The Role of Monocyte Count in Predicting the Severity of Necrotizing Enterocolitis in Egyptian Preterm Neonates: A Multicenter Study

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Abstract

Background: Necrotizing enterocolitis (NEC) is one of the most serious and common gastrointestinal medical/surgical emergencies in preterm neonates.

Aim of Study: To determine the diagnostic accuracy of monocyte count in diagnosis of NEC and whether the degree of monocyte count drop at the onset of necrotizing enterocolitis correlates with severity of disease which in turns will help in early diagnosis and treatment of the disease.

Patients and Methods: It was conducted on 150 preterm neonates in Neonatal Intensive Care Units of Abu El Reesh El Mounira Children Hospital, Cairo University Hospital and Misr University for Science and Technology Hospital.

Results: The data first highlighted the presence of metabolic acidosis in both groups. Significantly more infants in the NEC group experienced metabolic acidosis (78.0%) compared to the control group (46.0%), with a highly significant *p*-value of less than 0.001. This indicated a strong positive association between metabolic acidosis and the development of NEC. Metabolic acidosis, which reflected an imbalance between acid production and elimination in the body, might indicate compromised perfusion and oxygenation of tissues, potentially contributing to the pathogenesis of NEC. The study revealed that monocyte values at the onset of NEC were lower in the NEC group compared to the control group. In this analysis, Stage I and Stage II NEC cases were compared in terms of their monocytes values. The mean monocyte count for Stage I NEC was 12.71, while for Stage II NEC, it was 10.83. The data indicated a statistically significant difference in monocyte count between these two stages (*p*-value = 0.010), with a mean difference of 1.881. The confidence interval of 95% ranged from 0.161 to 3.6, which suggested that the true difference in monocyte count between the two stages could lie within this interval with 95% confidence. This result implied that Stage II NEC cases had a lower mean monocyte count compared to Stage I NEC cases.

Conclusion: The findings collectively suggest that interrupted feeding, formula feeding, sepsis, specific bacterial strains, and the use of inotropes and blood transfusions are strongly linked to the incidence of NEC in preterm neonates. On the other hand, certain factors such as gender, gestational age, and Apgar scores may not be significantly linked to NEC. Additionally, the analysis highlights the ROC of monocytes values with a diagnostic accuracy of 0.636 for NEC and 0.793 for stage II NEC which demonstrates excellent sensitivity and specificity. These insights contribute to a deeper understanding of the multifaceted nature of NEC development and highlight the importance of targeted interventions, infection control measures, and close monitoring to mitigate its risk in neonatal care settings.

Key Words: Monocyte count – Necrotizing enterocolitis – Preterm neonates.

Introduction

NECROTIZING enterocolitis (NEC) is one of the most serious and common gastrointestinal medical/surgical emergencies in preterm neonates, which affects 6%-7% of extremely low birth weight neonates. NEC is one of the major causes of mortality in neonates born prior to 32 weeks of gestation or with birth weight less than 1500 grams [1].

The clinical presentation can be insidious or fulminant and ranges from abdominal distension and bloody stools to intestinal perforation, peritonitis, sepsis, shock, and death. The severity is often categorized into "medical" and "surgical" NEC. At present, the "diagnosis" relies on a constellation of clinical signs, radiologic findings and laboratory data (taken together, called "modified Bell's staging criteria") [2].

In the premature intestine, developmental limitations in both the innate and adaptive arms of the mucosal immune system increase the risk of inflammatory injury and NEC. Systemic inflammation during NEC has been associated with several hematological abnormalities with altered counts of platelets, leukocytes including monocytes, neutrophils, and lymphocytes; and coagulopathy, and

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these changes may convey important diagnostic and prognostic information [3].

Circulating immune cells likely contribute to illness progression and may be helpful biomarkers in diagnosing infants suspected of having NEC and/ or stratifying disease severity. Prior studies have shown that an acute drop from baseline in monocyte count is suggestive of NEC diagnosis [4].

It has been suggested that this reduction is secondary to intestinal pathology. Monocyte-derived intestinal macrophages participate in the gut wall infiltration classically seen in NEC and once infiltration occurs, peripheral blood monocytes may be called upon to replete the intestinal monocyte pool [5].

Awareness of diagnostic problems and lack of a valid and measurable biomarker for early diagnosis of NEC forces specialists to treat neonates with abdominal and perilous symptoms, which is an expensive and unpleasant approach. Therefore appropriate and rapid diagnostic methods are needed to prevent unnecessary treatments [1].

Aim of the work:

To determine the diagnostic accuracy of monocyte count in diagnosis of NEC and whether the degree of monocyte count drop at the onset of necrotizing enterocolitis correlates with severity of disease which in turns will help in early diagnosis and treatment of the disease.

Patients and Methods

Study design:

Observational retrospective case-control study.

Population and location of the study:

This study was conducted on 50 neonates with a gestational age less than 37 weeks who are diagnosed with necrotizing enterocolitis according to Bell's staging, and for each case, 2 controls were identified based on gestational age, birth weight, date of admission and the presence of any medical condition other than NEC, in Neonatal Intensive Care Units of Abu El Reesh El Mounira Children Hospital, Cairo University Hospital and Misr University for Science and Technology Hospital during a period of 6 consecutive months from April 2023-September 2023.

Inclusion criteria in the NEC Group:

Neonates with a gestational age of less than 37 weeks who are admitted in the previously mentioned neonatal intensive care units (NICUs) and diagnosed with necrotizing enterocolitis according to Bell's staging.

Inclusion criteria in the Control Group:

Two controls were identified for each case based on gestational age, birth weight, date of admission and the presence of any medical condition other than NEC.

Exclusion criteria for both groups:

Neonates with congenital or acquired anomalies of the gastrointestinal tract (omphalocele, gastroschisis, tracheoesophageal, gastrointestinal perforation, intestinal obstruction).

Neonates in both groups will be subjected to the following: (a) Full medical history: Personal History (name, gender and order of birth), prenatal history (maternal diseases, infections or medications), natal history (PROM >18 hours, maternal fever >38 c, prolonged second stage of labour and any risk factors of infection), gestational age, mode of delivery (normal vaginal delivery [NVD] or cesarean section), postnatal history (Apgar score at 1 and 5 minutes, aggressive resuscitation, respiratory distress, cyanosis, fever, jaundice), family history (maternal age, consanguinity, similar conditions, other sibling deaths), present history which includes most common symptoms of necrotizing enterocolitis and history of antibiotics given (type-number of doses-duration). (b) Clinical examination: Confirmation of gestational age by Ballard score, in addition to birth weight assessment and measuring of abdominal girth, general examination and vital signs and complete systemic examination including cardiovascular, respiratory, abdominal and neurological examination. (c) Laboratory Investigations: 4mL of blood were collected and sent for: Complete blood count with differential leucocytic count and platelet count (the first one as a baseline prior to disease onset and the other one at time of illness onset), CRP quantitative assay, electrolytes and blood gases and blood culture using blood culture vials (BD BactecPeds Plus TM/F culture vials; Becton Dickinson, Mary-land, USA). (d) Imaging to neonates in NEC group only: Erect Abdominal X-ray at time of illness onset and other ones for follow-up that should be ordered every 8-12 hours for the first 48 hours in stage 1 and every 6-8 hours for the first 24-48 hours in stage 2 and 3.

Research involve: Human participants and biological samples.

Type of consent of study participants: Written consent.

Potential risks: Risk of pain at injection site.

Confidentiality of data: The Confidentiality of the Research Participants will be preserved in our research by: Data will be collected from the patients after signing written consents by any of the parents, any data that makes the patient identifiable will be omitted, patients will be numerically coded during the data collection phase, these codes will be used in all subsequent research phases and files will be locked and secured.

Study outcomes: Primary outcomes: To determine diagnostic accuracy of monocyte count in neonates with necrotizing enterocolitis and to correlate the extent of monocyte count change with the severity of NEC. Secondary outcome parameters: Identify different risk factors that may lead to NEC, identify comorbidities associated with NEC, identify feeding characteristics associated with NECand identify other biomarkers that may aid in the diagnosis of NEC.

Sample size: Using G power program for sample size calculation at power 80%, alpha error at 5% and after reviewing pervious study results Tajalli et al. [1] and using the following equation. Sample Size = [z2 * p(1-p)] / e2 / 1 + [z2 * p(1-p)] / e2 * N]. N = population size. z = z-score. e = margin of error. p=Standard of deviation. So the least sample size was found to be 50 NEC cases and 100 neonates in the control group.

Statistical analysis: Data was collected, coded then entered as a spread sheet using Microsoft Excel 2016 for Windows, of the Microsoft Office bundle; 2016 of Microsoft Corporation, United States. Data was analyzed using IBM Statistical Package for Social Sciences software (SPSS), (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp). The Kolmogorov-Smirnov test was used to verify the normality of distribution. Continuous data was expressed as mean ± standard deviation, median & IQR while categorical data as numbers and percentage. A statistical value <0.05 was considered as significant.

Analytic statistics: Chi-square test; used to study the association between two qualitative variables. Student *t*-test: For normally distributed quantitative variables, to compare between two studied groups. Mann Whitney test: For abnormally distributed quantitative variables, to compare between two studied groups. Correlation analysis (using Spearman's method): To assess the strength of association between two quantitative variables. The correlation coefficient denoted symbolically "*r*" defines the strength and direction of the linear relationship between two variables.

The ROC Curve (receiver operating characteristic) provides a useful way to evaluate the Sensitivity and specificity for quantitative diagnostic measures that categorize cases into one of two groups.

Results

This study was conducted on 150 neonates who were admitted to neonatal intensive care units of Abu El Reesh El Mounira Children Hospital, Cairo University Hospital and Misr University for Science and Technology Hospital. The cases were divided into two groups: NEC group: Included 50 neonates with necrotizing enterocolitis (NEC). Control group: Included 100 healthy neonates.

This table shows comparison between the two studied groups regarding demographic data. The mean gestational age in NEC group and control group were 30.98±2.14 weeks and 31.04±2.13 weeks respectively. Regarding gender, male gender tended to be lower in NEC group. There was higher prevalence of CS as a mode of delivery in both groups (84% Vs 76 respectively). But yet, there was no statistically significant difference between the two groups regarding gestational age, gender as well as mode of delivey (*p*>0.05).

This table shows the median level of APGAR score at 1, 5 & 10min. in NEC group was 2, 5 & 7 respectively while in control group was 2, 5 & 8 respectively. The median birth weight in NEC group and control group was 1300gm and 1435 gm respectively. There was no significant differences between the two studied groups regarding APGAR score at 1, 5 & 10 min and birth weight (*p*>0.05).

This table shows interrupted feeding significantly higher in NEC group compared to control group (88% vs 20, *p*<0.001) while start of feeding and delay of feeding did not differ significantly between the two groups. There was a statistically significant difference between them regarding type of feeding (*p*=0.001) as formula use was significantly higher in NEC group. The median (range) total NPO days before diagnosis of NEC was 4 (0-8 days).

This table shows no significant differences between the two groups regarding the mentioned comorbidities except for PDA that was significantly higher in NEC group (*p*=0.002) and sepsis that was also significantly higher in NEC group (100% vs 34%, *p*<0.001).

This table shows Bell's staging for NEC, 14 (28%) neonates had stage I NEC (10 cases with stage IA and 4 cases with stage IB) while 36 (72%) neonates had stage II (22 cases had stage IIA and 14 cases with stage IIB). The median (range) age at onset of NEC was 10 (3-21) days and the median (range) weight was 1330 (850-2660) gm.

This table shows that the baseline monocytes and monocytes at NEC onset were significantly declined in NEC group compared to control group (*p*=0.006, *p*<0.001 respectively). In addition, the incidence of metabolic acidosis was significantly higher in NEC group compared to control group (78% vs 46%, *p*<0.001). A higher significant proportion of neonates with culture-positive for pathogens in NEC group was also observed (*p*<0.001).

This table shows asignificant decline in monocytes at NEC onset compared to its level at baseline (*p*=0.016) in NEC group while there was significant increase in monocytes at follow up compared to its level at baseline (*p*=0.003) in control group.

This table shows that monocyte countwas significantly declined in stage II NEC compared to stage I NEC (*p*=0.010).

Receiver operating characteristic (ROC) analysis was performed to determine the value of monocyte count in detection and prediction of NEC. Monocyte count can differentiate NEC group from control group with 74% sensitivity and 51% specificity at a threshold value of 12 with AUC was 0.636 and was highly significant (*p*=0.004).

Receiver operating characteristic (ROC) analysis was performed to determine the value of monocyte count in staging of NEC. Monocyte count can differentiate stage I NEC from stage II NEC with 57.1% sensitivity and 94.4% specificity at a threshold value of 11 with AUC was 0.793 and was highly significant (*p*<0.001).

This table shows a higher significant proportion of neonates needed inotropic support and blood transfusion was found in NEC group (*p*<0.001).

Table (1): Demographic characteristics among the two studied groups.

	NEC group (No. = 50)		Control group (No. = 100)		Test value	*p*-value
	No.	%	No.	%		
Gender:					X^2=1.345	0.246
Male	24	48	58	58		
Female	26	52	42	42		
Gestational age (weeks):					ZMWU=0.381	0.704
Mean ± SD	30.98±22.14		31.04±2.13			
Median (IQR)	31 (30-32)		31 (30-32)			
Range	27-36		27-36			
Mode of delivery:					X^2=1.271	0.260
NVD	8	16	24	24		
CS	42	84	76	76		

*p≤0.05 is statistically significant.
**p≤0.01 is high statistically significant.
SD : Standard deviation.
IQR : Interquartile range.
NVD: Normal vaginal delivery.
CS: Cesarean Section.
X^2: Chi- Square test.
zMWU: Mann-Whitney U test.

Table (2): Comparison between the two studied groups regarding APGAR score and birth weight.

	NEC group (No. = 50)							Control group (No. = 100)							Mann-Whitney U Test	
	Mean	± SD	Median	IQR		Min.	Max.	Mean	± SD	Median	IQR		Min.	Max.	Test value	*p*-value
APGAR score 1 min.	2.04	1.07	2	1	3	0	5	2.38	1.39	2	1	3	1	7	1.193	0.233
APGAR score 5 min.	4.79	1.2	5	4	5	3	9	4.9	1.53	5	4	6	2	9	0.343	0.732
APGAR score 10 min.	7.19	1.3	7	6	8	5	10	7.42	1.41	8	6	9	5	10	1.161	0.246
Birth Weight (gm)	1379.2	390.7	1300	1180	1500	850	2800	1455.1	356.3	1435	1220	1660	830	2300	1.855	0.064

*p≤0.05 is statistically significant.
**p≤0.01 is high statistically significant.
SD : Standard deviation.
IQR : Interquartile range.

Table (3): Feeding characteristics among the two studied groups.

	NEC group (No. = 50)		Control group (No. = 100)		Test value	p-value
	No.	%	No.	%		
Start of feeding (days) :						
Mean ± SD	2.44±0.97		2.16±1.07		t=1.556	0.122
Median (IQR)	2 (2-3)		2 (2-2)			
Range	1-5		1-5			
Delay of feeding:						
No	28	56	62	62	X^2=0.5	0.480
Yes	22	44	38	38		
Interrupted feeding:						
No	6	12	80	80	X^2=63.01	<0.001**
Yes	44	88	20	20		
Total NPO days before diagnosis of NEC (days):						
Mean ± SD	3.82±1.72				–	–
Median (IQR)	4 (3-5)					
Range	0-8					
Type of feeding:						
Breast	10	20.4	48	48	X^2=10.531	0.001**
Formula	39	79.6	52	52		

*p≤0.05 is statistically significant.   SD : Standard deviation. X^2: Chi- Square test.
**p≤0.01 is high statistically significant.   IQR : Interquartile range.

Table (4): Comorbidities among the two studied groups.

	NEC group (No. = 50)		Control group (No. = 100)		Chi-Square test	
	No.	%	No.	%	X2	p-value
Placental Insufficiency	26	52	42	42	1.345	0.246
Antenatal steroids:						
No	44	88	76	76	3	0.083
Yes	6	12	24	24		
Congenital Heart Disease (CHD)	31	62	46	46	3.416	0.065
PDA	15	30	10	10	9.6	0.002**
Intracranial hemorrhage (ICH):						
No	29	58	66	66	3.537	0.472
Grade I IVH	16	32	24	24		
Grade II IVH	1	2	4	4		
Grade III IVH	3	6	6	6		
Grade IV IVH	1	2	0	0		
Sepsis proven by blood culture:						
No	0	0	66	66	58.93	<0.001**
Yes	50	100	34	34		

*p≤0.05 is statistically significant.**p≤0.01 is high statistically significant. X^2: Chi- Square test.

Table (5): Patients characteristics among NEC group.

		NEC group (No. = 50)	
		No.	%
NEC stage I	IA	10	20
	IB	4	8
II	IIA	22	44
	IIB	14	28
Age at Onset (days)	Mean ± SD	10.84±4.54	
	Median (IQR)	10 (7 - 12)	
	Range	3 – 21	
Weight at Onset (gm)	Mean ± SD	1423.9±401.6	
	Median (IQR)	1330 (1200 - 1500)	
	Range	850 - 2660	

SD: Standard deviation. IQR: Interquartile range.

Table (6): Laboratory data and monocytes data among the two studied groups.

	NEC group (No. = 50)		Control group (No. = 100)		Test value	p-value
	No.	%	No.	%		
Metabolic acidosis:					X^2=13.9	<0.001**
No	11	22	54	54		
Yes	39	78	46	46		
Blood culture:					66.45	<0.001**
No Growth	0	0.0	66	66.0		
Acinetobacter MDR	6	12.0	2	2.0		
CONS	22	44.0	18	18.0		
E.coli	5	10.0	0	0.0		
GBS	5	10.0	2	2.0		
Klebsiella MDR	11	22.0	12	12.0		
MRSA	1	2.0	0	0.0		
Baseline monocyte count:					ZMWU=2.726	0.006**
Mean t SD	11.36t2.82		12.99±3.96			
Median (IQR)	10.5 (9 - 13)		13 (10 - 15)			
Range	8 – 19		6 – 28			
Monocytes at NEC Onset (follow up in controls):					ZMWU=5.506	<0.001**
Mean t SD	10.54t6.26		14.26t3.73			
Median (IQR)	9 (7 - 14)		14.5 (11 - 17)			
Range	3 – 34		7 – 25			

*p≤0.05 is statistically significant.   MDR : Multi drug resistant.   SD : Standard deviation.
**p≤0.01 is high statistically significant. CONS: Coagulase negative staphylococci. IQR: Interquartile range.
                                        E.coli : Escherichia coli. X^2 : Chi- Square test.
                                        GBS : Group beta streptococci.   zMWU: Mann-Whitney U test.

Table (7): Monocyte count at baseline and at NEC onset among the two studied groups.

	Baseline Monocytes	Monocytes at NEC Onset	Difference	Test value	p-value
NEC group:					
Mean t SD	11.36t2.82	10.54t 6.26	–7.2%	z=2.414	0.016*
Median (IQR)	10.5 (9 - 13)	9 (7 - 14)			
Range	8 – 19	3 – 34			
Control group:					
Mean t SD	12.99±3.96	14.26t 3.73	9.8%	z=3.004	0.003**
Median (IQR)	13 (10 - 15)	14.5 (11 - 17)			
Range	6 – 28	7 – 25			

*p≤0.05 is statistically significant.   SD : Standard deviation. X^2 : Chi- Square test.
**p≤0.01 is high statistically significant.   IQR: Interquartile range.   z : Wilcoxon Signed Ranks Test.

Table (8): Relation between NEC stage and monocyte count at diagnosis.

	Stage I NEC	Stage II NEC	Test value	Mean difference	95% CI		p-value
Monocyte count:							
Mean t SD	12.71t2.55	10.83t2.77	ZMWU=2.582	1.881	0.161	3.600	0.010**
Median (IQR)	12.5 (11-13)	10 (9-12)					
Range	9 – 18	8 – 19					

*p≤0.05 is statistically significant.   CI : Confidence interval.   IQR: Interquartile range.
**p≤0.01 is high statistically significant.   SD: Standard deviation.   zMWU: Mann-Whitney U test.

Table (9): Accuracy of monocyte count in prediction and detection of NEC.

	Best cut off	Sensitivity	Specificity	PPV	NPV	AUC	p-value
Monocyte count	12	74%	51%	60.2%	66.2%	0.636	0.004

AUC: Area Under a Curve.
p-value: Probability value.
NPV: Negative predictive value.
PPV: Positive predictive value.
* Statistically significant at *p*≤0.05.

Table (10): Accuracy of monocyte count in staging of NEC.

	Best cut off	Sensitivity	Specificity	PPV	NPV	AUC	p-value
Monocyte count	11	57.1%	94.4%	91.1%	68.8%	0.793	<0.001 **

AUC: Area Under a Curve.
p-value: Probability value.
NPV: Negative predictive value.
PPV: Positive predictive value.
p≤0.05 is statistically significant.
** *p*≤0.01 is high statistically significant.
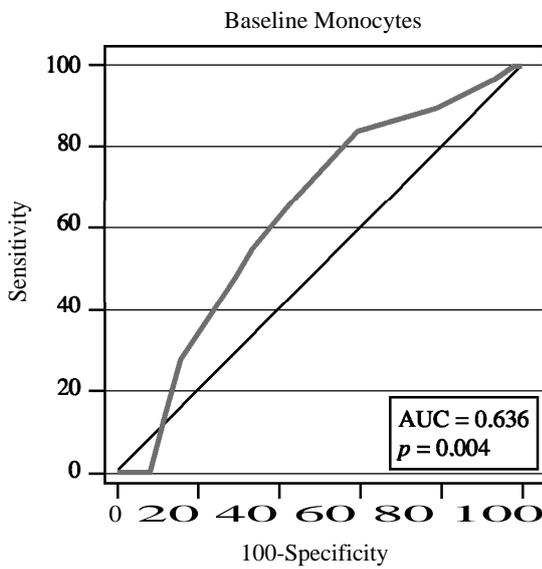
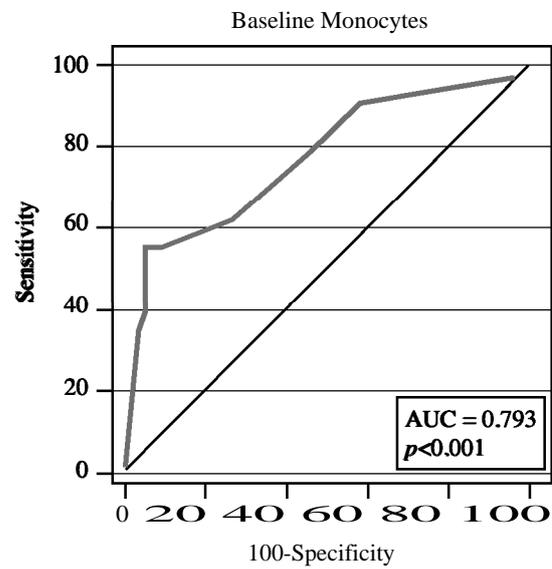Fig. (1): ROC curve of monocytes count in prediction of NEC.

Fig. (2): ROC curve of monocyte count in staging of NEC.

Table (11): Management among the two studied groups.

	NEC group (No. = 50)		Control group (No. = 100)		X2	p-value
	No.	%	No.	%		
Inotropes:						
Never used	32	6	86	86	0.487	<0.001**
Used	18	64	14	14		
Invasive mechanical ventilation:						
No	24	48	42	42	0.487	0.485
Yes	26	52	58	58		
Blood transfusion:						
No	19	38	72	72	16.15	<0.001**
Yes	31	62	28	28		
UVC Insertion:						
No	13	26	22	22	0.298	0.585
Yes	37	74	78	78		

p≤0.05 is statistically significant.
**p*≤0.01 is high statistically significant.
X2 : Chi- Square test.

Discussion

NEC is a serious gastrointestinal disease that primarily affects preterm neonates, particularly those with very low birth weights. It is characterized by inflammation and damage to the intestinal tissue, often leading to tissue necrosis (cell death) in severe cases. Predicting the severity of NEC in preterm neonates is crucial for timely intervention and management [6]. The monocyte count, which is a type of white blood cell, has been investigated as a potential biomarker for predicting the severity of NEC [1].

The current study aimed to determine the value of monocy count in diagnosis of NEC and whether the degree of monocyte count drop at the onset of necrotizing enterocolitis correlates with severity of the disease.

In our study, the gender, gestational age and mode of delivery were not statistically different between the NEC group and the control group. These results agreed with Tajalli et al. [1].

Our results showed that there was no statistically significant difference in the Apgar scores between the two groups. This indicates that the early status neonates in the delivery room, as assessed by Apgar score, did not significantly vary between those who developed NEC and those who did not which agreed with Tajalli et al. [1].

Although birth weight was slightly lower in NEC group versus control group (means 1379gm and 1455gm respectively) but the results were not statistically significantly different (*p*=0.064). This suggests that birth weight might not be a strong independent predictor of NEC in this study which agreed with Tajalli et al. [1].

The current study results revealed that neither the proper day of life for initiation of enteral feeding nor the delay of feeding affected the incidence of NEC. However, interrupted feeding presented a stark contrast. A significant majority of the NEC group experienced interrupted feeding (88%), compared to only 20% in the control group. This difference was highly statistically significant (*p*-value <0.001), indicating that interrupted feeding might be a significant risk factor for NEC and this agreed with Thompson-Branch and Havranek [7].

Another possibility is that neonates in the NEC group might have experienced early feeding intolerance before onset of NEC that necessitated interruption of feeding.

Furthermore, the type of feeding was also explored in relation to NEC. The data revealed that a higher proportion of infants in the NEC group were fed with formula (79.6%) compared to the control group (52%). This difference was statistically significant (*p*-value = 0.001), implying that formula feeding might be associated with an increased risk of NEC. It's worth noting that breastfed infants seemed to have a lower occurrence of NEC in this study which agreed with Good et al. [8].

The provided data distribution of NEC stages in the studied group indicated varying levels of severity. The majority of cases fell into stages IIA and IIB, comprising 44% and 28% respectively, followed by stages IA (20%) and IB (8%). This distribution suggested diversity in disease progression within the NEC group.

Similarly, Tajalli et al. [1] revealed that among NEC cases, 57.5% were classified as Stage II and 42.5% as Stage III. These findings emphasize the predominance of more severe stages compared to our study.

Additionally, the age and weight at onset of NEC provided insights into the presentation of NEC. The mean age at onset was approximately 10.84 days, with a median of 10 days and a range spanning from 3 to 21 days which agreed with Tajalli et al. [1] who revealed that the mean onset age of NEC was found to be 14.14 days.

This variability suggests that NEC could manifest at different points after birth, possibly influenced by various factors such as feeding practices, underlying health conditions, and other clinical factors. The mean weight at onset was around 1423.9 grams, with a median of 1330 grams and a range extending from 850 to 2660 grams. The relatively low mean weight at onset, along with the wide range, might reflect the vulnerability of premature infants to NEC, given their underdeveloped gastrointestinal systems.

The data highlighted the presence of metabolic acidosis in both groups of preterm infants. Significantly more infants in the NEC group experienced metabolic acidosis (78%) compared to the control group (46%), with a highly significant *p*-value of less than 0.001. This indicates a strong positive association between metabolic acidosis and the development of NEC. Metabolic acidosis, which reflects an imbalance between acid production and elimination in the body, might indicate compromised perfusion and oxygenation of tissues, potentially contributing to the pathogenesis of NEC.

This agreed with Clark and Munshi [9] whorevealed that NEC was associated with metabolic acidosis and was often sustained which might contribute to the pathogenesis of NEC.

Our data indicated that there was no significant difference between the NEC group and the control group regarding low APGAR scores and placental insufficiency. These results suggested that these fac-

tors might not be strong contributors to NEC development in this study population.

Our data indicated fewer cases in the NEC group received antenatal steroids compared to the control group. Although not statistically significant, this finding could still be of clinical interest, as antenatal steroids were known to have potential benefits in reducing the risk of certain complications in preterm infants.

Moving on to congenital heart disease (CHD) and patent ductusarteriosus (PDA), the data suggested no significant difference in the prevalence of CHD between the two groups ($p=0.065$) though the incidence was higher in NEC group compared to control group (62% versus 46%). This difference could be explained by changes in blood flow in CHD might decrease the blood flow to the intestine causing NEC. However, PDA was notably more prevalent in the NEC group (30.0%) compared to the control group (10.0%), with a statistically significant *p*-value of 0.002. This result highlighted the potential role of PDA in contributing to the risk of NEC which agreed with Zvizdić et al. (2015) and this was probably due to the influence of PDA on compromising mesenteric perfusion.

Sepsis was presented in all cases in the NEC group, in contrast to 34% of those in the control group. This stark difference was highly statistically significant (*p*-value <0.001), suggesting a strong association between sepsis and NEC which agreed with Lu et al. *[11]*. Sepsis, a systemic response to infection, can lead to compromised blood flow and inflammation, potentially contributing to the development of NEC. The data emphasized the importance of considering sepsis prevention and management as a potential strategy to mitigate the risk of NEC in neonates.

Regarding blood culture results, the data indicated a notable difference between the two groups. While the control group had a significant proportion (66%) of cases with no growth in blood cultures, the NEC group displayed various bacterial strains, including multidrug-resistant Acinetobacter, Coagulase-negative Staphylococci (CONS), Escherichia coli (E.coli), Group B Streptococcus (GBS), multidrug-resistant Klebsiella (Klebsiella MDR), and Methicillin-resistant Staphylococcus aureus (MRSA). These differences were highly significant (*p*-value <0.001) which agreed with Lin et al. *[12]* who revealed that positive rate for blood culture in NEC infants was found to be higher than the average positive rate indicating a potential association between specific bacterial strains and the development of NEC. The presence of drug-resistant bacteria in the NEC group might suggest a potential link between microbial colonization and the occurrence of NEC, emphasizing the need for infection control measures in neonatal care units.

Blood transfusion was another noteworthy factor. A higher proportion of cases in the NEC group (62%) received blood transfusions compared to the control group (28%). This difference was highly significant (*p*-value <0.001) which agreed with Lu et al. *[11]*. This could imply that blood transfusions might be linked to the risk of NEC, possibly due to the introduction of external factors or changes in immune response associated with transfusion or because NEC might be associated with severe anemia necessitating blood transfusion.

No significant difference was observed between the groups in terms of UVC insertion (*p*-value = 0.585), suggesting that this factor might not play a strong role in NEC development.

A significantly higher proportion of cases in the NEC group (36.0%) required inotropes compared to the control group (14%) with (*p*-value <0.001) which agreed with Zvizdic et al. *[13]* suggesting a strong association between the use of inotropes and the occurrence of NEC. The increased need for inotropes might indicate compromised cardiovascular function which could potentially contribute to the development of NEC. This emphasized the importance of closely monitoring cardiovascular stability in neonates, especially those at risk for NEC.

Moving on to invasive mechanical ventilation, the data suggested that there was no significant difference between the two groups in terms of its use (*p*-value = 0.485) which agreed with Zvizdic et al. *[13]*. While this suggested that mechanical ventilation might not be a significant factor specifically associated with NEC in this dataset, it was important to note that mechanical ventilation was a commonly used intervention in critically ill neonates and could potentially contribute to the development of NEC if not managed appropriately.

The study examined two readings for monocytes, baseline and at the onset of NEC or follow up for the control group. The baseline monocyte values were lower in the NEC group compared to the control group, with a mean value of 11.36 versus 12.99 respectively. This difference was statistically significant (*p*-value = 0.006), indicating that a lower baseline monocyte count might be associated with a higher risk of NEC. Similarly, the monocyte count at the onset of NEC was lower in the NEC group than the follow up value in the control group, with a significantly lower mean and median values in the NEC group (*p*-value <0.001). This could suggest that a lower monocyte count might be indicative of a predisposition to NEC development or might be a consequence of the condition. Moreover, the monocyte count at NEC onset showed a statistically significant decline from the baseline value by 7.2% in the NEC group (*p*-value 0.016) while the follow up monocyte count showed a highly statistically significant increase than the baseline value by 9.8% (*p*-value 0.003). This could suggest that monocyte

count could be a useful diagnostic marker in early detection of NEC.

In the same line Tajalli et al. *[1]* and Wang et al. *[10]* founded that the reduction of monocyte count was associated with the severity of preterm NEC.

In a study done by Wang et al. *[10]* revealed that monocyte count decreased sharply in NEC infants at onset, and the degree of decline was associated with the severity of NEC.

In this analysis, Stage I and Stage II NEC cases were compared in terms of their monocytes values. The mean monocyte count for Stage I NEC was 12.71, while for Stage II NEC, it was 10.83. The data indicated a statistically significant difference in monocyte count between these two stages (*p*-value = 0.01), with a mean difference of 1.881 and confidence interval of 95% ranged from 0.161 to 3.6. This result implied that Stage II NEC cases had a lower mean monocyte count compared to Stage I NEC cases.

Monocytes play a role in the immune response, particularly in inflammatory reactions. The lower monocyte count observed in Stage II NEC could potentially reflect a change in the immune response during disease progression. The decline in monocyte count might be associated with a diminished capacity to combat inflammation and infection, which could be a contributing factor to the severity of NEC.

In the same line Wang et al. *[10]* who noted that among different stages of NEC, stage III had the lowest monocyte count at onset and the largest percentage decrease in monocyte count.

The provided data assessed accuracy of the monocyte count in predicting and detecting NEC. The area under the curve (AUC) value of 0.636 signified a moderate level of diagnostic accuracy which indicated that the monocyte count had a fair capacity to discriminate between NEC-positive and NEC-negative cases. Sensitivity at 74% suggested that the monocyte countcan correctly identify approximately three-quarters of true positive cases, making it useful in ruling out the presence of NEC. However, the specificity of 51% implied that the test's ability to accurately classify true negative cases was somewhat limited, possibly leading to an increased rate of false positives. Despite this limitation, the positive predictive value (PPV) of 60.2% indicated that among those testing positive for anmonocyte countof 12, around 60.2% were indeed positive cases for NEC. Similarly, the negative predictive value (NPV) of 66.2% suggested that among those testing negative for this threshold, approximately 66.2% were truly negative for NEC. The provided *p*-value of 0.004 added significant weight to the results, indicating that the diagnostic performance of the monocyte countwas unlikely to

have occurred by chance. In sum, while the monocyte countmight not be a stand-alone definitive diagnostic tool, its moderate accuracy and significant p-value suggested its potential as a complementary element in NEC prediction and detection.

It's important to interpret these validity metrics in the context of clinical practice. The moderate AUC value indicated that the monocyte countcould provide valuable information but should not be solely relied upon for diagnosis. The higher sensitivity compared to specificity suggested that the monocyte countwas better at correctly identifying true positive cases than true negative cases, which was advantageous in ruling out NEC. However, the lower specificity could potentially lead to unnecessary further investigations or interventions in cases where the test yielded false positive results. The relatively modest positive predictive value implied that while a positive test result increased the likelihood of NEC, it was not a guarantee. Similarly, the negative predictive value suggested that a negative test result did not definitively rule out NEC.

In the same line Remon et al. *[14]* revealed that the ROC analysis, yielding a diagnostic accuracy of 0.76 for monocyte count, emphasized the potential of monocyte count as a predictive marker for NEC. The finding that monocyte count drop of >20% is indicative of NEC in infants with feeding intolerance is note worthy, as it indicated that monocyte count could be a valuable tool for identifying NEC cases early. The sensitivity and specificity of around 0.70 suggested a reasonably reliable ability to differentiate NEC cases in this context.

The provided data assessed the accuracy of the monocyte countin the staging of (NEC).

The analysis revealed that the monocyte countat a cutoff value of 11 demonstrates diagnostic accuracy when used to stage NEC cases. The sensitivity of 57.1% indicated that monocyte countcan identify a significant portion of true positive cases, which was crucial for correctly identifying those with NEC. The remarkable specificity of 94.4% emphasized the test's ability to accurately classify true negative cases, minimizing the chances of misdiagnosis. The (PPV) of 91.1% suggested that among those testing positive for monocyte countof 11, approximately 91.1% were truly positive for stage II NEC. Similarly, the (NPV) of 68.8% indicated that among those testing negative for this threshold, around 68.8% were indeed negative for NEC. The notably high AUC value of 0.793 reflected the overall diagnostic accuracy of the monocyte count, signifying its ability to discriminate between different stages of NEC. The very low *p*-value of <0.001 highlighted the strong statistical significance, indicating that the ability of the monocyte countto differentiate between stage I and stage II NEC was highly unlikely to be due to random chance.

In consistent with our study results Desiraju et al. **[5]** showed a clear correlation between the extent of monocyte count change and the severity of NEC, with significantly larger drops in monocyte count observed in more severe stages of NEC. The study suggested that percent of monocyte count change could be a useful marker for identifying NEC at onset and predicting disease severity.

At the end of our discussion results of the study met our aim to determine the accuracy of monocyte count in diagnosis and staging of NEC.

Conclusion:

The findings collectively suggest that interrupted feeding, formula feeding, sepsis, specific bacterial strains, and the use of inotropes and blood transfusions are strongly linked to the incidence of NEC in preterm neonates. On the other hand, certain factors such as gender, gestational age, and Apgar scores may not be significantly linked to NEC. Additionally, the analysis highlights the ROC of monocyte count with a diagnostic accuracy of 0.636 for NEC and 0.793 for stage II NEC which demonstrates excellent sensitivity and specificity. These insights contribute to a deeper understanding of the multifaceted nature of NEC development and highlight the importance of targeted interventions, infection control measures, and close monitoring to mitigate its risk in neonatal care settings.

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دور عدد الخلايا الوحيد فى توقع شدة التهاب الأمعاء والقولون الناخر فى الخدج المصريين: دراسة متعددة المراكز

الخلفيــة: يعد التهاب الأمعاء والقولون الناخر أحد أكثر حالات الطوارئ الطبية/الجراحية المعدية المعوية خطورة وشائعة عند الولدان المبتسرين.

الهـدف مـن العمـل: تحديد الدقة التشخيصية للخلايا الوحيدة فى تشخيصا لو ما إذا كانت درجة انخفاض عدد الوحيدات فى بداية التهاب الأمعاء والقولون الناخر يرتبط بخطورة المرض والذى بدوره سيساعد فى التشخيص المبكر والعلاج من المرض.

المرضى وطرق العمـل: تم إجراء هذه الدراسة على ١٥٠ التهاب الأمعاء والقولون الناخر طفلاً حديث الولادة فى وحدات العناية المركزة لحديثى الولادة بمستشفى أبوالريش المنيرة للأطفال ومستشفى جامعة القاهرة ومستشفى جامعة مصر للعلوم والتكنولوجيا.

النتائـج: تسلط البيانات الضوء أولاً على وجود الحماض الأيضى فى كلا المجموعتين. تعرض عدد أكبر بكثير من الرضع فى مجموعة التهاب الأمعاء والقولون الناخر للحماض الأيضى (٧٨٪) مقارنة بالمجموعة الضابطة (٤٦٪)، مع قيمة p هامة للغاية أقل من ١ ٠,٠٠. يشير هذا إلى وجود علاقة إيجابية قوية بين الحماض الأيضى وتطور التهاب الأمعاء والقولون الناخر. قد يشير الحماض الأيضى، الذى يعكس عدم التوازن بين إنتاج الحمض وإزالته فى الجسم، إلى ضعف التروية والأكسجين فى الأنسجة، مما قد يساهم فى التسبب فى التهاب الأمعاء والقولون الناخر. كشفت الدراسة أن قيم عدد الخلايا الوحيدة فى بداية التهاب الأمعاء والقولون الناخر كانت أقل فى مجموعة التهاب الأمعاء والقولون الناخر مقارنة بالمجموعة الضابطة. فى هذا التحليل، تتم مقارنة حالات التهاب الأمعاء والقولون الناخر من المرحلة الأولى والمرحلة الثانية من حيث قيم عدد الخلايا الوحيدة الخاصة بها. متوسط عدد الخلايا الوحيدة للمرحلة I من التهاب الأمعاء والقولون الناخر هو ١٢,٧١، بينما بالنسبة للمرحلة II من التهاب الأمعاء والقولون الناخر هو ١٠,٨٣. تشير البيانات إلى وجود فرق ذو دلالة إحصائية فيعدد الخلايا الوحيدة بين هاتين المرحلتين قيمة ($p=0,01$)، بفارق متوسط قدره ١,٨٨١. يتراوح فاصل الثقة ٩٥٪ من ٠,١٦١ إلى ٣,٦، مما يشير إلى أن الفرق الحقيقى فى عدد الخلايا الوحيدة بين المرحلتين يمكن أن يقع ضمن هذا الفاصل الزمنى بثقة ٩٥٪. تشير هذه النتيجة إلى أن حالات المرحلة الثانية من التهاب الأمعاء والقولون الناخر لها متوسط عدد الخلايا الوحيدة أقل مقارنةً بحالات المرحلة الأولى.

الاستنتاج: تشير النتائج مجتمعة إلى أن التغذية المتقطعة، والتغذية الصناعية، والإنتان، وسلالات بكتيرية معينة، واستخدام مقويات التقلص العضلى وعمليات نقل الدم ترتبط ارتباطًا وثيقًا بحدوث التهاب الأمعاء والقولون الناخر عند الولدان المبتسرين. من ناحية أخرى، قد لا تكون بعض العوامل مثل الجنس وعمر الحمل ودرجات أبغار مرتبطة بشكل كبير بالتهاب الأمعاء والقولون الناخر. بالإضافة إلى ذلك، يسلط التحليل الضوء على قيم ROC لعدد الخلايا الوحيدة بدقة تشخيصية تبلغ ٠,٦٣٦ لالتهاب الأمعاء والقولون الناخر ٠,٧٩٣ للمرحلة الثانية من التهاب الأمعاء والقولون الناخر مما يوضح حساسية ونوعية ممتازة. تساهم هذه الأفكار فى فهم أعمق للطبيعة المتعددة الأوجه لتطوير التهاب الأمعاء والقولون الناخر و تسلط الضوء على أهمية التدخلات المستهدفة، وتدابير مكافحة العدوى، والمراقبة الدقيقة للتخفيف من مخاطرها فى أماكن رعاية الأطفال حديثى الولادة.