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THE TRANSFORMATION OF MULTI-MODAL 3 COMPONENT PREVENTIVE SCHEME INTO TREATMENT PROTOCOL FOR NECROTIZING ENTEROCOLITIS IN NEWBORNS.

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Abstract

Necrotizing enterocolitis (NEC) is the leading cause of death from gastrointestinal disease in premature infants treated in neonatal intensive care unit (NICU).

At the present time the unified universally accepted set of informative diagnostic criteria has not been developed yet to allows specifically determine the stage of the disease for the diagnose and treatment strategy.

That is why comparing the results of various NEC treatment programs is very difficult. At the Department of Pediatric Surgery and Division of Neonatology of Medical University of Graz (Austria) the implementation of NEC prevention protocol resulted in a very low incidence of NEC. The Graz protocol consists originally of enteral application of a Probiotic (Lactobacillus rhamnosus), in combination with enteral application of an antibiotic (Gentamicin) and an antifungal substance (Nystatin).

Methods: The preventive multi-modal 3-component NEC prophylaxis scheme was implemented for NEC treatment in December of 2016 in Neonatology Intensive Care Unit of "Muratsan" clinical complex of Yerevan State Medical University.

The retrospective observational study was performed to assess the effectiveness of multi-modal 3-component NEC prophylaxis scheme used as part of the treatment among patients with NEC during 01.12.2015–30.11.2016 (period A), 01.12.2016–30.11.2017 (period B) and 01.12.2017–30.11.2018 (period C).

All observed patients were divided in to intervention and control groups.

Results: The mortality rate in control group was 38 infants (40%) compared to the intervention group was 13 infants (13%) (p=0·0001). The implementation of multi-modal 3-component NEC prophylaxis scheme into treatment protocol for NEC resulted in reduction of NEC development from 37% to 12%. Newborns' total mortality decreased from 11% to 6%, wherein the NEC mortality decreased from 16% to zero, and mortality with NEC diagnose included decreased from 34% to 14%. The number of advanced NEC decreased from 33% to 2% and it should be noted that the number of surgical interventions decreased from 21 to zero. The complete recovery at discharge increased from 50% to 86%.

Conclusion: The implementation of multi-modal 3-component NEC prophylaxis scheme into treatment protocol for NEC shows significantly reduction in NEC associated morbidity and mortality. The NEC development process in NICU also shows the positive dynamic after multi-modal 3-component NEC prophylaxis scheme administration among newborns with high risk of NEC development.

Keywords: *necrotizing enterocolitis, newborns, multi-modal scheme, NEC-prophylaxis, Gentamicin sulfate, Nystatin, LactoG (synbiotic)*

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Introduction

Bacterial infections are a major cause of death in newborn infants. They are linked to complications including: sepsis (an over exaggerated immune response to infection) and necrotising en-

terocolitis (a potentially fatal inflammatory bowel disease) [*Berrington JE, 2017*].

Necrotizing enterocolitis (NEC) is the leading cause of death from gastrointestinal disease in premature infants, affecting newborn babies at a rate of 1–3 per 1000 births per year in North America [*Papillon S et al., 2013; Stoll BJ et al., 2015*], with an average total treatment cost of US$500,000 per patient in the USA in current charges [*Bisquera JA et al, 2002; Stey A et al., 2015*]. Importantly, the mechanisms leading to the development of NEC in premature infants and the lessons learned from management of patients with NEC could have broad implications to other neonatal inflammatory processes [*Salhab WA et al., 2004; Neu J, Walker WA., 2011*]. Despite several decades of experience in treating patients with NEC, the overall mortality and approach to treatment have remained largely unchanged since the initial descriptions of the disease [*Sharma R, Hudak ML, 2013; Stey A et al., 2015*].

The usual onset of this disease is between 7th and 14th day of life, although later onset of NEC was documented in literature [*MacKendrick W, Caplan M., 1993; Kosloske AM, 1994; Rayyis SF et al., 1999*]. The clinical presentation of NEC includes variable signs, which are often non-specific for gastrointestinal dysfunction [*Claud CE et al., 2009; Carol L., 2019*]. According to Bell MJ, NEC is classified into 3 stages [*Bell MJ et al., 1978*]. Especially stages 3A and 3B are advanced stages of disease, and are associated with a high mortality, since they lead to intestinal perforation with peritonitis, to septic shock and the need for surgical interventions.

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 CE et al., 2001; Gephart SM.et al., 2012*].

The pathogenesis refers to the interaction of three aspects: intestinal ischemia, inflammation and necrosis [*Schmolzer G et al., 2006*]. 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 AM. 1994*].

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

Suspicion of NEC is frequently based on clinical presentation, which can include feeding intolerance, abdominal distention, bloody stools, emesis, and gastric retention. These signs lead to further workup including blood work to detect potential thrombocytopenia or metabolic acidosis and imaging studies to identify dilated loops of bowel, intestinal perforation, pneumatosis intestinalis, and portal venous gas. Depending on imaging findings and clinical presentation, surgical intervention is considered [*Alganabi M., 2019*]. Abdominal X-ray and ultrasound have been shown to be useful in helping to monitor the progression of the disease and detecting the presence of NEC [*Janssen ML et al., 2018.*]. In general, for Bell MJ stage I (suspected NEC), supportive medical management alone is provided. For Bell stage II (proven NEC), medical management is usually tried first. This includes antibiotic treatment, nasogastric decompression, and total parenteral nutrition. If the patient fails to respond to medical treatment, surgical management is considered [*Rees CM et al., 2005*]. Patients with Bell stage III (advanced NEC) can be treated medically and may require inotropic support. However, neonates who develop intestinal perforation, have suspected bowel necrosis, or fail to respond to medical treatment require surgical treatment. Among VLBW infants, 27–52% require surgical intervention [*Robinson JR et al., 2017*].

Several studies have identified interventions that resulted in *reductions of the incidence* of *NEC* - such as breast milk feeding, use of probiotics, progression of enteral feeds, and enteral anti-

biotic prophylaxis [*Updegrove K., 2004; Schmolzer G et al., 2006; Yeo SL., 2006; AlFaleh K, Anabrees J, 2014; Alganabi* M et al., *2019*].

In 1977 an article was published in "The Lancet" about *prevention of necrotizing enterocolitis with oral gentamicin.* A double-blind controlled study of prophylactic oral gentamicin was undertaken. The rationale was a previous report that prophylactic Kanamycin prevented NEC in babies under *1500 g* at birth [*Egan E A et al., 1976*]. 42 babies at risk of NEC were included. 20 in the treatment group received *2.5 mg/kg gentamicin* every 6 h for 1 week. The control group of 22 babies received dextrose and water placebo in an equivalent small volume. None of the 20 gentamicin-treated babies had NEC. 4 of the control babies were affected; 2 died and the diagnosis was confirmed at necropsy. The two groups did not differ significantly in terms of birth weight, gestational age, Apgar scores, incidence of hypotensive or hypoxic episodes, breast feeding, respiratory-distress syndrome, or the need for respiratory assistance. These results, in a random, double-blind trial, and in accord with a previous study, suggest a policy of treatment with oral gentamicin for all babies under *1500 g*, all babies needing umbilical catheters, and all premature babies with a history of fetal distress, Apgar score less than 7 at 1 or 5 min, or an episode of hypotension and/or hypoxia after birth [*Lawrence Grylack L, Scanlon JW.,1977*].

At the Department of Pediatric Surgery and Division of Neonatology of Medical University of Graz (Austria) the NEC prevention protocol was used over the last 20 years, resulted in a very low incidence of NEC of around 1% in preterm neonates less than *1500g* [*Schmolzer G.et al., 2006*]. The Graz protocol of multi-modal 3-component regimen for NEC prevention consists originally of enteral application of a Probiotic (Lactobacillus rhamnosus), in combination with enteral application of an antibiotic (Gentamicin) and an antifungal substance (Nystatin). No prospective randomized trials with this protocol have been performed due to ethical norms [*Schmolzer G et al., 2006*].

There are several experimental studies about the effects of use of parenteral antibiotics on gut function and preventing NEC. Oral Gentamycin, Ampicillin and Metronidazole showed marked effect on modulating intestinal immunity and prevent NEC in preterm neonatal piglets. Moreover, oral antibiotics increase gut metabolism and antioxidant proteins and decrease acute phase response in experimental NEC [*Jiang P et al., 2012; Jensen ML et al., 2014; Birck M.M et al., 2016*].

The oral antibiotic selection based on such factors as pharmacokinetics of enteral absorption. If antibiotic can have absorbed in gastrointestinal tract, it can impact on parenteral antibiotics cross-interaction and dose with effect of overdoses and side-effects. Ampicillin and Metronidazole absorbed in gastrointestinal tract and that is why they cannot be used for NEC enteral prevention. The choice for oral Gentamycin is based on such factors as no-absorption in gastrointestinal tract with high antimicrobial effect in topical use with no metabolism to active forms [*Abu-Basha E.A.et al., 2013*]. An antifungal substance Nystatin and Probiotic (Lactobacillus rhamnosus), like Gentamycin, do not absorbed through gastrointestinal tract during oral use and show the positive effects locally.

The "Muratsan" clinical complex of Yerevan State Medical University is main and one of the biggest pediatric clinic in Republic of Armenia with specialized neonatal care division. Therefore, the majority of NEC cases (65 – 75%) in Armenia are admitted to "Muratsan" clinical complex. The statistical data for 5 years (2011-2016) period evidenced a lot of NEC cases and a high incidence of NEC- related mortality in NICU of YSMU [*Harutyunyan AS, 2017*]. During 5 years (2011-2016) 3028 newborns were admitted to NICU of "Muratsan" clinical complex of YSMU with 213 cases of NEC (7%). 77 newborns with NEC (36.2%) have died, 136 (63.8%) NEC patients discharged or transferred to other departments after treatment. The age of majority cases were 1-4 days old with average mortality maximum in 1-3 days old. Weight range of majority (78.4%) cases 500 – 2000 grams with average mortality maximum *1000-1500 gr*. 12 newborns (5.6 %) were operated with 83.3% of mortality. 22 newborns (10.3%) were drainaged with 90.9% of mortality. 187 newborns (87.8%) were treated conservative without surgical interventions with 29.4% of mortality. The conclusion showed a visible tendency of increase of cases number of NEC, high rate of mortality, difficulty in diagnostic [*Harutyunyan AS, 2017*].

For a more detailed understanding the problem

of diagnosis and tactics of management of newborns with necrotizing enterocolitis it was necessary to combine clinical data with the results of histological examination [*Harutyunyan AS et al., 2019a*]. The retrospective study was conducted at "Muratsan" clinical complex of Yerevan State Medical University, department of autopsy of medical centre "Arabkir" and the records of mortal cases of 21 delivery hospitals of Republic of Armenia. For the purpose of analysis and compare of NEC clinical incidence with histology findings, the data from the NICU of "Muratsan" clinical complex medical records, department of autopsy of pathohistology department of medical centre "Arabkir" and the records of mortal cases of 21 delivery hospitals of Republic of Armenia for the time period 12.01.2016 – 27.12.2017 were analyzed. Records included information regarding demographics, prescribed medications, laboratory results, procedures and diagnoses of newborns and the histology findings in autopsy or part of intestines removed by operations. Demographic data included gender, birth weight (BW), gestational age (GA) and Apgar score. For the comparison of data the referral clinical diagnose compared to hospital or autopsy from delivery and/or NICU with clinical and/or histological diagnose of autopsy or NICU for the 2 years periods of 01.01.2016– 31.12.2017. A retrospective analysis was performed to assess the consilience and inconsistency in clinical and histology diagnosis of NEC. The results of study denote that a high proportion of the incompatibilities of NEC diagnosis can be attributed to diagnostic limitations and are potentially avoidable with use of modern diagnostic technics [*Harutyunyan AS et al., 2019a*].

The results led to change the strategy and use the new approaches for NEC diagnose and treatment. Moreover, we decided that it was better to use the NEC prevention strategy combining well known strategies to one concept including enteral administration of antibiotics, antifungal agent and probiotics. The early trophic feeding with human breast milk in NICU was not possible because of absence of donor mother milk's bank.

Since December 2016 the Graz (Austria) protocol of multi-modal 3-component regimen for NEC prophylaxis was taken as a base for NEC prevention and NEC treatment at the NICU of "Muratsan" clinical complex YSMU [*Harutyunyan A.S et al., 2018*]. The retrospective observational study was performed to assess the effectiveness of multimodal 3-component NEC prophylaxis scheme used as part of the treatment in NICU of "Muratsan" clinical complex YSMU among patients with NEC during periods 01.12.2016–30.11.2017 (period B) and 01.12.2017–30.11.2018 (period C) compare the results to the time period 01.12.2015–30.11.2016 (period A) without multi-modal 3-component NEC prophylaxis scheme. The intervention group composed of newborns who received multimodal 3-component NEC prophylaxis scheme during period B (45 newborns) and period C (59 newborns). The control group composed of newborns who didn't receive multi-modal 3-component NEC prophylaxis scheme during period A (70 newborns) and period B (26 newborns).

The "Austrian" protocol was revised and probiotic L. rhamnosus was replaced with a locally available product (with GMP standards): Synbiotic "LactoG". LactoG consists of prebiotic (fructooligosaccharide) and probiotics containing the following strains: Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium infantis and Lactobacillus acidophilus.

After receiving an ethics approval, the decision was also to use modified multi modal 3 component scheme not only as a prevention for NEC, but also as a component in complex treatment of NEC (implemented first time). The modified preventive multi-modal 3-component NEC prophylaxis scheme were implemented for NEC treatment in December of 2016 in Neonatology Intensive Care Unit of "Muratsan" clinical complex of YSMU. Apart from introduction of modified "Graz's NEC multi-modal prevention protocol" nothing else has been changed in the treatment of children with NEC as compared to the previous year. The following medication was administered enterally (via nasogastric tube): an enteral antibiotic Gentamicin sulfate (*15 mg/kg/day – in 2 doses*), an antifungal agent Nystatin (*10000 IU/kg/day – in 4 doses*), and a synbiotic (LactoG bodyweight < *2000 gr – 2 x ¼ caps. pulveris*; bodyweight > *2000 gr – 2 x ½ caps. pulveris*) [*Harutyunyan AS et al, , 2018*].

The introduction of multi-modal 3 component NEC prophylaxis scheme as a component in complex treatment of NEC has significantly improved

the outcome of disease and resulted in visible reduction of infant mortality not only due to NEC complications but other severe conditions as well.

It must be mentioned, that multi-modal 3-component regimen was given during Period B and Period C not only to newborns with NEC, but also it was prescribed to newborns who were in high risk group of NEC development (NoNEC group). 64 newborns with NoNEC during Period B and 142 NoNEC newborns during Period C 64 received modified multi modal 3 component scheme as prophylaxis for NEC development.

The prospective observational study performed to reveal the x-ray dynamics of intestinal pneumatosis in newborns at NICU of "Muratsan" clinical complex of Yerevan State Medical University, Republic of Armenia. The inclusion criteria were newborns admitted to NICU of YSMU who received the multi-modal 3 component enteral NEC prophylaxis scheme [*Harutyunyan AS et al., 2019b*]. Newborns divided in 2 groups. First group were newborns with diagnose NEC, who received the multi-modal 3 component enteral NEC prophylaxis scheme as part of the complex treatment of NEC. The second group were newborns who *had no clinical diagnose* of NEC, but has received the multi-modal 3 component enteral NEC prophylaxis scheme as NEC prevention, because of high risk of NEC development. In both groups the multi-modal 3 component enteral NEC prophylaxis scheme was prescribed at the first day of admittance to NICU. Abdominal imaging radiographs were done on second day of multi-modal 3 component enteral NEC prophylaxis scheme prescription, and were repeated on 3rd and 5th days. The results of this study showed that multi-modal 3 component NEC prophylaxis per oral scheme (Gentamicin + Nystatin + LactoG synbiotic) has a positive effect on the resolution of process of intestinal damage manifested in the form of intestinal pneumatosis in newborns with necrotizing enterocolitis. Also the multi-modal 3 component NEC prophylaxis per oral scheme has a clear effect on the prevention of NEC developmental process [*Harutyunyan AS et al., 2019b*].

The final recommendation was to include multi-modal 3 component NEC prophylaxis per oral scheme (Gentamicin + Nystatin + LactoG synbiotic) in complex treatment of patients with necrotizing enterocolitis in places, where the early breastfeeding is impossible due to different reasons (donor milk bank absent and etc.) and high risk of nosocomial infection where could be present [*Harutyunyan AS et al., 2018*].

Materials and Methods

The study was conducted at "Muratsan" clinical complex of Yerevan State Medical University. We analyzed the data from the "Muratsan" clinical complex NICU medical records for the time period 2016–2018. 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.

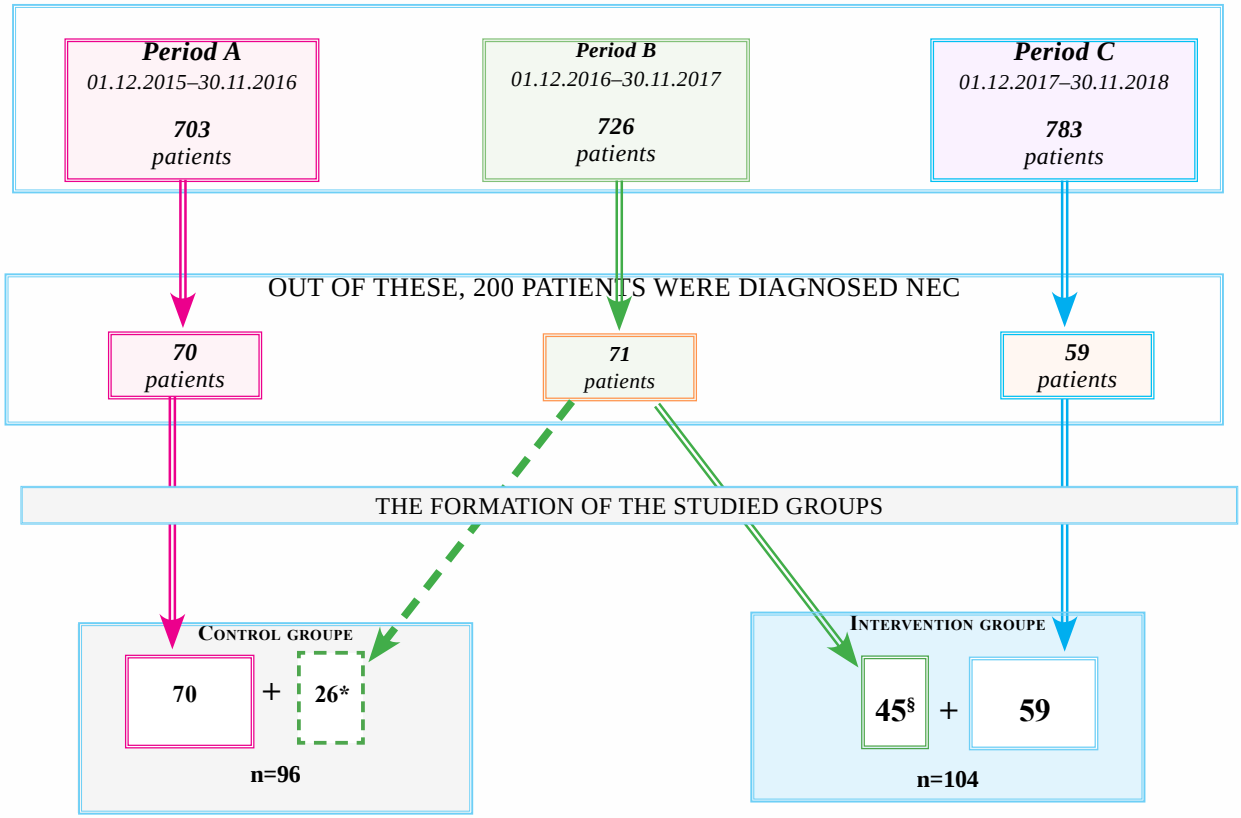
The following medication was administered enterally (via nasogastric tube): an enteral antibiotic Gentamicin sulfate (*15 mg/kg/day – in 2 doses*), an antifungal agent Nystatin (*10000 IU/kg/day – in 4 doses*), and a synbiotic (LactoG bodyweight < *2000 gr – 2 x ¼ caps. pulveris*; bodyweight > *2000 gr – 2 x ½ caps. pulveris*).

The retrospective observational study was performed to assess the effectiveness of multi-modal 3-component NEC prophylaxis scheme used as part of the treatment in NICU of "Muratsan" clinical complex YSMU among patients with NEC during periods 01.12.2016–30.11.2017 (period B) and 01.12.2017–30.11.2018 (period C) compare the results to the time period 01.12.2015–30.11.2016 (period A) without multi-modal 3-component NEC prophylaxis scheme. During period B newborns with NEC were divided in 2 groups: first group of 45 newborns received the multi-modal 3-component NEC prophylaxis scheme as part of the treatment, and the second group of 26 newborns didn't received the multi-modal 3-component NEC prophylaxis scheme as part of the treatment due to absent of parent's consent. The intervention group composed of newborns who received multi-modal 3-component NEC prophylaxis scheme during period B (45 newborns) and period C (59 newborns). The control group composed of newborns who didn't receive multi-modal 3-component NEC prophylaxis scheme during period A (70 newborns) and period B (26 newborns) – (*Scheme 1*).

Ethical Approval. The study was approved by Ethics Committee of IRB (Study reference number 12/SC/0416) and Ethics Committee of YSMU

SCHEME 1:

Patients' distribution scheme by years' periods and nosology with group creation.



NOTE: * - *newborns of period B who didn't receive multi-modal 3-component prophylaxis scheme,*
§- newborns of period B who receive multi-modal 3-component prophylaxis scheme.

(Study reference № 8, 19.04.2018).

Statistical analysis. Fisher's exact test was performed to analyse the group differences and threshold probability, value of $p<0{\cdot}05$ was used to indicate statistical significance. Microsoft Excel and Epi Info programs were used for statistical analyses.

RESULTS

The total number of newborns admitted to NICU of "Muratsan" clinical complex YSMU during periods A, B and C were 2212. The total number of newborns with NEC were 200 (Period A – 70, Period B – 71 and Period C – 59). Control group composed of 96 newborns without multi-modal 3-component NEC prophylaxis scheme during periods A and B. Intervention group composed of 104 newborns who recieded multi-modal 3-component NEC prophylaxis scheme during periods B and C (*Scheme 1*).

During periods A, B and C the number of admitted preterm newborns were 316 (45% - Period A), 351 (48% - Period B) and 327 (42% - Period C).

During periods A, B and C the number of newborns with NEC treated in NICU were 70 (10% - Period A), 71 (10% - Period B) and 59 (8% - Period C). During Period A the number of newborns admitted with NEC (*Income NEC*) was 44 (63%); and the number of newborns with NEC developed in NICU was 26 (37%). During Period B the number of newborns admitted with NEC (*Income NEC*) was 58 (82%); and the number of newborns with NEC developed in NICU was 13 (18%). During Period C the number of newborns admitted with NEC (*Income NEC*) was 52 (88%); and the number of newborns with NEC developed in NICU was 7 (12%). (Table 1).

The number of all lethalis cases were 77 (11%) out of 703 (100%) newborns during Period A, 65 (9%) out of 726 (100%) during Period B and 45 (6%) out of 783 (100%) during period C.

During Period A 24 newborns died with diagnose NEC included (34% of 70 NEC clinically diagnosed and 31% of all 77 lethalis cases). Clinically and histologically NEC-perforation was de-

scribed in 12 cases (50% of lethalis cases with NEC diagnose included and 16% of all lethalis cases). All 12 infants with intestinal perforation were treated surgically - underwent abdominal drainage and/or laparotomy (in some cases surgical interventions performed more than one time).

During Period B 19 newborns died with diagnose NEC included (27% of 71 NEC clinically diagnosed cases and 29% of all 65 lethalis cases). Clinically NEC-perforation was not described in any cases. Histologically (autopsy) intestinal micro perforations were described in 3 cases, who didn't receive multi-modal 3-component NEC prophylaxis scheme – control group (16% of lethalis cases with NEC diagnose included and 5% of all lethalis cases). No infants were treated surgically.

During Period C 8 newborns died with diagnose NEC included (14% of 59 NEC clinically diagnosed cases and 18% of all 45 lethalis cases). Clinically NEC-perforation was not described in any cases. Histologically (autopsy) intestinal micro perforations were no described in any cases. No infants were treated surgically. (Table 2, Table 3).

During Period A (Control group) 31 (44%) newborns were diagnosed advanced NEC clinically and/or instrumentally (operation and/or his-

Table 1:

NEC patients' distribution by gestation age and NEC development origin

	Period A	Period B	Period C
Number of newborns	703	726	783
Clinically diagnosed NEC	70 (9, 96 %)	71 (9, 78 %)	59 (7, 53 %)
Preterm	62 (88, 57%)	62 (87, 32%)	51 (86, 44%)
Term	8 (11, 43%)	9 (12, 68%)	8 (13, 56%)
NEC developed 1st – 4th days	44 (62, 86%)	58 (81, 69%)	52 (88, 14%)
NEC developed after 5th day	26 (37, 14%)	13 (18, 31%)	7 (11, 86%)

Table 2:

NEC patients' distribution by mortality, histological findings and surgical interventions.

	Control group		Intervention group	
	Period A	Period B	Period B	Period C
Lethals				
with diagnose NEC included	24	14	5	8
% of NEC clinically diagnosed	34,29% of 70	53,85 % of 26	11,11 % of 45	13,55 % of 59
% of all lethalis cases	31,17 % of 77	21,54 % of 65	7,69 % of 65	17,78 % of 45
NEC (3B) perforation / micro perforations				
% of NEC diagnose included	12 (50%)	3 (21.43%)	0	0
% of all lethalis cases	15.58 %	21.43%	-	-
Surgical interventions (Drainage/laparotomy)	21	0	0	0

Table 3:

NEC patients' distribution by clinical and histological data, total and group mortality and surgical interventions.

	Control group		Intervention group	
	Period A	Period B	Period B	Period B
NEC clinically diagnosed	70	26	45	59
All lethalis cases	77	65	65	45
Lethalis with DS NEC included	24	14	5	8
NEC (3B) perforation / micro perforations	12	3	0	0
Surgical interventions	21	0	0	0

tology). NEC 3A were diagnosed in 19 newborns (27%) with mortality in 14 cases. NEC 3B were diagnosed in 12 newborns (17%) with mortality in 9 cases. (Table 4, Table 5).

During Period B (both Control and Intervention groups) 5 (7%) newborns were diagnosed advanced NEC clinically and instrumentally (operation and/or histology). NEC 3A were diagnosed clinically in 5 newborns (7%) with mortality in 4 cases. NEC 3B was not diagnosed clinically (0%). During Period B (control group) the advanced NEC were diagnosed in 4 newborns with mortality in all 4 cases. NEC 3A clinically in 4 cases, NEC 3B clinically was not diagnosed. After histological study the intestinal microperforations were discovered in 3 cases. During Period B (Intervention group) the advanced NEC was diagnosed in 1 newborn with recovery discharge. NEC 3A clinically diagnosed in 1 case, NEC 3B clinically was not diagnosed. [Table 4, Table 5].

During Period C (Intervention group) 4 (7%) newborns were diagnosed advanced NEC clinically and/or instrumentally (operation and/or histology). NEC 3A were diagnosed in 4 newborns (7%) with mortality in 2 cases. NEC 3B was not diagnosed clinically and/or instrumentally (0%) (Table 4, Table 5).

During Period A (Control group) 45 (64%) newborns of 70 NEC cases had no clinical presentation of NEC at admittance – they developed NEC during stay at NICU.Out of these 45 newborns 26 developed NEC after 5th day of admittance ("the NEC of NICU, not delivery department").The clinical presentation of NEC at admittance was revealed in 25 (36%) newborns out of 70. NEC degree 1A and 1B were present at admittance in 17 cases; NEC degree 2A and 2B were present at admittance in 8 cases and NEC degree 3A and 3B were not present at admittance. The maximal degrees of NEC during stay in NICU were: 1AB in 3 (4%) cases; 2AB in 36 (51%) cases and 3AB in 31 (45%) cases. The full recovery with no NEC signs at discharge was in 35 (50%) cases. At discharge (and/or transfer to other departments or death) NEC 1AB degrees were diagnosed in 7 (10%) cases; NEC 2AB degrees were diagnosed in 5 (7%) cases and NEC 3AB degrees were diagnosed in 23 (33%) cases. (Table 6).

During Period B (Control group) 20 (77%) newborns of 26 NEC cases had no clinical presentation of NEC at admittance – they developed NEC during stay at NICU; 13 newborns of these 20 developed NEC after 5th day of admittance ("*the NEC of NICU, not delivery department*"). The clinical presentation of NEC at admittance was revealed in 6 (23%) newborns out of 26. NEC degree 1A and 1B were present at admittance in 8 cases; NEC degree 2A and 2B were not diagnosed

TABLE 4.

NEC patients' distribution by Bell's degree.

NEC stage	Period A			Period B			Period C		
	Number	% of 70	% of 703	Number	% of 71	% of 726	Number	% of 59	% of 783
1 A	1	1.43 %	0.14 %	0	0	0	8	13.56%	1.02%
1 B	2	2.86 %	0.29 %	13	18.31%	1.79 %	23	38.98%	2.94%
2 A	24	34.29 %	3.41 %	30	42.25%	4.13 %	9	15.25%	1.15%
2 B	12	17.14 %	1.71 %	23	32.40%	3.17 %	15	25.42%	1.92%
3 A	19	27.14 %	2.70 %	5	7.04%	0.69 %	4	6.78%	0.51%
3 B	12	17.14 %	1.71 %	0	0	0	0	0	0

Table 5: Patients with advanced NEC distributed by clinical and histological data with mortality rate.

	Control group				Intervention group			
	Period A		Period B		Period B		Period C	
NEC stages	3A	3B	3A	3B	3A	3B	3A	3B
Sorted by clinical diagnose	19	12	4	0	1	0	4	0
Sorted by clinical and/or autopsy diagnose	19	12	1	3	1	0	4	0
Mortality	14	9	1	3	0	0	2	0

Table 6:

Patients' distribution by NEC degree at admittance, maximal degree and degree at discharge.

NEC parameters	Control group		Intervention group	
	Period A	**Period B**	**Period B**	**Period C**
NEC degree at admittance and developed				
degree 0 at admittance	45 (64%)	20 (77%)	30 (67%)	25 (42%)
developed after 5th day of admittance	26 (58% of 45)	13 (65% of 20)	12 (40% of 30)	7 (28% of 25)
present at admittance	25 (36%)	6 (23%)	15 (33%)	34 (58%)
The admitted NEC degrees				
degree 1AB at admittance	17 (68%)	5 (83%)	6 (40%)	10 (29%)
degree 2AB at admittance	8 (32%)	0	8 (53%)	22 (65%)
degree 3AB at admittance	0	1 (17%)	1 (7%)	2 (6%)
Total by groups	***25 (100%)***	***6 (100%)***	***15 (100%)***	***34 (100%)***
The maximum NEC degree				
maximum degree 1AB	3 (4%)	6 (23%)	7 (16%)	31 (53%)
maximum degree 2AB	36 (51%)	16 (62%)	37 (82%)	24 (41%)
maximum degree 3AB	31 (45%)	4 (15%)	1 (2%)	4 (6%)
NEC degree at discharge				
degree 0 at discharge	35 (50%)	12 (46%)	40 (89%)	51 (86%)
degree 1AB at discharge	7 (10%)	1 (4%)	0 (%)	5 (9%)
degree 2AB at discharge	5 (7%)	9 (35%)	5 (11%)	2 (3%)
degree 3AB at discharge	23 (33%)	4 (15%)	0 (%)	1 (2%)
Total by groups	**70 (100%)**	**26 (100%)**	**45 (100%)**	**59 (100%)**

at admittance and NEC degrees 3A and 3B were present at admittance in 1 case. The maximal degrees of NEC during stay in NICU were: 1AB in 6 (23%) cases; 2AB in 16 (62%) cases and 3AB in 4 (15%) cases. The full recovery with no NEC signs at discharge was in 12 (46%) cases. At discharge (and/or transfer to other departments or death) NEC 1AB degrees were diagnosed in 1 (4%) case; NEC 2AB degrees were diagnosed in 9 (35%) cases and NEC 3AB degrees were diagnosed in 4 (15%) cases. (Table 6).

During Period B (Intervention group) 30 (67%) newborns of 45 NEC cases had no clinical presentation of NEC at admittance – they developed NEC during stay at NICU. 12 newborns of these 30 developed NEC after 5th day of admittance ("*the NEC of NICU, not delivery department*"). The clinical presentation of NEC at admittance was revealed in 15 (33%) newborns out of 45. NEC degree 1A and 1B were present at admittance in 6 cases; NEC degree 2A and 2B were present at admittance in 8 cases and NEC degrees 3A and 3B were present at admittance in 1 case. The maximal degrees of NEC during stay in NICU were: 1AB in 7 (16%) cases; 2AB in 37 (82%) cases and 3AB in 1 (2%) case. The full recovery with no NEC signs at discharge was in 40 (89%) cases. At discharge (and/or transfer to other departments or death) NEC 1AB degrees were not diagnosed; NEC 2AB degrees were diagnosed in 5 (11%) cases and NEC 3AB degrees were not diagnosed. (Table 6).

During Period C (Intervention group) 25 (42%) newborns of 59 NEC cases had no clinical presentation of NEC at admittance – they developed NEC during stay at NICU. 7 newborns of these 25 developed NEC after 5th day of admittance ("*the NEC of NICU, not delivery department*"). The clinical presentation of NEC at admittance was revealed in 34 (58%) newborns out of 59. NEC degree 1A and 1B were present at admittance in 10 cases; NEC degree 2A and 2B were present at admittance in 22 cases and NEC degrees 3A and 3B were present at admittance in 2 cases. The maximal degrees of NEC during stay

in NICU were: 1AB in 31 (53%) cases; 2AB in 24 (41%) cases and 3AB in 6 (6%) cases. The full recovery with no NEC signs at discharge was in 51 (86%) cases. At discharge (and/or transfer to other departments or death) NEC 1AB degrees were diagnosed in 5 (9%) cases; NEC 2AB degrees were diagnosed in 2 (3%) cases and NEC 3AB degrees were diagnosed in 1 (2%) case. (Table 6).

Discussion:

The first case report of necrotizing enterocolitis probably dates back to 1825 when Charles-Michel Billard used the term "gangrenous enterocolitis" or "malignant enteritis" to describe necrosis and inflammation of the intestinal tract in a small infants. *(Billard CM: De la membrane muqueuse gastro-intestinale dans l'état sain et dans l'état inflammatoire ou recherches d'anatomie pathologique sur les divers aspects sains et morbides que peuvent présenter l'estomac et les intestins. Ouvrage couronné par l'Athénée de médecine de Paris. Paris, Gabon, 1825, p IX.)*

Despite of history of about 200 years necrotizing enterocolitis is still remains a major concern for neonatologists, pediatric surgeons and gastroenterologists due to its high morbidity and mortality. These infants often have poor developmental outcome, and contribute to significant economic burden resulting in marked stress in these families.

By developing and adhering to strict feeding protocols, encouraging human milk feeding preferably from the infant's mother, use of probiotics, judicious antibiotic use, instituting blood transfusion protocols, the occurrence of NEC may possibly be reduced. However, because of its multifactorial etiology, it cannot be completely eradicated in the NICUs, particularly in the extremely premature infants. Ongoing surveillance of NEC and quality improvement projects may be beneficial.

NEC, as an acquired disease, has a multifactorial etiology and the pathogenesis has not fully been elucidated and remain controversial. Scientific findings suggest the importance of ischemia and microcirculatory disorders in the pathogenesis of NEC with presence of inflammation and bacterial overgrowth. 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. It is known that majority of all patients with NEC are preterm. According to Bell MJ, NEC is classified into 3 stages. Especially stages 3A and 3B (severe NEC) are advanced stages of disease, and are associated with a high mortality, since they lead to intestinal perforation with peritonitis, septic shock and other complications, in a situation with need for surgical interventions. 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.

Treatment applied during the first days of life plays a crucial role in prevention of NEC complications. The principal indication for surgical intervention in NEC is a perforated or necrotic intestine. Other indications include clinical deterioration and severe abdominal distention causing abdominal compartment syndrome (organ dysfunction or failure due to a severe increase in the pressure within the abdomen). Two surgical approaches are usually done depending on clinical presentation. Laparotomy with resection (removal) of necrotic bowel and/or primary peritoneal drainage.

The mortality rate reported for preterm newborns who are diagnosed with NEC is 10-50%, and this did not significantly decrease over the past 30 years especially in operated, were the mortality rate is 60-70%. Newborns with NEC have not only increased risk of death, but also significantly longer hospitalization days, and significantly higher treatment costs compared to newborns without NEC.

Breast milk feeding, enteral antibiotic prophylaxis, use of probiotics, nosocomial infection control and slow progression of enteral feeds resulted in reductions of the incidence of NEC. All these approaches are used for NEC prevention and are not studied for already NEC diagnosed treatment, particularly, where "nullius per os" system is in use.

Over years high incidence and mortality rates of NEC have been registered in NICU of "Muratsan" clinical complex of Yerevan State Medical University. In a struggle with high mortality due to NEC since December 2016, for the first time, we decided to use modified protocol of multi-modal 3-component regimen for NEC prophylaxis of the Department of Pediatric Surgery and Division of Neonatology of Medical University of Graz (Aus-

tria), as a part of treatment for necrotizing enterocolitis in NICU of "Muratsan" clinical complex of YSMU. Apart from introduction of modified "Graz's NEC multi-modal prevention protocol" nothing else has been changed in the treatment of children with NEC as compared to the previous year. The results were analyzed between 3 "timeline" groups of Period A (01.12.2015–30.11.2016) – before scheme implementation, Period B (01.12.2016–30.11.2017) and Period C (01.12.2017–30.11.2018). Newborns were divided in 2 "intervention" groups. The Control group composed of newborns who didn't receive multimodal 3-component NEC prophylaxis scheme during period A (70 newborns) and period B (26 newborns, who hadn't parental consent). The Intervention group composed of newborns who received multi-modal 3-component NEC prophylaxis scheme during period B (45 newborns) and period C (59 newborns).

During 3 periods there was no significant differences between numbers of admitted newborns. The ratio of preterm and term babies was the same. Changes were discovered in number of NEC patients – during Period C the number of NEC newborns decreased in 2,5 %, what is associated with a decrease of NEC development in the department (preventive properties of the applied scheme). Interesting results were observed when comparing the data of newborns who were admitted to the department with an already developed NEC with children whose NEC developed in the department. It turned out that NEC development in department decreased twice in period B and three times in period C compared with period A. This clearly indicates the prophylaxis properties of the applicable scheme.

The newborns' total mortality rate in NICU decreased almost 2 times from 19% (period A) to 6% (period C) *(p=0·001)*. When analyzing the data of deaths where the diagnosis of NEC was present, it turned out that in the period C the number of deaths was 18% in the group with a clinical diagnosis of NEC and 11% in the intervention group of period B. Whereas in the group of period A the number of fatal cases diagnosed with NEC was 34% and 22% in the control group of period B. It should be noted that NEC perforation was not detected both clinically and instrumentally in period C and in the intervention group of period B, while perforation and microperforation of the intestine were detected in half of all deaths diagnosed with NEC of period A and in three cases out of 14 in the intervention group of period B. Mortality due to NEC perforation decreased to zero in period C and in the intervention group of period B (where all patients received the regimen), compared with period A, where perforative NEC mortality was 16% of total mortality.

As it known, the only acceptable treatment approach for intestinal perforation is surgery. During period A 21 surgical operations (laparotomy and abdominal drainage) were performed on advanced NEC. In the period B and C, no surgical interventions were performed, due to the absence of perforating complications. These data indicate the high efficiency of the applied scheme in the process of preventing the development of complications in necrotizing enterocolitis, since there were no other changes in the treatment of NEC during periods B and C. There were 3 cases of microperforations in control group of period B without clinical signs of perforation – intestinal destruction identified postmortem.

A thorough assessment on changes in the ratio of NEC degrees was also carried out. The obtained data clearly indicate a positive effect of multi-modal 3-component regimen on NEC inflammatory process development. So, in period A the second (51% of all NEC patients) and especially third (45%) stages of NEC were prevailed, with a small number of patients of the first (4%) stage. During period C, there is a sharp increase in the mild suspicious forms of NEC - (53%) in the first stage, (40%) of the second stage, with a small number of patients in the third (7%) stage. This shift indicates an improvement in NEC indicators with an increase in borderline cases, where NEC in most cases is diagnosed based on assumptions and suspicions.

At the intensive care unit, doctors are faced with two types of NEC. Newborns admitted with a clinic of necrotizing enterocolitis or developed in the first 4 days – "income" NEC. And necrotizing enterocolitis developed in the department. During period A the "income" NEC was in 63% of cases, while during period C the "income" NEC was in 88% of cases. This indicates a sharp decrease in

NEC development in the department due to preventive characteristics of multi-modal 3-component regimen. It should be noted that in period A, at the time of admission, in most cases (68%) first degree NEC were prevailed, while in period C - the second degree prevailed (65%). At the same time, the maximum degree of NEC in most cases of period A was the second (51%) and third (45%), while in period C in most cases the maximum degree of NEC was the first (53%) and second (41%). That is, due to multi-modal 3-component scheme, NEC did not reach the advanced stages and in most cases regressed.

And finally, complete recovery and absence of NEC symptoms at discharge observed in 86% of cases during period C and in 89% of cases of intervention group of period B, while complete recovery of NEC observed in 50% of period A and in 46% of control group of period B. The percent of advanced NEC at the discharge (at the time of death) was completely opposite: 33% during period A and 15% of control group of period B compare to 2% of cases during period C and in 0% of cases of intervention group of period B.

CONCLUSION:

The implementation of multi-modal 3-component NEC prophylaxis scheme into treatment protocol for NEC resulted in reduction of NEC development in NICU from 37% to 12%. Newborns' total mortality decreased from 11% to 6%, wherein the NEC mortality decreased from 16% to zero, and mortality with NEC diagnose included decreased from 34% to 14%. The number of advanced NEC decreased from 33% to 2% and it should be noted that the number of surgical interventions decreased from 21 to zero. The complete recovery at discharge increased from 50% to 86%.

The implementation of multi-modal 3-component NEC prophylaxis scheme into treatment protocol for NEC shows significantly reduction in NEC associated morbidity and mortality. The NEC development process in NICU also shows the positive dynamic after multi-modal 3-component NEC prophylaxis scheme administration among newborns with high risk of NEC development.

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Charles-Michel Billard.

Pioneer of neonatal medicine.

Charles-Michel Billard was a French physician, best known for his research of pediatric diseases.

Billard was born in Pellouaille, France, in 1800. He studied medicine, first in Angers and then, between 1824-1828, in Paris. During this time, he was very poor and supported himself by writing for medical journals and undertaking translations, having learned German, English, and Italian.

The first case report of necrotizing enterocolitis probably dates back to 1825 when Charles Billard used the term "gangrenous enterocolitis" or "malignant enteritis" to describe necrosis and inflammation of the intestinal tract in the small infants. (Billard CM: De la membrane muqueuse gastro-intestinale dans l'état sain et dans l'état inflammatoire ou recherches d'anatomie pathologique sur les divers aspects sains et morbides que peuvent présenter l'estomac et les intestins. Ouvrage couronné par l'Athénée de médecine de Paris. Paris, Gabon, 1825, p IX.).

In 1828, the year he became Docteur en Medecine de la Faculte de Paris, he published his masterpiece "Traiti des Maladies des Enfants Nouveau-Nis et I la Mamelle".

Sadly, this brilliant and modest man then contracted phthisis and returned with his wife and child to Angers where he died at the early age of 32.

Billard's book was the first systematic clinical/pathological text on the newborn infant. Indirectly the fetus was also studied; for, as he wrote: 'During intrauterine life man often suffers many affectations, the fatal consequences of which are brought with him into the world ... children may be born healthy, sick, convalescent, or entirely recovered from former diseases'.

Among the many now familiar disorders included the first description of neonatal gangrenous enterocolitis (today's necrotizing enterocolitis), and description of kernicterus in four infants, Billard is also credited with determining the precise age of ductus arteriosus closure, and an infant he described with colonic hypertrophy likely had Hirschsprung's disease.

[PERINATAL LESSONS FROM THE PAST.
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