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Research

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The TNF, IL-1, IL-6 and HNP Peritoneal Fluid Concentrations in Premature Infants Treated with Peritoneal Drainage for Intestinal Perforation-Preliminary Study

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ABSTRACT

Purpose: The purpose of the study was to analyze the pro-inflammatory cytokines (TNF, IL-1, IL-6 and HNP) profile in the peritoneal fluid in preterm infants with intestinal perforation due to Necrotizing enterocolitis (NEC) or Spontaneous Intestinal Perforation (SIP) treated with percutaneous peritoneal drainage.
Methods: 6 infants with intestinal perforation due to NEC or SIP treated with peritoneal drainage were analyzed prospectively. Samples of peritoneal fluid were drawn for 36 hours after drain placement. Concentrations of cytokines: TNF, IL-1, IL-6 and Human Neutrophil Peptide (HNP-1) were determined using enzyme like immunoassay technique.
Results: The peritoneal fluid concentrations of TNF were 0-11,963 pg/mL (mean 960±2.249 pg/mL), IL-1 were 0-10,390 pg/mL (mean 968±2.219 pg/mL), IL-6 were 0.3-1.660 ng/mL (mean 184±335 ng/mL), HNP were 0.04-18.36 µg/mL (mean 3.18±4.32 µg/mL). Peritoneal fluid TNF level>1.000 pg/mL, IL-1>500-1.000 pg/mL and IL-6>200-500 ng/mL were associated with fatal outcome.
Conclusions: In analyzed material the pattern of pro-inflammatory cytokines concentrations in peritoneal fluid after intestinal perforation in preterm infants was not identified. The highest peritoneal fluid TNF, IL-1 and IL-6 concentrations were related to fatal outcome in the most premature infants with unspecified extent of intestinal necrosis.

KEYWORDS: Peritoneal drainage; Intestinal perforation; Tumor necrosis factor; Interleukin 1**;** Interleukin 6**;** Human neutrophil peptide (HNP-1).

ABBREVIATIONS: NEC: Necrotizing enterocolitis; SIP: Spontaneous Intestinal Perforation; HNP-1: Human Neutrophil Peptide; NICU: Neonatal Intensive Care Unit; TNF: Tumor Necrosis Factor; IL-1: Interleukin 1; IL-6: Interleukin-6; IL-18: Interleukin-18; IVH: Intraventricular hemorrhages; HR: Heart Rate; MAP: Mean Arterial Pressure; HNP: Human Neutrophil Peptide.

INTRODUCTION

Necrotizing enterocolitis (NEC) and spontaneous intestinal perforation (SIP) are common gastrointestinal diseases in the preterm infants – in 1-3% of Neonatal Intensive Care Unit (NICU) patients.[1] Traditional surgical management of perforated intra-abdominal viscus includes laparotomy with debridement of the offending intestinal segment and formation of stomas.[2] In 1975, Marshall introduced peritoneal drainage as a way to stabilize and improve

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the systemic status of premature infants with intestinal perforation due to NEC or SIP before formal laparotomy.[3] In 1977 Ein proved significant impact of bedside peritoneal drainage on premature infants'survival.[4] Experience in utilizing peritoneal drainage in numerous centers is growing; however its mechanism of action remains unknown.

Inflammation ongoing within intestinal wall tissues in NEC or SIP activates inflammatory cascade and releases numerous pro-inflammatory cytokines, which have also systemic effect.[5] The number of cytokines and their role in the pathogenesis of hemodynamic instability in patients with NEC or SIP are still undefined.[6] Elevated plasma levels of Tumor Necrosis Factor (TNF), Interleukin 1 (IL-1), Interleukin 6 (IL-6) and Interleukin18 (IL-18) in infants with NEC reflect their local production in damaged intestinal tissue.[7,8] Relation with symptoms of hemodynamic instability in infants with NEC treated with traditional laparotomy and pro-inflammatory mediators like TNF and IL-6 (which were similar to those in bacterial sepsis) was confirmed in many studies.[8] Correlation between cytokines (TNF, IL-1, IL-6) concentrations and severity of systemic inflammation was reported in many *in vitro* and *in vivo* studies.[9,10] The role of inflammatory response mechanism in NEC and SIP pathogenesis was defined in animal model studies, in which intraperitoneal injection of anti-TNF antibodies resulted in increased survival of rat infants with NEC.[11]

The evaluation of serum cytokines profile requires multiple drawing of blood samples, which is unacceptable in very low birth weight infants. The use of peritoneal fluid obtained during abdominal drainage to determine cytokines concentration is non-invasive method compared to evaluation in serum. Data concerning peritoneal fluid concentrations of pro-inflammatory cytokines in NEC is uncommon.[6] Whereas data concerning cytokines profile in the peritoneal fluid in NEC or SIP newborns is not available.

The aim of the study was to assess the pro-inflammatory cytokines profile in the peritoneal fluid as a result of bowel perforation in newborns with NEC or SIP treated with bedside peritoneal drainage.

PATIENTS AND METHODS

During last three-year period, 10 infants with suspected intestinal perforation related to NEC or SIP were treated with bedside peritoneal drainage. Only 6 patients were prospectively analyzed (3 males and 3 females) as in 3 cases consents weren't obtained, in 1 case intestinal perforation wasn't confirmed by radiological examination.

Laparotomy is preferred method of treatment of bowel perforation due to NEC or SIP in author's department. However, it cannot be performed in infants with symptoms of hemodynamic instability. In these rare cases peritoneal drainage is used as initial treatment before formal laparotomy.

All analyzed patients were born prematurely (gestational age: 23-29 weeks, mean 25.5±2.4 weeks), with birth weight 580-1.300 g (mean 823±263 g) and with asphyxia (mean APGAR score at first minute of life 3.5±1.7). Mechanical ventilation after birth was required in 5 infants. Intraventricular hemorrhages (IVH) were revealed in all patients, in 4 children IVH grade 4.

Intestinal perforation was confirmed by radiological examination in all infants.

Peritoneal drain was placed between 6-21 days of age (mean 12.5±5.9 days). During drain placement 4 infants presented symptoms of metabolic acidosis (pH arterial blood: 7.07-7.285, mean 7.17±0.03). All patients required ventilation supporting: 4 infants were ventilated in SIMV mode, remaining 2 in HFO mode. Saturation (SaO_2) was 87-96% (mean 92.5±3%), Heart Rate (HR) was 147-206 per minute (mean 173±20) and Mean Arterial Pressure (MAP) was 16.78 mm Hg (mean 37.5±19.3 mm Hg) at the moment of drain placement. 4 infants required administration of at least one catecholamine, in one patient diuretic therapy was necessary (Table 1).

In four cases drain was placed in local NICU due to patient's hemodynamic instability. Drain placement was performed under sterile conditions, sedation and local anesthesia in all patients. Peritoneal fluid contained gas in 5 cases and meconium was observed in 4 of them. 3 infants died (in day 1., 2. and 5. after drain placement), 2 of them presented clinical symptoms of NEC, one of SIP confirmed in X-ray and ultrasound examinations, however autopsy wasn't performed. 3 patients survived and underwent laparotomy 19-48 hours after the drain placement. SIP was confirmed in 2 patients and segmentary NEC in

Patient	GA	birth weight	Apgar SCORE	IVH	pH	SaO_2	MAP	NEC/SIP	survive
p1	26	940	6	IV	7,195	87	36	SIP	+
p2	28	930	4	II	7	94	29	NEC	+
p3	23	580	1	IV	7,12	94	29	SIP	died
p4	23	590	2	IV	7,166	94	16	NEC	died
p5	24	600	3	IV	7,285	96	78	NEC	died
p6	29	1300	5	III	7,28	90	37	SIP	+

Table 1: Baseline characteristics of the patients treated with peritoneal drainage.

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one child during surgical intervention. In all survived patients resection of perforated bowel were performed and stomas were formed (Table 1).

Samples of peritoneal fluid were drawn every 3 hours during initial 12 hours followed by every 6 hours later on. Collection of samples was continued for 36 hours after drain placement. Drawn peritoneal fluid in portions of 0.2-1.0 mL was centrifuged and stored at -86°C.

Quantikine ELISA kits R&D (Mineapolis, MN, USA) were used to determine TNF, IL-1 and IL-6 concentrations using enzyme-linked immunoassay technique. Concentration of Human Neutrophil Peptide (HNP) was measured using Human Neutrophil Peptide 1-3 ELISA kit Hycult Biotechnology (Uden, Netherlands). Concentrations of cytokines: TNF, IL-1, IL-6 and HNP were determined in all collected samples.

Juxtaposition of peritoneal fluid volume of all collected samples and outcome of treatment with bedside peritoneal drainage is shown in the Table 2.

RESULTS

TNF

Concentrations of TNF in peritoneal fluid were 0-11,963 pg/mL (mean 960±2,249 pg/mL). The highest concentrations were observed in patient 4, who symptoms of NEC presented and died in day 2, after drain placement. The lowest concentrations were observed in patient 1 in whom SIP was confirmed during laparotomy (Table 3).

IL-1

Concentrations of IL-1 in peritoneal fluid were 0-10,390 pg/mL (mean 968±2,219 pg/mL). The highest concentrations were observed in patient 4. The lowest concentrations were observed in patient 2 in whom segmentary NEC was confirmed during surgical intervention after 24 hours treatment with bedside peritoneal drainage (Table 4).

		time (h)									follow-up
	patient	0	3	6	9	12	18	24	30	36	
Volume (mL)	p1	5	6	3,5	4	2	4	2	3,5	1,7	laparotomy in 48. hours
	p2	18	4	1	4	0	0	laparotomy			-
	p3	3,2	1,2	1	0	1,2	0	0	death		-
	p4	5	1	0.5	0	1,2	2,5	death			-
	p5	0,5	1	1	3	1,5	5	8	3	1,5	death in day 5.
	p6	18	2,5	0	1	1,5	5,5	laparotomy			-

Table 2: Sample volume summary and treatment outcome.

	patient	time (h)									NEC/SIP	survive
		0	3	6	9	12	18	24	30	36		
TNF (pg/mL)	p1	0	16	0	22	27	0	0	0	40	SIP	+
	p2	70	128	53	90						NEC	+
	p3	1465	3903	5499		721					SIP	died
	p4	11963	2934	512		320	132				NEC	died
	p5	0	222	20	216	2752	1096	67	38	36	NEC	died
	p6	716	458		354	414	302				SIP	+

Table 3: Concentrations of TNF, diagnosis and survival in patients treated with peritoneal drainage.

	patient	time (h)									NEC/SIP	survive
		0	3	6	9	12	18	24	30	36		
IL-1 (pg/mL)	p1	0	0	11	28	28	165	193	185	238	SIP	+
	p2	90	16	48	34						NEC	+
	p3	1467	1100	982		449					SIP	died
	p4	10390	7834	5456		2139	565				NEC	died
	p5	0	13	10	102	741	1190	363	101	105	NEC	died
	p6	475	212		93	31	27				SIP	+

Table 4: Concentrations of IL-1, diagnosis and survival in patients treated with peritoneal drainage.

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IL-6

Ranges of IL-6 concentrations were 0.3-1,660 ng/mL (mean 184±335 ng/mL). The highest concentrations were observed in patient 4 and the lowest in patient 6, who underwent laparotomy in 19th hour after drain placement and SIP was confirmed (Table 5).

HNP

Concentrations of peritoneal fluid HNP were 0.04-18.36 µg/mL (mean 3.18±4.32 µg/mL). The highest concentrations were observed in patient 6 and the lowest in patient 2 (Table 6).

Any particular tendency for TNF, IL-1, IL-6 and HNP wasn't observed throughout drainage duration. Furthermore the maximum concentrations were noted in different time points for each patient.

DISCUSSION

Inflammation ongoing within intestinal wall tissues in NEC or SIP releases numerous mediators including the most pro-inflammatory cytokines like: TNF, IL-1 and IL-6. These cytokines released in the intestine, implicated in the hepatic production of acute-phase proteins, in the enhancement of T-cell proliferation and in the promotion of B-cell antibody secretion. Pro-inflammatory cytokines also stimulate macrophages and neutrophils to produce large quantities of reactive and cytotoxic species of oxygen and nitrogen, which are important element of inflammatory response. However, these cytotoxic compounds may also cause substantial host tissue damage and multiple organ dysfunction, especially when their production remains unchecked when the host defense system is immature, as commonly seen in stressed neonates in the NICU.[5] In these cases cytokines and cytotoxic species like Nitrogen Oxide (NO) could affect hemodynamic instability in preterm infants. Symptoms of progressive multiple organ dysfunction described in patients with NEC or SIP result from inflammatory damage in intestinal tissue but could be also consequence of large production of pro-inflammatory mediators which have systemic effect.

Additionally intestinal inflammation with secondary failure of the gut barrier expedites bacterial translocation from the gut into the systemic circulation and releases human neutrophil peptides (HNP) from neutrophil granules. Furthermore, antimicrobial activity HNP participates in the regulation of chemotaxis and triggering of the inflammatory reactions. Correlation of elevated HNP in peritoneal fluid with pro-inflammatory cytokines like TNF, IL-6 and IL-8 was described in patients with endometriosis.[12]

The inflammatory process within intestinal wall due to NEC or SIP concerns also the capillaries and may result in leaking of plasma cytokines into the peritoneum enhancing the levels of peritoneal fluid cytokines. The study comparing the cytokines profile in peritoneal fluid and serum requires multiple drawing of blood samples, which is unacceptable in very low birth weight infants.

In our study, the infants who died during treatment with peritoneal drainage had lower gestational age, birth weight and Apgar scores than infants who survived and underwent subsequent laparotomy. Each patient who died required mechanical ventilation in the first minute after birth and every patient had IVH IV° detected. Differences in clinical condition weren't observed (Table 1).

IL-6 (ng/mL)	patient	time (h)									NEC/SIP	survive
		0	3	6	9	12	18	24	30	36		
	p1		88,6	89,1	97,8	106,5	154,1	67,1	33,3	54,8	SIP	+
	p2	95	90	233	32,9						NEC	+
	p3	508	90	30	25,1						SIP	died
	p4	1022	1660			24	39				NEC	died
	p5	0,3	9,2	12,8	53,3	396	732	248	120,3	109,6	NEC	died
	p6	5	12		35	6	2				SIP	+

Table 5: Concentrations of IL-6, diagnosis and survival in patients treated with peritoneal drainage.

HNP (µg/mL)	patient	time (h)									NEC/SIP	survive
		0	3	6	9	12	18	24	30	36		
	p1		0,35	1,03	0,19	0,3	0,64	0,19	0,46	0,24	SIP	+
	p2	0,21	0,18	0,21	0,24						NEC	+
	p3	10,46	6,47	6,11		1,17					SIP	died
	p4	11,8	10,81	0,04		2,6	1,3				NEC	died
	p5		0,16		0,43	1,01	3,16	2,2	2,1	1,13	NEC	died
	p6	18,36	7,04		8,03	2,85	3,6				SIP	+

Table 6: Concentrations of HNP, diagnosis and survival in patients treated with peritoneal drainage.

The analysis presented revealed higher concentrations of TNF, IL-1 and IL-6 in patients who died comparing to patients who survived, although correlation between cytokines concentrations and treatment outcome wasn't defined.

The highest concentrations of TNF, IL-1 and IL-6 were observed in patient 4, who presented clinical and radiological symptoms of NEC and died in second day after drain placement. He was born in the 23rd gestational week as extremely low birth weight infant with asphyxia. The autopsy wasn't performed and therefore correlation of cytokines concentration and the extent of intestinal damage couldn't be determined.

The lowest concentrations were observed in patient 1 for TNF, in patient 2 for IL-1 and HNP and in patient 6 for IL-6. All of these patients survived and underwent laparotomy. During surgical intervention SIP in patients 1 and 6 and segmentary NEC in patient 2 was confirmed. Their gestational week, birth weight and Apgar score were higher than in patient 4.

In our study peritoneal fluid TNF level>1,000 pg/mL, IL-1>500-1,000 pg/mL and IL-6>200-500 ng/mL were associated with fatal outcome. Similar differences were described by Morecroft in analysis of 18 infants with NEC. In his study plasma IL-6 levels were significantly higher in infants with Bell stage III disease compared to infants with Bell II stage disease (3,127 pg/mL *vs.* 127 pg/mL), nonetheless there was no discernible pattern in plasma TNF concentration in both groups.[13] In another study of 24 infants with NEC, Morecroft described plasma IL-6 levels tended to be higher in infants who died compared to survivors (2,835 pg/mL *vs.* 1,023 pg/mL).[9]

The maximum rather than decreasing concentrations of these cytokines would have prognostic value in analyzed material. Tendency of cytokines concentrations as well as time point of maximum concentration weren't relevant. In presented analysis, HNP concentration seems not to have any value in children with NEC or SIP.

Furthermore, our study revealed higher mean peritoneal fluid concentrations of TNF, IL-1 and IL-6 comparing to the mean plasma concentrations of these cytokines in patients with bacterial Gram-positive sepsis. The results presented by de Bont were: TNF 560 pg/mL, IL-1 18 pg/mL, IL-6 79.7 ng/mL comparing to 960 pg/mL, 968 pg/mL, 184 ng/mL in our material, respectively.[14]

The results of peritoneal fluid cytokines levels demonstrated in this study are considerably higher than plasma cytokines levels in patients with NEC described in literature. Sharma described data of 27 children- mean plasma concentrations of cytokines in NEC which were accordingly: for TNF: 277 pg/mL *vs.* 1,147 pg/mL, for IL-1: 499 pg/mL *vs.* 1,622 pg/mL and for IL-6: 316 pg/mL *vs.* 286,900 pg/mL in presented NEC patients.[5] Higher peritoneal fluid level of these cytokines compared to plasma levels may suggest an advantage of their local production in damaged intestinal tissues.

The analysis of cytokine profiles presented in this study is an attempt to explain beneficial effect of using bedside peritoneal drainage in newborn with bowel perforation as a result of NEC or SIP. Regardless this is a pilot study, an evacuation of cytokines with peritoneal fluid seems to be not the only factor effecting beneficially (gradual decrease of cytokines concentrations wasn't observed clearly). The relationship between cytokines concentration and the degree of infants immaturity as well as the extent of intestinal damage remains unknown.

This study was approved by Local Ethics Committee.

CONCLUSIONS

In analyzed material the pattern of pro-inflammatory cytokines concentrations in peritoneal fluid after intestinal perforation in preterm infants was not identified.

The highest peritoneal fluid TNF, IL-1 and IL-6 concentrations were related to fatal outcome in the most premature infants with unspecified extent of intestinal necrosis.

CONFLICTS OF INTEREST: None.

CONSENT

We hereby confirm that all patients' parents participating in study "The TNF, IL-1, IL-6 and HNP peritoneal fluid concentrations in premature infants treated with peritoneal drainage for intestinal perforation- preliminary study" were consented relevantly and all signed consent forms are retained.

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