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THE INCONSISTENCY OF CLINICAL AND HISTOLOGICAL DIAGNOSIS IN NEONATES WITH NECROTIZING ENTEROCOLITIS.

Harutyunyan A. S.[1*], Badalyan A. R.[2], Lorenc D. V.[3], Babloyan A. S.[4], Hovhannisyan M.G.[5], Eyramjyan G.G.[6]

[1] Department of Pediatric Surgery, "Muratsan" Hospital Complex, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia
[2] Department of Epidemiology, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia.
[3] Neonatal Intensive Care Unit, "Muratsan" Hospital Complex, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia
[4] Faculty of Pediatric Surgery, Yerevan State Medical University after M. Heratsi, "Arabkir" Medical Center, Yerevan, Armenia.
[5] Faculty of Public Health, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia.
[6]Faculty of Pediatric Surgery, Yerevan State Medical University after M. Heratsi, Yerevan, Armenia.

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Abstract

Despite decades of active research, the exact cause (etiology) and pathogenesis of Neonatal Necrotizing Enterocolitis (NEC) remains unknown. The mechanism of disease development and the progression of intestinal injury remain areas of ongoing research and controversy.

NEC is a major cause of mortality and significant morbidity, often in premature infants with an inverse relationship to gestation, and may occur uncommonly in term infants. Despite advances in neonatal intensive care, its incidence, morbidity and mortality has remained unchanged. NEC remains a major concern for neonatologists, pediatric surgeons and gastroenterologists due to its high morbidity and mortality.

The etiology and pathogenesis of NEC remain controversial. The classic histological finding is coagulation necrosis present in over 90% of specimens.

With NEC, \the most commonly affected areas are the terminal ileum and the proximal ascending colon. The pattern of disease may involve a single isolated area or multiple discontinuous lesions. The most common histologic findings are associated with mucosal injury. These include coagulation necrosis of the mucosa with active and chronic inflammation, mucosal ulceration, edema, hemorrhage, and pneumatosis of the submucosa.

The clinical presentation of NEC is nonspecific, broad and includes variable symptoms which are often nonspecific signs of gastrointestinal dysfunction.

The aim of the study is to compare the histological (autopsy) and clinical data in neonates with necrotizing enterocolitis.

The retrospective study was performed to assess and compare histological (autopsy) and clinical data among newborns with necrotizing enterocolitis over 2016-2017 periods – born in 21 deliveries hospitals, newborns admitted to NICU of "Muratsan" clinical complex of Yerevan State Medical University aft. M. Heratsi and autopsy data of medical centre "Arabkir".

In 73 (86.9% out of 84 cases) newborns the NEC was diagnosed during autopsy and histological examination. The histological diagnose NEC matched with referral diagnose in 27 cases – 37% (out of 73 cases). In 46 (63% out of 73 newborns) cases NEC was present during autopsy and histological examination, but didn't manifested clinically or was not diagnosed before death. In other findings 42 cases out of 142 lethal newborns during the autopsy the histological signs of different stages of NEC were detected, but were not included in clinical diagnose.

The results of our study denote that a high proportion of the incompatibilities of NEC diagnosis can be attributed to diagnostic limitations and are potentially avoidable with the use of modern diagnostic technics.

Keywords: *Necrotizing enterocolitis, newborns, perforation.*

Address for Correspondence:
Dr. Arman S. Harutyunyan,
"Muratsan" Hospital Complex, Yerevan State Medical University, 114 Muratsan Street, Yerevan 0075, Republic of Armenia
Tel.: (+374 10453302), Fax: (+374 10565247)
E-mail: armansgn@yahoo.com

Introduction

Neonatal Necrotizing Enterocolitis (NEC) is a major cause of mortality and significant morbidity, often in premature infants with an inverse relationship to gestation, and may occur uncommonly in term infants. Despite advances in neonatal intensive care, its incidence, morbidity and mortality

has remained unchanged. NEC remains a major concern for neonatologists, surgeons, and gastroenterologists due to its high morbidity and mortality [*Huda S et al., 2014*]. NEC is a multifactorial nonspecific inflammatory condition - which characterized by variable damage to the newborns' intestinal tract, ranging from mucosal injury to full-thickness necrosis and perforation [*Springer S et al., 2017*]. NEC is the most common acquired emergency of neonates.

The etiology and pathogenesis of NEC remain controversial. It is believed that NEC is secondary to a complex interaction of multiple factors, notably prematurity, that result in mucosal damage, which leads to intestinal ischemia and necrosis [*Lee JS, Polin RA., 2003; Nowicki PT, 2005*]. The mucosal injury may be due to infection, intraluminal contents, immature immunity, release of vasoconstrictors and inflammatory mediators [*Caplan MS, MacKendrick W., 1994; Vieten D. et al., 2005*]. The loss of mucosal integrity allows 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 classic histological finding is coagulation necrosis present in over 90% of specimens [*Ballance W. et al., 1990*].

With NEC, the areas most commonly affected are the terminal ileum and the proximal ascending colon. The pattern of disease may involve a single isolated area or multiple discontinuous lesions. The most common histologic findings are associated with mucosal injury. These include coagulation necrosis of the mucosa with active and chronic inflammation, mucosal ulceration, edema, hemorrhage, and pneumatosis of the submucosa - Fig. 1A, 1B. [*Springer S et al., 2017*].

Advanced disease may result in full-thickness necrosis of the intestinal wall. Regenerative changes with epithelial regeneration, granulation tissue formation and fibrosis are seen in as many as two thirds of patients. This indicates an inflammatory process lasting several days, with concurrent areas of continuing injury and healing - **Fig. 1C.** [*Springer S et al., 2017*].

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*].

Necrotizing enterocolitis model was described in experiment on premature piglets received parenteral nutrition for 48-hours after delivery, followed by enteral feeds every three hours until death or euthanasia at 96-hours. Necropsy was performed on all animals immediately after death for NEC piglets and after euthanasia for piglets completing the 96-h

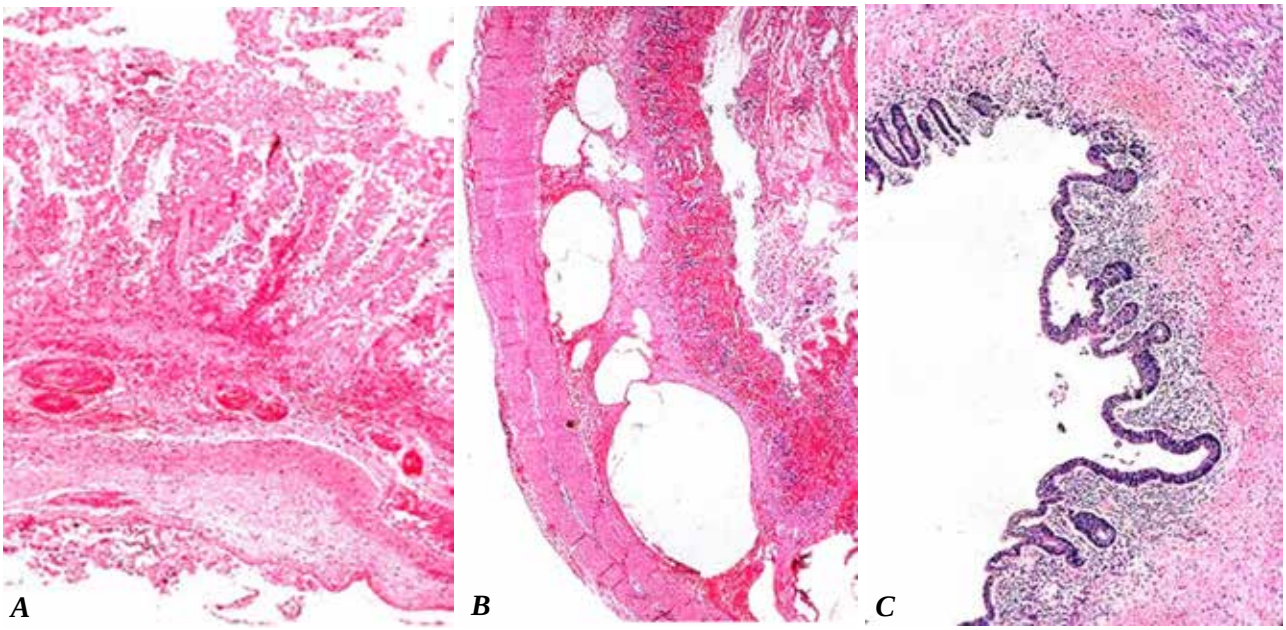

Figure 1. *- Histologic section of bowel mucosa, showing A - transmural necrosis, B - wall demonstrating pneumatosis, C- regeneration of normal cellular architecture. Photos courtesy of the "Department of Pathology, Cornell University Medical College" [Springer S et al., 2017].*

study period. Tissue samples were harvested from the stomach, the liver and the small bowel at proximal, middle, and distal regions between the proximal jejunum and colon for histologic analysis. Classification of NEC severity was based on a combination of clinical, histologic and pathological features [*Gay AN, et al., 2011; Zamora IJ., et al., 2015*]. Piglets were classified as fulminant-NEC (f-NEC), non-fulminant-NEC (nf-NEC) and NoNEC according to the severity of clinical and histologic features (Fig. 2 A - F) [*Gay AN, et al., 2011*].

The clinical manifestations of neonatal necrotizing enterocolitis in premature neonates include abdominal distension, ileus and bloody stool at several days of age. Compared to normal bowel at the left, bowel involved by NEC at the right shows hemorrhagic necrosis, beginning in the mucosa and extending to involve the muscular wall, with the potential for perforation. With NEC, the intestinal villi are seen to be disintegrating, with loss of cell nuclei, and reddish cytoplasm (Fig. 3).

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, Urlesberger B et al., 2006*].

The symptoms of necrotizing enterocolitis may resemble other digestive conditions or medical problems. Bell Staging continues to be used as the standard of practice to diagnose, stage, and treatment of NEC in the NICU. For descriptive purposes and for disease stratification, the Bell scoring system has been widely utilized, which assesses the degree of NEC severity as mild (Bell stage I), moderate (Bell stage II) or severe (Bell stage III) [*Niño D et al., 2016*].

BELL STAGE I–SUSPECTED DISEASE

Stage IA characteristics are as follows:

- Mild, nonspecific systemic signs such as apnea, bradycardia, and temperature instability are present
- Mild intestinal signs such as increased gastric

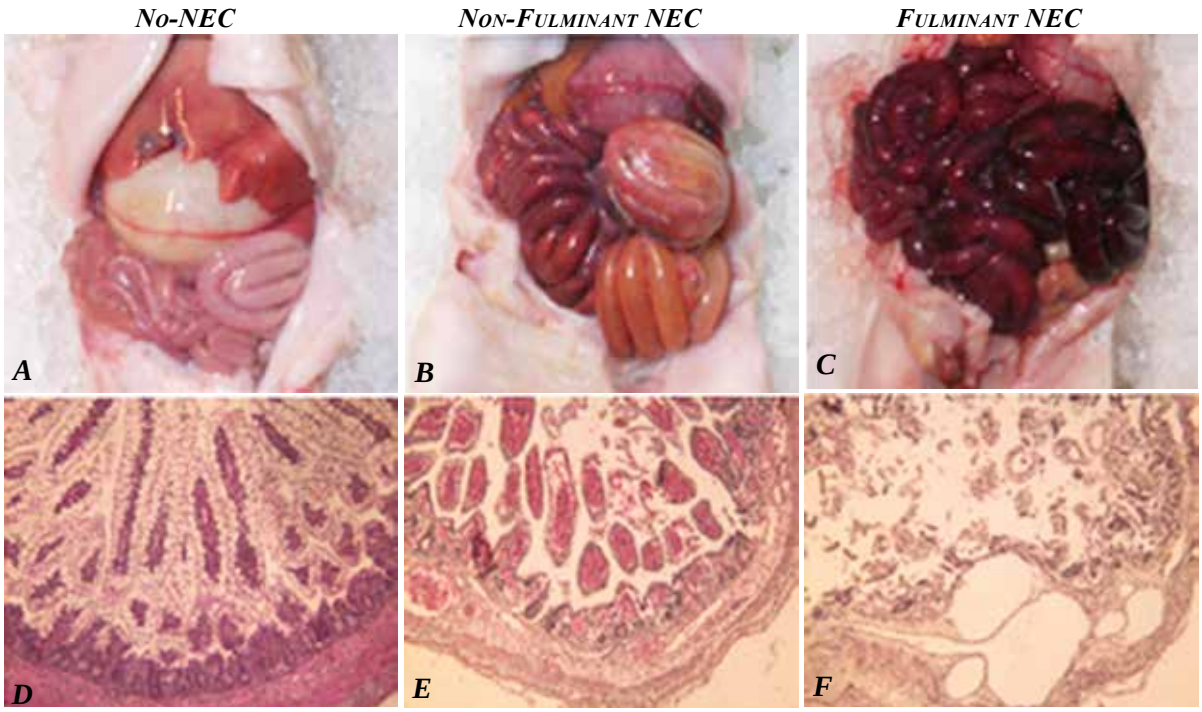

FIGURE 2. *Premature Pig Model of NEC—Gross and Histological Examination. A, Gross examination of the abdominal viscera of healthy No-NEC piglet. B, Gross examination of piglet with non-fulminant NEC shows inflamed and congested small bowel with areas of focal necrosis. C, Gross examination of piglet with fulminant NEC shows diffuse necrosis throughout the entire bowel. D, Histologic examination of the normal small intestine of a No-NEC piglet. E, Histologic examination of the small intestine of a piglet with non-fulminant NEC demonstrating moderate mucosal injury, blunting of villi and separation of the basement membrane. F, Histologic examination of the small intestine of a piglet with fulminant NEC demonstrating severe mucosal injury and diffuse pneumatosis [Gay AN, Lazar DA, Stoll B, Naik-Mathuria B, Mushin OP, Rodriguez MA, et al. Near-infrared spectroscopy measurement of abdominal tissue oxygenation is a useful indicator of intestinal blood flow and necrotizing enterocolitis in premature piglets. J Pediatr Surg. 2011; 46(6):1034–40. doi: 10.1016/j. jpedsurg. 2011.03.025 PMID: 21683194.].*

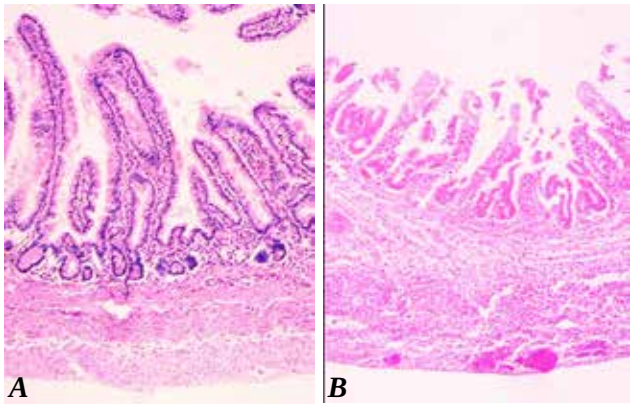

Figure 3. *Histology of normal bowel at the left and bowel involved by NEC at the right -shows hemorrhagic necrosis, beginning in the mucosa and extending to involve the muscular wall, with the potential for perforation.The Internet Pathology Laboratory for Medical Education, hosted by the University of Utah Eccles Health Sciences Library [Springer S et al., 2017].*

residuals and mild abdominal distention are present
- Radiographic findings can be normal or can show some mild nonspecific distention.

Stage IB diagnosis is the same as stage IA, with the addition of grossly bloody stool.

Bell stage II–definite disease

Stage IIA characteristics are as follows:
- Patient is mildly ill.
- Diagnostic signs include the mild systemic signs present in stage IA
- Intestinal signs include all of the signs present in stage I, with the addition of absent bowel sounds and abdominal tenderness
- Radiographic findings show ileus and/or pneumatosis intestinalis

This diagnosis is sometimes referred to as "medical" necrotizing enterocolitis as surgical intervention is not needed to successfully treat the patient.

Stage IIB characteristics are as follows:

Patient is moderately ill
- Diagnosis requires all of stage I signs plus the systemic signs of moderate illness, such as mild metabolic acidosis and mild thrombocytopenia
- Abdominal examination reveals definite tenderness, perhaps some erythema or other discoloration, and/or right lower quadrant mass
- Radiographs show portal venous gas with or without ascites

Bell stage III–advanced disease

This stage represents advanced, severe NEC that has a high likelihood of progressing to surgical intervention.

Stage IIIA characteristics are as follows:
- Patient has severe NEC with an intact bowel
- Diagnosis requires all of the above conditions, with the addition of hypotension, bradycardia, respiratory failure, severe metabolic acidosis, coagulopathy, and/or neutropenia
- Abdominal examination shows marked distention with signs of generalized peritonitis
- Radiographic examination reveals definitive evidence of ascites

Stage IIIB designation is reserved for the severely ill infant with perforated bowel observed on radiograph in addition to the findings for IIIA.

Although many serologic markers have been suggested for diagnosis of necrotizing enterocolitis, there is little consensus on which of these is potentially clinically useful [*Evennett N et al., 2009*]. 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*]. Based on literature one of the most promising seems to be intestinal fatty acid-binding protein, which is a cytoplasmic protein (part of enterocyte lipid metabolism) [*Heida FH, et al., 2015*]. In case of damage of enterocyte, the fatty acid binding protein is getting released into circulation and can be detected in urine. As enterocyte damage draw a parallel with intestinal necrosis, this biomarker has been recommended as useful noninvasive measure in the prediction of NEC [*Heida FH, et al., 2015*]. As NEC lasts to be one of the most critical areas of need 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 NECs development, prognosis, diagnosis, prevention and treatment monitoring including all promising biomarkers and possible analysis methods for strong outcome data [*Ng PC., 2014*].

The statistical review of NEC incidence was performed in NICU of "Muratsan" clinical complex of Yerevan State Medical University [*Harutyunyan A.S., 2017*]. We do not have official statistical data regarding the incidence of NEC in Armenia, but in our institution we had evidenced a lot of NEC cases and a high incidence of NEC- related mortality annually [*Harutyunyan A.S., 2017; Harutyunyan A.S. et al., 2018]*].

For a more detailed understanding the problem

of diagnosis and tactics of management of newborns with necrotic enterocolitis, it is necessary to combine clinical data with the results of histological examination.

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 No 8, 19.04.2018).

Study design and setting: The study was conducted at "Muratsan" Hospital 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, we analyzed 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. 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, we compared the referral clinical diagnose 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 discrepancy index (Di) was calculated using the following formula.

$$Di = \frac{N_i}{N_t} x100$$

where Di - discrepancy index, Ni - Number of incompatible cases, Nt - Total number of cases in the study

RESULTS

84 newborns with referral diagnose of NEC (from delivery hospital to NICU) and/or histologically proved NEC underwent autopsy at pathohistology department of medical center "Arabkir" during the time period of 12.01.2016 – 27.12.2017. Out of 84 (100%) – the number of newborns with referral diagnose of NEC were 38 (45.2% out of 84 cases). In 73 (86.9% out of 84 cases) newborns the NEC was diagnosed during autopsy and histological examination. The histological diagnose NEC matched with referral diagnose in 27 cases – 37% (out of 73 cases). In 46 (63% out of 73 newborns) cases NEC was present during autopsy and histological examination, but didn't manifested clinically or was not diagnosed before death. Diagnose NEC were not described histologically in 11 (13.1% out of 84 cases) cases of newborns with referral clinical diagnose of NEC. **(Chart)**

The rate of discordance (Di) between clinical suspicion and pathology diagnosis is 63% (Table).

1429 newborns were admitted to NICU of "Muratsan" clinical complex of YSMU during 2016 and 2017. The overall mortality rate was 9.9% (142) cases. NEC was clinically diagnosed in 141 (9.9%) patients. Out of these 142 newborns NEC was clinically described in 43 (30.3%) cases. 37 newborns underwent autopsy in pathohistology department of medical centre "Arabkir". The autopsy was not done to 6 newborns because parents didn't give their consent (but one case out of these 6 was proven NEC 3B by operation). Out of 37 newborns autopsy didn't reveal NEC histological changes in 11 cases.

CHART

Characteristics of patients according to histological investigation and clinical manifestation of NEC

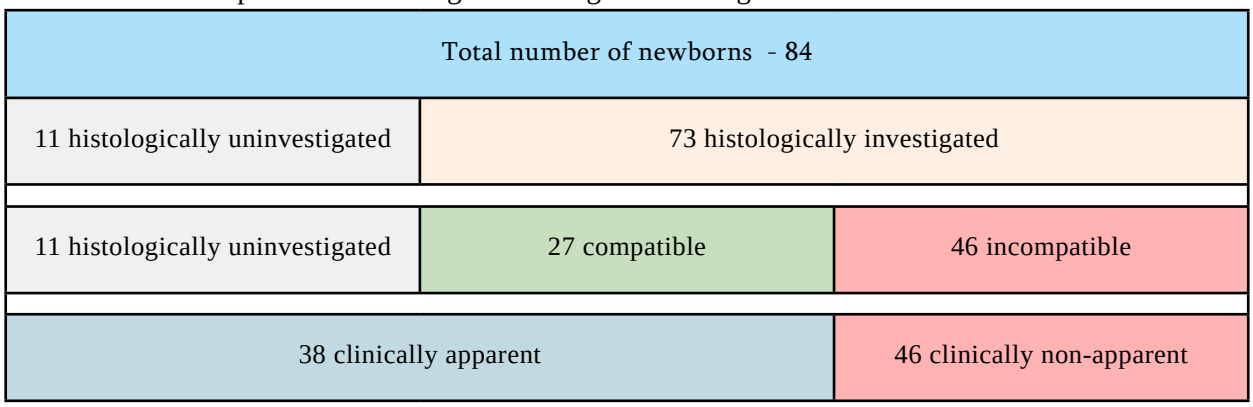

TABLE.

The diagnostic concordance distribution of histological examination during the period of review (n=73)

Clinical versus pathology diagnoses	Number of cases	
	(%)	Abs.
Compatible	37	27
Incompatible	63	46
Total	100	73

The rate of discordance (Di) between clinical suspicion and pathology diagnosis is 29%.

However, in 42 cases out of 142 lethal newborns during the autopsy the histological signs of different stages of NEC were detected, but were not included in clinical diagnose.

DISCUSSION

This is the first study to report on autopsy findings in a NEC patients and to compare the clinical and post-mortem diagnoses. We found that the rate of diagnostic discrepancies is very high (63%). Additionally, our results show the same tendency in other studies (29%). Also, another study has definitely shown that in the general structure of overall mortality, an estimated 30% was NEC at the stages which are not clinically manifested, which were relatively "missed" by physicians. There is an urgent need to emphasize that with current ability for diagnosis of NEC at clinically unapparent stages is very difficult.

One of the promising trends in early diagnosis of NEC is use of biomarkers.

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 addressed only on 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 K et al., 2014 a; b*].

Amongst this variety of molecules, based on literature one of the most promising seems to be intestinal fatty acid-binding protein, which is a cytoplasmic protein (part of enterocyte lipid metabolism) [*Heida FH, et al., 2015; Schurink M, et al., 2015*]. In case of damage of enterocyte, the fatty acid binding protein is getting released into circulation and can be detected in urine. As enterocyte damage draw a parallel with intestinal necrosis, this biomarker has been recommended as useful noninvasive measure in the prediction of NEC [*Schurink M, et al., 2015*]. As NEC lasts to be one of the most critical areas of need 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 NECs development, prognosis, diagnosis, prevention and treatment monitoring including all promising biomarkers and possible analysis methods for strong outcome data.

CONCLUSION

The results of our study denote that a high proportion of the incompatibilities of NEC diagnosis can be attributed to diagnostic limitations and are potentially avoidable with the use of modern diagnostic technics.

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