоритму на основі даних, які автоматично визначають стадію некротичного ентероколіту за Bell, і прогнозують ризик прогресування хвороби до необхідності хірургічного втручання.

Висновки. Є необхідність уточнювати останні думки клініцистів про патогенез, діагностику і лікування некротичного ентероколіту, а також роль мікрофлори в патогенезі цієї патології. При лікуванні некротичного ентероколіту необхідно узагальнювати різні моделі клінічного прогнозування та біомаркерів в етіопатогенезі цієї хвороби, які могли бути предикторами точної діагностики та вибору відповідної терапії.