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THE INTRODUCTION AND RESULTS OF MULTIMODAL 3-COMPONENT PROPHYLAXIS SCHEME BOTH IN PREVENTION AND TREATMENT OF NECROTIZING ENTEROCOLITIS IN NEWBORNS

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Abstract

The retrospective study was performed to assess the effectiveness of multimodal 3-component necrotizing enterocolitis prophylaxis scheme used among patients with necrotizing enterocolitis during 01.12.2016-30.11.2017 (period B) compared to the results of 01.12.2015-30.11.2016 (period A) without necrotizing enterocolitis prophylaxis. The investigation included 71 newborns with necrotizing enterocolitis. According to the hospital records, 45 newborn infants were exposed to multimodal 3-component necrotizing enterocolitis prophylaxis scheme and 26 were not. The descriptive study was performed to evaluate the dynamics of necrotizing enterocolitis mortality and stages over 2016-2017 among the newborns admitted to "Muratsan" university hospital of Yerevan State Medical University after M. Heratsi.

In total, 1429 medical records, registered during periods A and B were investigated. Necrotizing enterocolitis rate was increasing from 2011 to 2017. Significant difference ($p<0.01$) was found between the multimodal necrotizing enterocolitis prophylaxis exposure and the number of necrotizing enterocolitis perforation cases (12 cases out of 70 in period A and 0 cases out of 45 in period B). During period B, after introduction of multimodal 3-component necrotizing enterocolitis prophylaxis to 45 newborns, no cases of surgical operations have been registered as compared with 14 cases among the 70 newborns not exposed to necrotizing enterocolitis prophylaxis scheme ($p<0.01$) during period A. Significant association was found in number of death cases ($p<0.01$) among 45 newborns during period B (5 cases without necrotizing enterocolitis) and non-exposed 70 newborns during period A (24 cases).

An introduction of multimodal 3-component necrotizing enterocolitis prophylaxis scheme among newborns with necrotizing enterocolitis is associated with significant decrease in the number of necrotizing enterocolitis cases at stages 3A, 3B and related deaths. Currently, the research continues to identify further positive effects of multimodal 3-component necrotizing enterocolitis prophylaxis to estimate the possible influence of necrotizing enterocolitis prophylaxis on condition of admitted newborns with different health problems.

Keywords: *Necrotizing enterocolitis, newborns, multi-modal scheme, necrotizing enterocolitis-prophylaxis, Gentamicin sulfate, Nystatin, LactoG.*

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Introduction

Necrotizing enterocolitis (NEC) i.e. an acquired multifactorial nonspecific inflammatory condition, is characterized by variable damage to the newborn intestinal tract, ranging from mucosal injury to full-thickness necrosis and perforation [*Springer C, 2016*].

The usual onset of this disease is between 7th and 14th day of life, although, the onset of NEC has also been documented several weeks after birth, particularly in very low birth weight newborns [*Mac Kendrick W et al.,1993; Kosloske A, 1994; Rayyis S et al.,1999*].

NEC occurs in one to three of 1,000 live births [*Kosloske A, 1984; Noerr B, 2003*], equally often in female and male [*Noerr B, 2003*]. NEC most commonly affects babies born between 30 and 32 weeks and is often diagnosed during the second week of life [*Kliegman R, Fanaroff A, 1981; Ladd A. et al., 1998*]. The mortality of NEC has been reported to make up 10-50% of all affected infants [*Henry M, 2004*]. The surgical mortality has decreased over the last several decades from 70% to 20 -50% [*Henry M, 2004*].

NEC does not occur in utero. NEC has a multifactorial etiology; its pathogenesis has not been fully elucidated and remains controversial [*Ballance W. et al., 1990; Epelman M. et al., 2007*]. The classic histological finding is coagulation necrosis present in over 90% of specimens [*Ballance W. et al., 1990*]. This finding suggests the importance of ischemia in the pathogenesis of NEC [*Henry M, 2004*]. Inflammation and bacterial overgrowth are also present [*Ballance W. et al., 1990*].

It is believed that NEC is secondary to a complex interaction of multiple factors, notably prematurity, that result in mucosal damage, leading to intestinal ischemia and necrosis [*Lee J et al. 2003; Nowicki P, 2005*]. The mucosal injury may be due to infection, intraluminal contents, immature immunity, release of vasoconstrictors and inflammatory mediators [*Caplan M. et al., 1994; Vieten D. et al., 2005*]. The loss of mucosal integrity allows the passage of bacteria and their toxins into the bowel wall and then into the systemic circulation, resulting in a generalized inflammatory response and overwhelming sepsis in the severe forms of NEC [*Vieten D. et al., 2005*].

The inflammatory process in NEC leads to increased blood flow in the affected bowel segment. Bacteria penetrate the mucosal defense, and their by-products of metabolism lead to the formation of intramural gas (Fig. 1a and 1b). As NEC progresses, platelet-activating factor produced by inflammatory cells and bacteria exacerbate the inflammatory cascade, mainly that of cytokines and complement, leading to extensive transmural involvement of NEC [*Gonzalez-Crussi F et al., 1983; Hsueh W et al., 1998*]. Eventually, ischemic changes to the tissue occur; nonperfused bowel wall undergoes necrosis, which may be so severe that sloughing of the bowel wall occurs, eventually resulting in bowel wall thinning and perforation (Fig.1c). NEC most commonly affects the terminal ileum, caecum and ascending colon.

There is an assumption that NEC occurs by the interaction of three events. Initially a mucosal injury occurs due to intestinal ischemia, followed by inflammation of the disturbed mucosal integrity with subsequent necrosis of the affected area. The further steps are colonization by pathogenic bacteria and excess protein substrate in the intestinal lumen. Furthermore, the immunologic immaturity of the neonatal gut has been implicated in the development of NEC [*Kosloske A. 1994*].

The clinical presentation of NEC is nonspecific, broad and includes variable symptoms which are often non-specific signs of gastrointestinal dysfunction [*Claud C. et al., 2009*].

Typical clinical signs include abdominal distension, bile- or blood-stained emesis or gastric aspirate, abdominal wall erythema and bloody stools. Diagnosis is based on radiographic evidence as bowel distension, ileus, pneumatosis intestinalis and/or bowel perforation [*Schmolzer G, et al., 2006*].

Bell Staging (Table 1) is still used as the standard practice to diagnose, to determine the stage and administer a treatment of NEC in the NICU (Table 1). For descriptive purposes and disease stratification, the Bell scoring system which assesses the degree of NEC severity as mild (Bell stage I), moderate (Bell stage II) or severe (Bell stage III) has been widely utilized [*Diego F. et al., 2016*].

There are several risk factors of NEC such as preterm birth, low birth weight, polycythemia, respiratory distress, congenital anomalies, bacterial colonization, hypoxia/altered intestinal blood flow, and formula feeding [*Claud E et al., 2001; Gephart M et al., 2012*]. The pathogenesis refers to the interaction of three aspects: intestinal ischemia, inflammation and necrosis [*Schmolzer G et al., 2006*].

Newborns with NEC have higher risk of death before discharge, significantly longer hospitalization period, and impose a significantly higher treat-

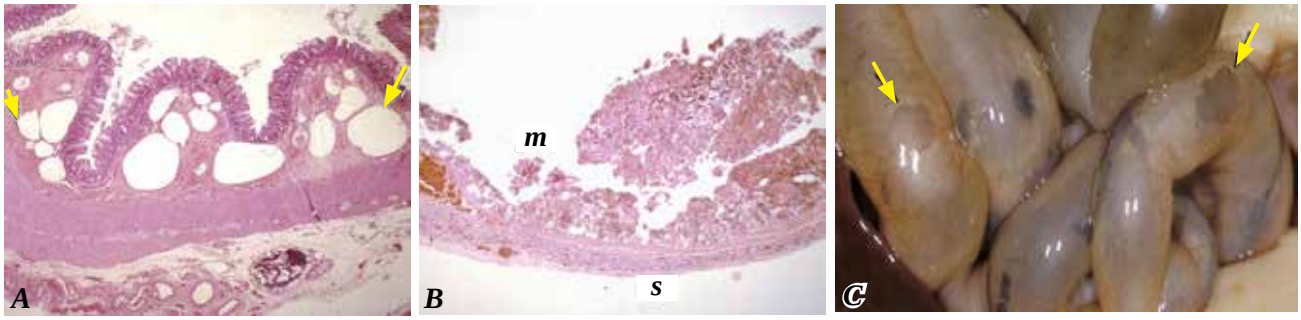


FIGURE *1. Pathologic findings in NEC (**A**) Histologic section of small bowel (original magnification, ×100; hematoxylin-eosin stain). Intramural gas is seen as rounded bubbles in the submucosa (arrows). There is hyperemia of the serosa. (**B**) Histologic section of small bowel (original magnification, ×100; hematoxylin-eosin stain). The bowel is affected much more severely than in a. There is necrosis of the mucosa, submucosa, and muscularis with intraluminal necrotic debris on the mucosal side of the bowel wall (m). Only the serosa appears intact. s = serosal surface of bowel wall. (**C**) Postmortem photograph of bowel involved with severe NEC. The arrows indicate areas of the bowel wall where there has been so much necrosis and sloughing of the mucosa, submucosa, and muscularis that only the serosa is intact. One can see through these areas of the serosa into the lumen of the bowel. [Necrotizing Enterocolitis: Review of State-of-the-Art Imaging Findings with Pathologic Correlation, Monica Epelman et al., 2007]*

ment costs as compared to newborns without NEC [*Holman R, et al., 2006; Stey A, et al., 2015*].

Large, population-based and hospital-based multicenter studies coordinated by neonatal research networks in Europe, North America, Australia and New Zealand have determined the incidence of NEC to be up to 13% among newborns born ≤33 weeks of gestation or whose birth weight is ≤2,500 g [*Horbar JD, et al., 2002; Sankaran K, et al., 2004; Luig M. et al., 2005; Guillet R, et al., 2006; Holman RC, et al., 2006; Kovacs L et al., 2007; Stoll BJ, et al., 2010; Yee WH, et al., 2012; Hossain S, et al., 2016*].

The mortality rate reported for preterm newborns classified as extremely low birthweight (<1000 grams) who are diagnosed with NEC is 35-50% [*Luig M. et al., 2005*]. Newborns classified as very low birthweight (<1500 grams) who are diagnosed

TABLE 1.

Bell Staging for necrotizing enterocolitis classification. [*Diego F. et al., 2016*].

Stage	Classification	Systeming signs	intestinal sisns	Radiologic signs
1A	Suspectid NEC	Temperature instability, apnea, bradycardia, lethargy	Increased pregavage residuals, mild abdominal distention, emesis, guaiac-positive stool	Normal or intestinal dilation, mild ileus
1B	Suspectid NEC	Same as above	Bright red blood from rectum	Same as above
2A	Proven NEC – mildly ill	Same as above	Same as above, plus absent bowel sounds, with or without abdominal tenderness	Intestinal dilation, ileus, pneumatosis intestinalis
2B	Proven NEC – mildly ill	Same as above, plus mild, metabolic acidosis, mild thrombocytopenia	Same as above, plus absent bowel sounds, definite abdominal tenderness, with or without abdominal cellulitis or right lower quadrant mass	Same as IIA, plus portal venous gas, with or without ascites
3A	Advanced NEC – severely ill, bowel intact	Same as IIB, plus hypotension, bradycardia, severe apnea, combined respiratory and metabolic acidosis, disseminated intravascular coagulation and neutropenia	Same as above, plus sings of generalized peritonitis, marked tenderness, and distention of abdomen	Same as IIB, plus definite ascites
3B	Advanced NEC – severely ill, bowel perforated	Same as IIIA	Same as IIIA	Same as IIB, plus pneumoperitoneum

with NEC have a mortality rate between 10 to 30%, and this mortality rate has not significantly decreased over the past 30 years [*Caplan MS et al., 2001; Carter B. et al., 2008*]. A large study of neonatal intensive care units (NICU) in Japan identified a 0.3% incidence of NEC, which is significantly lower than what is reported in similar patient populations in the United States. [*Kawase Y et al., 2006*]

Several studies have identified interventions that resulted in reductions in the incidence of NEC (such as breast milk feeding, enteral antibiotic prophylaxis, use of probiotics, and slow progression of enteral feeding), but additional approaches to NEC prevention are needed [*Updegrove K., 2004; Bin-Nun A et al., 2005; G. Schmolzer, B. Urlesberger et al., 2006; Yeo SL., 2006; Dharmesh S et al., 2012*].

There is no official statistical data available regarding the incidence of NEC in Armenia, but in our hospital, we annually deal with numerous of NEC cases and a high incidence of NEC- related mortality [*Harutyunyan A.S., 2017*].

History

In 2016 The American Austrian Foundation (www.aaf-online.org) sponsored and supported Arman S. Harutyunyan, one of the YSMU "Muratsan" university hospital doctors to undergo one-month observership program under the supervision of OA. Priv.-Doz. Emir Q. Haxhija at University Clinic of Graz, Austria (Universitätsklinik für Kinder- und Jugendchirurgie). Afterwards the program was implemented at "Muratsan" university hospital. During the observership theoretical and practical approaches to the NEC prevention scheme were presented. With the consent of both parties, it was decided to apply the NEC prophylaxis scheme both in prophylaxis and treatment of necrotizing enterocolitis in newborns in YSMU "Muratsan" university hospital NICU. All data transfer and long-term collaboration between professionals of University Clinic of Graz and medical staff of YSMU "Muratsan" university hospital was ensured by The American Austrian Foundation. In a struggle with NEC as a fatal disease we spent an observership period at the Department of Pediatric Surgery and Division of Neonatology of the Department of Pediatrics of the University Clinic in Graz, Austria, and experienced their NEC prevention protocol (multimodal 3-component scheme) which is used there, with minor changes, over the last 20 years resulting in a very low NEC incidence of 1% in children less than 1500 *gr*. This protocol consisted of oral Gentamicin, oral Nystatin and Probiotic [*Schmolzer G, Urlesberger B et al., 2006*]. No prospective randomized trials with this protocol have been performed due to ethical norms.

In 2016, the Austrian protocol was revised and probiotic L. rhamnosus was replaced with a locally available product: Synbiotic "LactoG". LactoG consists of prebiotic (fructooligosaccharide) and probiotics containing the following strains: Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium infantis and Lactobacillus acidophilus. We decided to use modified multimodal 3- component scheme not only as NEC prevention, but also as a component of complex treatment of NEC (implemented first time). After receiving an ethics approval to implement this protocol in newborns with diagnosed NEC and at risk for NEC development we decided to initially compare our results during the one-year period prior to the implementation of the new protocol with the one-year period after the implementation of the new NEC protocol. In addition, we analyzed the NEC incidence and outcome of these newborns for the period of 2016-2017 so that the reader of this article can see the tendency of this fatal disease in our Institution over the last years. Apart from introduction of "Graz's NEC multimodal prevention protocol" nothing else has been changed in the treatment of children with NEC as compared to the previous year.

Material and Methods

Ethical Approval: The study was approved by Ethics Committee of IRB (Study reference number 12/SC/0416) and Ethics Committee of YSMU (Study reference №8, 19.04.2018)

Study design and objective: The study was conducted in "Muratsan" university hospital of Yerevan State Medical University. "Muratsan" university hospital is the main and one of the biggest pediatric clinics in Armenia with specialized neonatal care division. Therefore, the majority of NEC cases in Armenia are admitted to "Muratsan" university hospital.

For the purpose of analysis of NEC incidence over the years we analyzed the data from the "Mu-

ratsan" university hospital medical records for the period 2016–2017. Hospital records included information regarding demographics, prescribed medications, laboratory results, procedures and diagnoses of newborns. Demographic data included gender, birth weight (BW), gestational age (GA) and Apgar score. The dynamics of the NEC cases among preterm newborns over the period 2012-2017 was also investigated.

In order to compare potential changes in incidence and outcome of newborns with NEC we collated the two one-year study periods 01.12.2015–30.11.2016 (**period A**) and 01.12.2016–30.11.2017 (**period B**). Overall 1429 medical records registered during periods A and B were investigated. During period A, 70 NEC cases were registered. Among 71 newborns who met the inclusion criteria to be treated as NEC cases during the period B 45 newborns were exposed to multimodal 3-component NEC prophylaxis scheme and 26 were not exposed because parents did not give their consent.

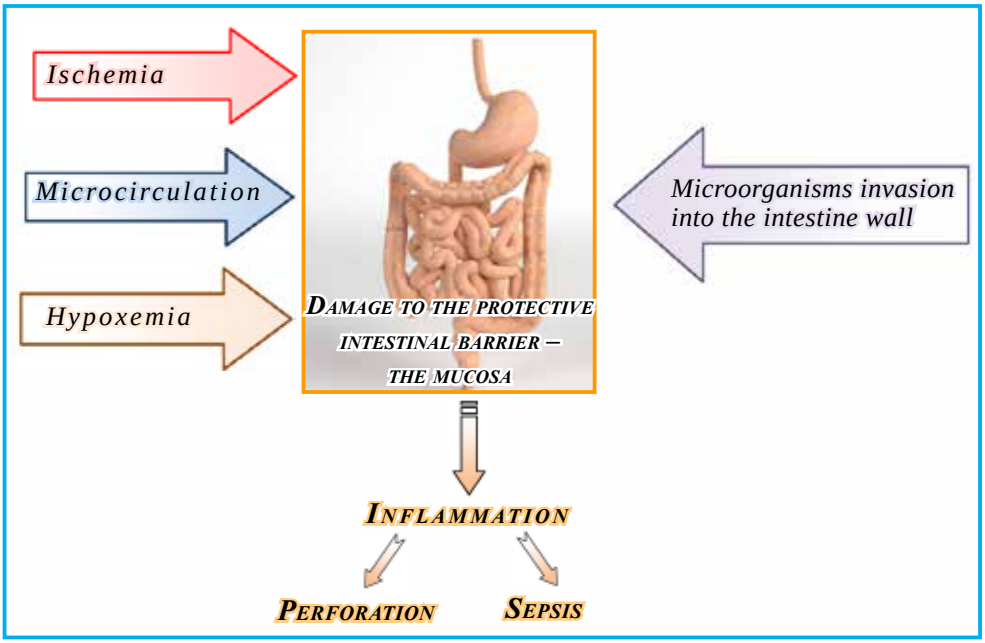
A retrospective analysis was performed to assess the effectiveness of enteral administration of a multimodal 3-component NEC prophylaxis scheme (oral Gentamicin sulfate 15 *mg/kg/day* – in 2 dozes), an antifungal agent (Nystatin 10000 *IU/kg/day* – in 4 dozes), and synbiotic (LactoG body weight < 2000 *gr* – 2 x ¼ *caps. pulveris*; > 2000 *gr* – 2 x ½ *caps. pulveris*) among patients with NEC and high-risk group newborns for NEC development. Inclusion criteria met the diagnose NEC and the following risk factors: low birth weight LBW (less than 2500 g), very low birth weight VLBW (less than 1500 g) and extremely low birth weight ELBW (less than 1000 g), gestation period less than 37 weeks and respiratory insufficiency with hypoxia at birth.

The retrospective study was performed to assess the differences in number of advanced NEC cases (3A and 3B stages) including bowel perforation related deaths and number of surgical interventions in 70 newborns with NEC registered during period A without multimodal 3-component NEC prophylaxis and 45 newborns with NEC exposed to a multimodal 3-component NEC prophylaxis scheme during period B.

The difference in the number of advanced NEC cases (3A and 3B stages) registered during period B between 26 NEC newborns who did not receive multimodal 3-component NEC prophylaxis and 45 NEC newborns who received multimodal 3-component NEC prophylaxis was also collated.

Statistical analysis: Incidence rates, confidence intervals and statistical significance of multimodal 3-component NEC prophylaxis results for NEC outcomes were defined. Bell Staging was used as the standard practice to diagnose, to determine the stage and administer a treatment of the NEC [*Bell MJ, et al., 1978*].

The multivariable linear regression model was



SCHEME: Possible NEC pathogenesis. Factors causing immune disorders and sepsis.

TABLE 2.

Comparative analysis of the clinically diagnosed necrotizing enterocolitis stages between periods A and B

Period A (01 12 2015 – 30 11. 2016) NEC – 70 newborns (9.96 % of 703 newborns)				***Period B*** (01 12 2016 – 30 11 2017) NEC – 71 newborns (9.78 % of 726 newborns)				The ratio of periods A and B
	No	70 NEC	of 703 all patients		No	71 NEC	of 726 all patients	
1 A	1	1.43 %	0.14 %	**1 A**	0	0	0	**1.4** ↓
1 B	2	2.86 %	0.29 %	**1 B**	13	18. 31%	1.79 %	**6.4** ↑
2 A	24	34.29 %	3.41 %	**2 A**	30	42. 25%	4.13 %	**1.2** ↑
2 B	12	17.14 %	1.71 %	**2 B**	23	32. 40%	3.17 %	**1.9** ↑
3 A	19	27.14 %	2.70 %	**3 A**	5	7. 04%	0.69 %	**3.9** ↓
3 B	12	17.14 %	1.71 %	**3 B**	0	0	0	**17** ↓

developed to assess the correlation between NEC 3B including bowel perforation and identified risk factors among the newborns who were exposed to a multimodal 3-component NEC prophylaxis.

The Fisher's exact test were performed to analyze the group differences and threshold probability, value of $p<0.05$ was used to indicate statistical significance.

Distribution of the weight and gestation met the World Health Organization classification [*WHO, 2004*.].

Statistical analised Microsoft Excel (Microsoft, Redmond WA, USA) and SPSS (IBM, Chicago, IL, USA, statistics version 22) were used for statistical analyses.

RESULTS

The number of NEC cases was sharply increasing over the 2013-2017 (Figure 2).

The total number of sick newborns admitted to ICU of "Muratsan" university hospital during the period A and period B was 703 and 726 respectively. 70 NEC cases were registered during period A and 71 NEC cases were registered during period B (Tables 2 and 3).

Based on this fact the estimated prevalence of NEC in Armenia comprised 1.2 per 1000 (CI: 0.7-1.7) live births, which is almost in the line with mentioned NEC rate [*Papillon S. et al., 2013; Stoll B, et al., 2015*].

The median age at baseline of all NEC cases including exposed to multimodal 3-component NEC prophylaxis and not exposed was 2 days during period B. The total number of male and female included in the study was 37 (52%) and 34 (48%) respectively. No relationship between gender and NEC was noted [*Carter. B et al., 2008*].

The frequent birth weight range of newborns exposed to a multimodal 3-component NEC prophylaxis was 1000-1499 gr. and 1500-1999 gr. (23% and 27% respectively) and the same weight for not exposed group (22.2% and 29% respectively).

Comparative analysis of the number of newborns with NEC cases and their weight over the years from 2016 to 2017 did not reveal the tendency of increasing the number of newborns with VLBW in the past year (Table 4).

The total number of NEC perforation with mortal outcome for 2016-2017 comprised 12 cases (Figure 3).

TABLE 3.

Comparative analysis of the NEC stages and newborns' weight between periods A and B

NEC stage	Birth weight			
	extremely low	very low	low	normal
Period A				
1A	-	-	-	1
1 B	-	-	2	-
2A	3	10	4	7
2 B	1	2	7	2
3A	5	8	5	1
3 B	1	6	2	3
Period B				
1A	-	-	-	-
1 B	1	2	8	2
2A	2	9	16	3
2 B	4	5	8	6
3A	3	1	-	1
3 B	-	-	-	-

Significant association ($p<0.01$) was found between the exposure to a multimodal 3-component NEC prophylaxis and the number of NEC 3A, 3B including bowel perforation: 31 cases in period A (19 cases 3A stage and 12 cases 3B stage) and 1 case 3A stage and 0 case 3B stage in period B (Figure 4).

Significant association was found related to number of death cases ($p<0.01$) among 45 newborns exposed to multimodal 3-component NEC prophylaxes during period B (5 cases not from NEC) and non-exposed 70 newborns during period A (24 cases).

Necrotizing enterocolitis often requires surgical intervention [*Gephart M et al., 2012*]. The implementation of multimodal 3-component NEC prophylaxes during period B significantly reduces the number of surgical interventions (Figure 5).

The principal indication for surgical intervention in NEC is a perforated or necrotic intestine [*Stey A et al., 2015*]. During period B after introduction of multimodal 3-component NEC prophylaxis among 45 newborns no cases of surgical operations has been registered compared with 14 cases among 70 newborns not exposed to NEC prophylaxis scheme ($p<0.01$) during period A (Figure 4). The same statistics (0 cases for period B and 8 cases for period A) has been registered regarding drainage cases ($p<0.05$).

According to medical records of "Muratsan" university hospital 87.5% of newborns with drainage and 75% of operated newborns with NEC have died during period A. The sharp decrease in the number of surgical interventions among newborns with NEC in "Muratsan" university hospital during period B after introduction of multimodal 3-component prophylaxis scheme indicates about positive effect of applied scheme.

Introduction of multimodal 3-component NEC prophylaxis led to significant decrease in the number of death cases during period B among newborns exposed to NEC prophylaxis scheme compared with newborns that did not received multi-

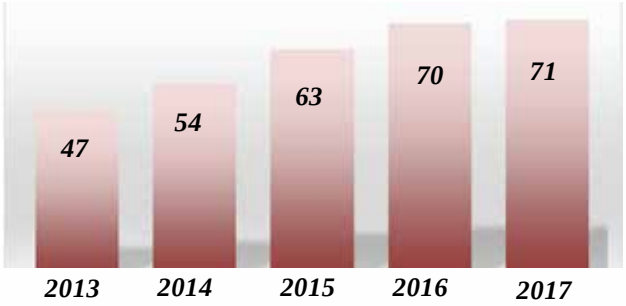


FIGURE 2. *Number of registered NEC cases in "Muratsan" university hospital (2013-2017)*

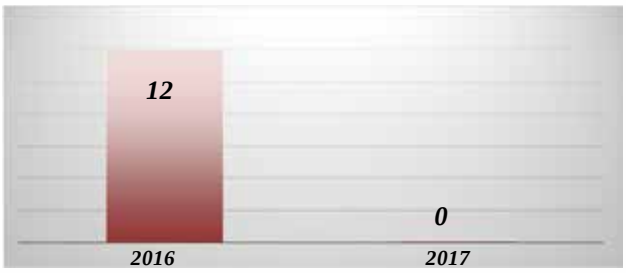


FIGURE 3. *Number of NEC 3B deaths in "Muratsan" university hospital (2016 and 2017)*

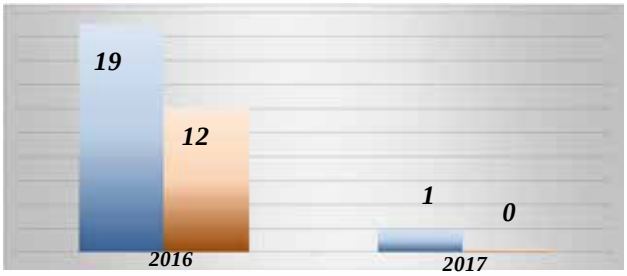


FIGURE 4. *Number of NEC 3A (left columns) and 3B (right columns) in "Muratsan" university hospital (2016 and 2017)*

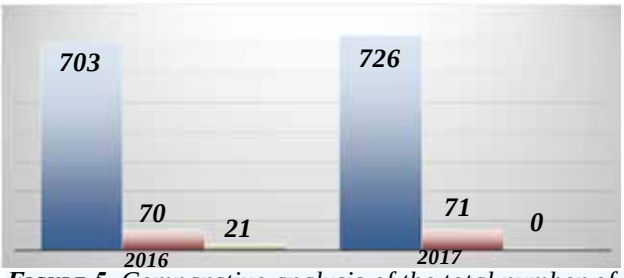


FIGURE 5. *Comparative analysis of the total number of newborns ICU (left columns), number of NEC patients (midle columns) and surgical interventions (right columns) from 2016-2017*

TABLE 4.

Birth weight comparative analysis in "Muratsan" hospital complex over 2016-2017

Year	Number of newborns with NEC	Birth weight			
		extremely low	very low	low	normal
2016	70	14.3% (10)	37.1% (26)	28.6% (20)	20% (14)
2017	71	14.1% (10)	23.4% (17)	45.1% (32)	16.9% (12)

modal NEC per oral prophylaxis. However, the death cases registered in period B were caused by conditions other than NEC (Figure 6).

It indicates that multimodal 3-component NEC prophylaxis per oral scheme decreases the risk of NEC complications and related death.

In addition, the number of death cases not from NEC complications (septic shock, pneumothorax and intracranial hemorrhages, etc.) among newborns with risk factors who received multimodal 3-component prophylaxis (11.1%) has sharply decreased as compared with newborns who were not exposed to multimodal NEC prophylaxis scheme ($p<0.01$) during period B (53.9%).

The multivariable linear regression model testifies the dependence between disease outcome as an independent value and risk factors among newborns who were exposed to a multimodal 3-component NEC prophylaxis (adjusted R^2 0.42). Independent variables age and sex were dropped from the model if they did not statistically significantly affect its fit, as defined by a p-value of > 0.05 on likelihood ratio testing.

Discussion

Neonatal necrotizing enterocolitis is still the most frequent lethal disease arising from the gastrointestinal tract in preterm newborns [*Fizan A. et al., 2008; Diego F. Niño et al., 2016*].

Analysis of hospital records of YSMU "Muratsan" university hospital has shown significant increase in NEC incidence and number of death cases during 2011 through 2016 that justified previous studies [*Llanos AR et al., 2002; Luig M, 2005; Harutyunyan A.S., 2017*].

It is known that approximately 90% of all patients with NEC are preterm [*Wilson R et al., 1981*]. The same tendency we observed in our study. Treatment applied during the first days of life plays a crucial role in prevention of NEC complications [*Schmolzer G., Urlesberger B., 2006; Gephart M. et al., 2012*]

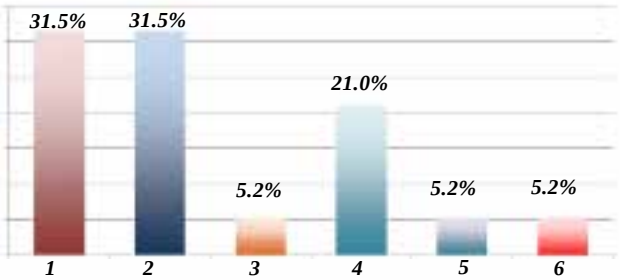


Figure 6. *Causes of death among newborns with NEC during period B. (1) septic shok, (2) pneumathorax, (3) cardic anomaly, (4) intracranial hemorrhage, (5) intestinal obstruction, (6) pneumonal bleeding.*

Injury in NEC usually begins with breach in the intestinal mucosal barrier leading to bacterial translocation across the epithelium, and exacerbation of the inflammatory cascade, resulting in the clinical signs of NEC. The most common complications of NEC are intestinal stricture, short-bowel syndrome, and the complications of difficulty providing adequate nutrition and parenteral nutrition-induced cholestasis [*Claud.C., 2009*].

Multiple randomized clinical trials now validated the empirical observation that breast milk statistically evidently reduces the incidence of NEC *[Diego F. Niño et al., 2016*]. Human milk has been shown to be protective against NEC [*Claud.C., 2009*]. Multiple factors in breast milk are hypothesized to prevent the development of NEC, including immunoglobulins, erythropoietin, IL-10, epidermal growth factor (EGF) and platelet-activating factor (PAF)-acetylhydrolase. It is notable that several investigators have failed to show a decrease in the incidence of NEC by using oral immunoglobulins [*Schmolzer G., Urlesberger B., 2006*]. Therefore, the leading role in prevention of NEC belongs to breast milk. This fact testifies about common approach to prevent NEC using breast milk feeding and Armenia is not an exception in this range.

It should be emphasized that the absence of mother's milk bank in NICU of "Muratsan" university hospital becomes a reason of artificial feeding in preterm newborns in cases when mother is not able to breastfeed. After introduction of multimodal 3 component NEC prophylaxis scheme that included oral administration of Gentamicin sulfate, an antifungal agent Nystatin, and synbiotic LactoG among newborns led to sharp decrease in the number of NEC severe stages, complications, surgical interventions and deaths.

Several studies have used different strains of probiotics and different administrative regimes. None of the trials has reported adverse effect, furthermore there was not observed any episode of pathogenic infection caused by probiotic organism. We used synbiotic (probiotic and prebiotic) LactoG for effective gut colonization and prevention

of probable intestinal sterilization due to oral antibiotic administration. Published data suggest that the use of enteral antibiotics may be effective as NEC prophylaxis. However, the risks of enteral antibiotics have not been quantified yet and this strategy has never been widely adopted, due to concerns about emergence of resistant bacteria and absorption of antibiotics from the gut. However, such adverse effects have not been reported so far [*Schmolzer G., Urlesberger B., 2006*]. Other randomized and quasi-randomized controlled trials where different antibiotic regimens were used for treatment of NEC provided insufficient evidence to recommend a particular antibiotic regimen for the treatment of NEC [*Dharmesh S., Sinn J., 2012*]. It is important to point out that specialists of "Muratsan" university hospital are not experienced with longterm usage of such approach but the investigated period B has not revealed any complications of administrated probiotics, antibiotics and antifungal agents as well.

Also, the important to note that in "Muratsan" university hospital only newborns with risk factors such as low birth weight <2500, gestation age <37 weeks and respiratory distress were included in the study. After introduction of multimodal 3-component NEC (antibiotic, antifungal agent and synbiotic) prophylaxis scheme in "Muratsan" university hospital the frequency of severe stages such as 3A and 3B of NEC decreased significantly over a period B compared with the period A. The same tendency was observed for surgical interventions and overall number of deaths.

As it was noted earlier during period B there were clinically diagnosed NEC in 71 newborns (9,8% from 726 newborns). Out of 71 newborns who met the inclusion criteria to be treated as NEC cases during the period B, 45 newborns were exposed to multimodal 3-component NEC prophylaxis scheme and 26 were not exposed because parents did not give their agreement. It should be noted, that during period B NEC 3A stage developed in 5 cases - 1 survived case in group of 45 newborns with "oral" treatment; and 4 cases in group of 26 patients - and they died. The difference between group is not statistically significant ($p>0.05$) but it also shows that 4 newborns out of 26 died of septic shock - 3 out of which had peritonitis and microperforations showed at autopsy, very suggestive of NEC as the reason for septic shock (Table 5). In 2 newborns out of 45 cases with death from septic shock peritonitis and/or microperforations weren't revealed at autopsy. We found a dramatic reduction of mortality in the group of 45 patients treated with the multimodal therapy, as compared to the group of 26 patients not receiving this therapy during the period B. Not only did not these 45 patients die from NEC, but significantly less was the number of deaths from other complications such as tension pneumothorax, intracranial hemorrhage and septic shock. We consider that maintenance of good bowel function is of a crucial importance for survival of these children!

Since the treatment and scientific study is carried out up to this day, we hope to provide answers to a number of interesting questions in future publications.

It is also important to understand the role of microbiota in pathogenesis of NEC [*Bhoomika P., Shah J., 2012*]. This study has shown that there is a lack of colonization in infant's intestine; therefore, further work is needed to investigate the role of bacterial adherence in NEC.

The biomarkers used in prognosis and diagnosis of NEC are relative nonspecific as other noninvasive and less invasive methods. Therefore, several biomarkers described in literature as detected in the blood need to be specifically assessed for their prognostic value [*Nantais-Smith L et al., 2015; Ng PC, Ma TP et al., 2015; Niemarkt HJ, et al., 2015*]. As this pilot study was aimed only at treatment of NEC, the whole pallet of biomarkers has not been measured.

Nevertheless, a number of blood markers are promising diagnostic and prognostic measures, including:

- acute-phase biomarker (C-reactive protein, TNF α, IL-6 and IL-8, etc.) [*Niemarkt HJ, et al., 2015*]
- organ-specific biomarkers (intestinal fatty acid-binding protein, liver fatty acid-binding protein, faecal calprotectin, trefoil factor 3 and claudin-3 etc.) [*Thuijls G, et al., 2010; Ng PC et al., 2014*]
- urine fibrinogen peptide used in combination with 27 clinical parameters (FGA1826, FGA1883 and FGA2659) [*Sylvester KG, et al., 2014; Sylvester KG, et al., 2014*].

Based on literature, intestinal fatty acid-binding protein, which is a cytoplasmic protein (part of enterocyte lipid metabolism) seems to be one of the most promising amongst this variety of molecules

TABLE 5.

Comparative table of the death causes among newborns with NEC during period B

Stage of NEC	Numper of patients	Mortality	Mortality - the reasons			
			Tension pneumothorax	Intracranial bleeding	Septic shock	Cardiac anomaly
Groups "26"						
1B	6	1	1			
2A	8	2		1	1	
2B	8	7	3	3	1	
3A	4	4	1	1	2	
Total	26	14	5	5	4	
		53.9%	35.7%*	35.70%	28.60%	
Groups "45"						
1B	7	0				
2A	22	4		1	2	1
2B	15	1	1			
3A	1	0				
Total	45	5	1	1	2	1
		11.1%	20%	20%	40%	20%

NOTE: ** - % of group total mortality*

[*Heida FH, et al., 2015; Schurink M, et al., 2015*]. In case of enterocyte damage, the fatty acid binding protein is released into circulation and is detected in urine. As enterocyte damage is accompanied with intestinal necrosis, this biomarker is recommended as a useful noninvasive measure to predict NEC [*Schurink M, et al., 2015*]. As NEC still remains the most challenging issue in the field of neonatology, we plan to enlarge our pilot study and to conduct a large investigation of NEC based on our treatment model and including NEC development, prognosis, diagnosis, prevention and treatment monitoring including all promising biomarkers and possible methods of analysis for positive outcome data.

CONCLUSION

For the first time multimodal 3-component NEC prophylaxis scheme was applied in NICU of YSMU "Muratsan" hospital complex. Introduction of multimodal 3 component NEC prophylaxis scheme along with breast feeding has significantly improved the disease outcome and raised new expectations for a possible reduction of infant mortality not only caused by NEC complications but other severe conditions as well. The current study has shown that multimodal 3-component NEC prevention strategy that includes enteral administration of antibiotics, antifungal agent and probiotics (Gentamicin + Nystatin + LactoG synbiotic) used only among newborns with high risk of NEC (birth weight, gestation age and respiratory insufficiency) significantly decreases the number of NEC cases including advanced stages, complications and related deaths, compared to the newborns without multimodal approach.

Positively, we can recommend to include multimodal 3-component NEC prophylaxis per oral scheme (Gentamicin + Nystatin + LactoG synbiotic) in complex treatment of the patients with necrotizing enterocolitis in medical facilities, where early breastfeeding is impossible due to different reasons (donor milk bank absence, etc.).

Future research must be conducted with enlarged cohort, investigating current prevention approach to NEC and its complications.

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