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Original Article

The Role of Absolute Monocytic Count in Early Diagnosis of Nercotizing Enterocolitis in Premature Neonates. A Prospective Analytical Study

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Abstract

Background: Necrotizing enterocolitis (NEC) is characterized by macrophage infiltration in the intestine. As intestinal macrophages are derived from recruitment of blood monocytes into gut mucosa, it in turn reduces blood monocytes concentration which can be helpful marker for NEC.

Aim: To evaluate the contribution of absolute monocytic numbers in separating NEC from other potential causes of preterm feeding intolerance.

Subjects and methods: This was a prospective research which was conducted (after exclusion of 44 (25.1%) neonates after their death) for 131 neonates (70.2% with feeding intolerance and 29.8% with NEC). All the included neonates were subjected to full history taking, clinical evaluation including also Ballard score , and full investigations including absolute monocytic count.

Results: Statistically substantial variation between preterm with FI and preterm with NEC regards nutritional history with p-value <0.001 most of FI preterm was on trophic feeding while most of NEC preterm were on TPN. Statistically significant decrease of Ballard score in NEC preterm than feeding intolerance with p-value=0.014 as infants in feeding intolerance group developed feeding intolerance at an earlier postnatal age than those with NEC. AMC after 3-5 days after FI had statistically significant difference between NEC and FI with p-value 0.000.

Conclusions: Absolute monocytic count in 3rd to 5th day after signs of feeding intolerance has predictive role in differentiation of NEC from other causes of feeding intolerance, but it had no significant difference between different stages of NEC.

Key words: Monocytic Count; Nercotizing Enterocolitis; Premature; Neonates

Introduction

Necrotizing enterocolitis (NEC), which may be deadly in the majority of seriously afflicted newborns, is thought to be the most prevalent acquired inflammatory bowel illness in preterm infants [1]**.** At the commencement of NEC, it is impossible to anticipate which babies would have the largest illness burden [2]**.** While some NEC cases begin with milder courses but have the potential to develop into severe illness, other instances begin with fulminant symptoms.

Surgery may be necessary in severe instances to remove necrotic bowel or remove pneumoperitoneum [3]**.** When such severe instances are discovered, it is possible to quickly move the babies who are impacted to higher levels of care where pediatric surgery is available [4]**.** However, no specific biomarker exists to identify which newborns would have the greatest severity of the condition [5].

Preterm children with NEC have a decrease in AMC at the start of the disease compared to baseline, which is caused by intestinal pathology such the gut wall infiltration seen in NEC is contributed to by intestinal macrophages produced from monocytes, and the intestinal monocyte pool may subsequently be replenished by peripheral blood monocytes. Thus, a decrease in peripheral blood monocyte count at the beginning of symptoms should be anticipated, and it has been seen in Modified Bell Stages 2 and 3 NEC [6]**.** In order to diagnose NEC in preterm neonates who had feeding intolerance and to distinguish NEC from other feeding intolerance reasons, we evaluated the effect of CBC parameters, including absolute monocytic count (AMC).

Aim of the Work: To evaluate the role of absolute monocytic numbers in differentiating cases with NEC from other potential causes of feeding intolerance.

Methods

This was a prospective study performed on preterm neonate's ≤ 32 weeks with feeding intolerance (including NEC and other causes) at NICU, Cairo university during 6 months, after approval by the institutional review board.

Inclusion criteria: Preterm patients aged 1 day-28 days that were admitted in the NICU with a diagnosis of feeding intolerance "at Cairo University Pediatrics Hospital during 2020.

Exclusion criteria: Full-term patients, age more than 28 days and major congenital anomalies

Data was collected from files: Full history taking including: Name, sex, age, birth weight, gestational age, and method of delivery, maternal history (DM, HTN, PROM), nutritional history (NPO, TPN ,Trophic feeding , Breast feeding), clinical examination: color, heart rate, grimace reaction, muscular tone, and breathing are all components of the Apgar score. Each component receives a score of 0 (zero), 1, or 2. All newborns have their scores recorded at one minute and five minutes. Ballard score, Weight, length, skull circumference, abdominal circumference, chest circumference, blood pressure, respiratory rate, heart rate.

Criteria of feeding intolerance: The criteria for diagnosing feeding intolerance. Meal intolerance was identified in preterm babies who had abdominal distension and a gastric residual volume more than 50% of the preceding feeding volume [6,7].

Investigations:

Laboratory investigations: White cell counts (WCC), absolute neutrophil counts (ANC), absolute lymphocyte counts (ALC), and the absolute monocytic count are all included in complete blood counts (CBC) (AMC).These data were done in 3 times: 1st one on admission, 2nd one at start of feeding intolerance and 3rd one from 3 days to 5 days after development of feeding intolerance.

C-reactive protein (CRP): Blood culture: Radiological investigations: Plain X Ray erect position, abdominal ultrasound (if possible) and echocardiography (if possible).

Ethical considerations

This study was approved from the ethics committee of faculty of Medicine, Cairo university and written informed consents were obtained from the parents and they informed about the nature and steps of the study.

Statistical analysis

Statistical Package for Social Sciences was employed to computerize and statistically analyze the gathered data (SPSS 24 Inc. Chicago, IL, USA). Utilizing the Shapiro Walk test, the distribution of the data was examined for normality. Frequencies and relative percentages were employed to depict qualitative data. The difference between the qualitative variables was calculated utilizing chi square test ($\chi 2$) and Fisher exact, as shown. Non-parametric data such as the median and range were employed to convey quantitative data. The Mann Whitney U (MWU) test was developed to determine the variance between quantitative variables in two sets of non-parametric data. Wilcoxon test for non-parametric data was used to compare the serial changes in markers' values within each group. The non-parametric variables were correlated using the Spearman's correlation test. Superior test performance is indicated by a greater area under a ROC curve (AUC), where 1 denotes 100% sensitivity and specificity and 0.5 denotes no discriminatory usefulness. Every statistical comparison used a two-tailed significance test. Level of P-value ≤ 0.05 denotes a substantial change, $p < 0.001$ denotes a very significant difference, and $P > 0.05$ denotes no difference.

Results

This is a prospective study performed on 175 preterm neonates with feeding intolerance. Forty four neonates (25.1%) were excluded due to death) while 131 neonates (70.2% with feeding intolerance

and 29.8% with NEC) completed the study (Figure 1).

Demographic data of the included preterm with median age 16 days ranged between 7 to 44 days, with male predominance (51.9%), and females accounts 48.1%. There was no statistically substantial variation between preterm with FI and preterm with NEC regards clinical data except for nutritional history with p-value <0.001 most of FI preterms were on trophic feeding while most of NEC preterms were on TPN (Table 1).

AMC after 3-5 days after FI had statistically substantial variation between NEC and FI with p-value 0.000(Table 2).

Most of NEC infants were stage IIA (48.7%), followed by stage IA, IIB, and IIIA (Figure 2).

Table 4 showed statistically positive correlation between AMC at admission, of infants with signs of FI, their chest circumference, DBP, and MAP and statistically significant negative correlation between AMC at admission and ANC 1st one on admission with p-value <0.05. There is statistically positive correlation between AMC at admission, of infants with NEC, and chest circumference with p-value= 0.011 and there was statistically substantial negative connection between AMC at admission, of infants with NEC, and SBP with p-value=0.018 as presented in table.

The validity of ALC, ANC, AMC and WCC levelS on admission with area under the ROC curve (AUC) as a diagnostic marker for NEC in the premature neonate (Figures 3-6).

Discussion

Preterm infants with NEC have been shown to have a decrease in peripheral absolute monocyte count at the time of disease start compared to baseline, although it hasn't yet been connected to the severity or prognosis [6].

This was a prospective study performed on preterm infants with feeding intolerance to evaluate the role of AMC in diagnosis and differentiation between

NEC and other causes of feeding intolerance.

In the present study; Preterm with median age 16 days ranged between 7 to 44 days, with male predominance (51.9%), and females accounts 48.1% with male to female ratio 1.1: 1. Gestational age, maternal history and mode of delivery did differ in NEC infants and others with picture of FI with p-value >0.05, which was similar to what was reported by a study of Chaaban et al. 2010 [7] aimed to evaluate Inter alpha inhibitor protein in predicting NEC and found no statistically substantial variation between both groups regards gestational age, maternal history, and mode of delivery.

In contrast to Pantalone et al. 2021 [8] which was conducted to estimate the role of gestational age specific CBC in NEC and found statistically significant decrease of GA in NEC infants than controls (signs of FI but not NEC) with p-value <0.001. This difference may be related to different sample size or different time of diagnosis.

There is statistically significant difference between preterm with FI and preterm with NEC regards nutritional history with p-value <0.001 most of FI preterm were on trophic feeding while most of NEC preterm were on TPN. This finding can explore that breast feeding can reduce risk of NEC in preterm infants [9].

Statistically significant decrease of Ballard score in NEC preterm than feeding intolerance with p-value=0.014 as infants in feeding intolerance group developed feeding intolerance at an earlier postnatal age than those with NEC. This goes in run with Remon et al. (2014) study [6] which was conducted on 69 preterm diagnosed with NEC and 257 preterm with feeding intolerance to investigate AMC and found statistically significant difference between both groups (NEC, FI) regards Bell staging as infants in control group (FI) developing feeding intolerance in earlier postnatal

age than NEC group with p-value <0.001.

In this study, there is no statistically significant difference between infants with FI and NEC infants regards results of blood culture although most of FI infants had positive blood cultures (Mostly klebsiella) (64.1%) more than infants with NEC (35.9%). The same was reported by Remon et al. 2014 [6] in which some of these babies' gastrointestinal symptoms may be explained by sepsis-related ileus, which may peak at an earlier postnatal age than NEC, since control infants had a greater prevalence of blood culture-positive sepsis and having had a central line [10].

Our study reported that most of NEC infant were stage IIA (48.7%), followed by stage IA, IIB, and IIIA. This goes in run with Henderson et al. 2007 [11] who assessed enteral feeding regimens and NEC in 53 preterm infants and found that most NEC infant were stage II to III (75.5%) followed by stage I (24.5%).

No statistically significant difference between different stages of NEC regards ALC, ANC, AMC, and WCC with p-value >0.05.

In contrast to Pantalone et al. 2021 [8] which revealed statistically significant decrease in ALC, ANC, and AMC in advanced stages of NEC (surgical NEC) than early stages of NEC (medical NEC) with p-value <0.001. This difference can be explained by the probability of immature immune system in preterm which in turn make preterm infants have already decreased blood cells including neutrophils, lymphocytes and monocytes [12].

Desiraju et al. 2020 [5] conducted their study on 29 (FI not NEC infants) and 76 NEC cases to estimate the role of AMC in differentiating different stages of NEC and found statistically significant difference between NEC infants and those with signs of feeding intolerance but not NEC as regards AMC being lower in NEC than FI.

There is statistically positive correlation between AMC at admission, of infants with NEC, and chest circumference with p-value= 0.011 and there was statistically significant negative correlation between AMC at admission, of infants with NEC, and SBP with p-value=0.018. As abdominal circumference is a great sign for development of NEC and differs also according to degree of [13].

Several factors, including resistance of the anterior abdominal wall, timing of feeding and urination, breathing phase, and amount of fat may affect abdominal circumference [14], this gives chance of that AMC can be a good prognostic marker for NEC diagnosis and progression.

No statistically significant difference between different stages of NEC regards ALC, ANC, AMC, and WCC with p-value >0.05.

In contrast to Desiraju et al. 2020 [5] which was performed for 29 (FI not NEC infants) and 76 NEC cases to estimate the role of AMC in differentiating different stages of NEC and found statistically significant difference in different stages of NEC regards AMC s AMC shows greater decline in stage 3 NEC than grade 2 NEC and grade 1 NECwith p-value <0.001

Roc curve analysis in our study to detect the ability of AMC to distinguish between NEC and those with benign symptoms of FI showed area under the curve was 0.593, cutoff of >9, with 66.67% sensitivity and 57.61% specificity, compared with Desiraju et al. 2020 [5] which was performed for 76 NEC cases, and 29 FI and found that AMC can differentiate between NEC and those with benign symptoms of FI showed area under the curve was 0.81, cutoff of 50%, with 51% sensitivity and 93% specificity

Conclusions

Absolute monocytic count in 3rd to 5th day after signs of feeding intolerance has predictive role in differentiation of NEC from other causes of feeding intolerance, but it had no significant difference

between different stages of NEC. The AMC is a compelling indicator that the practitioner already has access to NEC at no additional expense.

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Author's contributions

All authors contributed equally in this work and approved the manuscript for publication

Conflict of interest

The authors have no conflict of interests to declare.

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References

1- Patel RM, Denning PW. Intestinal microbiota and its relationship with necrotizing enterocolitis. Pediatr Res 2015; 78:232–238.

2- Hackam D, Caplan M. Necrotizing enterocolitis: pathophysiology from a historical context. Semin Pediatr Surg 2018; 27:11–8.

3- Mara MA, Good M, Weitkamp J-H. Innate and adaptive immunity in necrotizing enterocolitis. Semin Fetal Neonatal Med 2018; 23: 394–9.

4- Niño DF, Sodhi CP, Hackam DJ. Necrotizing enterocolitis: new insights into pathogenesis and mechanisms. Nat Rev Gastroenterol Hepatol 2016; 590–600.

5- Desiraju S, Bensadoun J, Bateman D, Kashyap S. The role of absolute monocyte counts in predicting severity of necrotizing enterocolitis. Journal of Perinatology 2020; 40(6):922-7.

6- Remon J, Kampanatkosol R, Kaul RR, Muraskas JK, Christensen RD, Maheshwari A. Acute drop in blood monocyte count differentiates NEC from other causes of feeding intolerance. J Perinatol 2014; 34:549–54.

7- Chaaban H, Shin M, Sirya E, Lim YP, Caplan M, Padbury JF. Inter-alpha inhibitor protein level in neonates predicts necrotizing enterocolitis. The Journal of pediatrics 2010; 157(5):757-61.

8- Pantalone JM, Liu S, Olaloye OO, Prochaska EC, Yanowitz T, Riley MM, et

al. Gestational Age-Specific Complete Blood Count Signatures in Necrotizing Enterocolitis. Frontiers in pediatrics 2021; 26:79:90.

9- Jones E, Spencer SA. Optimising the provision of human milk for preterm infants. Archives of Disease in Childhood-Fetal and Neonatal Edition 2007; 92(4):F236-8.

10- Namachivayam K, Blanco CL, Frost BL, Reeves AA, Jagadeeswaran R, MohanKumar K, et al. Preterm human milk contains a large pool of latent TGF-β, which can be activated by exogenous neuraminidase. American Journal of Physiology-Gastrointestinal and Liver Physiology 2013; 304(12):G1055-65.

11- Henderson G, Craig S, Brocklehurst P, McGuire W. Enteral feeding regimens and necrotising enterocolitis in preterm infants: a multicentre case–control study. Archives of Disease in Childhood-Fetal and Neonatal Edition 2009; 94(2):F120-3.

12- Tröger B, Müller T, Faust K, Bendiks M, Bohlmann MK, Thonnissen S, et al. Intrauterine growth restriction and the innate immune system in preterm infants of ≤ 32 weeks gestation. Neonatology 2013; 103(3):199-204.

13- Manogura AC, Turan O, Kush ML, Berg C, Bhide A, Turan S, et al. Predictors of necrotizing enterocolitis in preterm growth-restricted neonates. American journal of obstetrics and gynecology 2008; 198(6):638-45.

14- Meldere I, Urtans V, Petersons A, Abola Z. Measurement of abdominal circumference in preterm infants. BMC research notes 2015; 8(1):1-4

Table 1: Clinico-demographic data with the prenatal history as regard the final diagnosis

Item		Diagnosis				Total		P-value
		F.I		NEC				
		N=92		N=39		N=131		
Age		16 (7-43)		17 (9-44)		16 (7-44)		0.501
Birth weight Kg		1.36 (0.86-1.90)		1.32 (0.79-1.80)		1.34 (0.79-1.90)		0.093
Gestational age,wks		31 (27-32)		31 (25-32)		31 (25-32)		0.183
Sex	**Female**	40	43.5%	23	59.0%	63	48.1%	0.105
	Male	52	56.5%	16	41.0%	68	51.9%	
Mode of delivery	**CS**	81	88.0%	32	82.1%	113	86.3%	0.362
	NVD	11	12.0%	7	17.9%	18	13.7%	
Maternal history of DM	**No**	79	85.9%	34	87.2%	113	86.3%	0.842
	Yes	13	14.1%	5	12.8%	18	13.7%	
Maternal history of HTN	**No**	71	77.2%	28	71.8%	99	75.6%	0.512
	Yes	21	22.8%	11	28.2%	32	24.4%	
Maternal history of PROM	**No**	57	62.0%	18	46.2%	75	57.3%	0.095
	Yes	35	38.0%	21	53.8%	56	42.7%	
Nutritional history	**NPO**	1	1.1%	14	35.9%	15	11.5%	<0.001
	TPN	13	14.1%	19	48.7%	32	24.4%	
	Trophic	48	52.2%	4	10.3%	52	39.7%	
	Breast	30	32.6%	2	5.1%	32	24.4%	

While qualitative data were represented as numbers and percentages and compared using the Chi-square X2 test, quantitative data were reported as Median (range) and compared using the Mann Whitney test.
DM: diabetes mellitus, PROM: premature rupture of membranes

Table 2: Comparison between serial measurements of AMC as regard the final diagnosis

Item	Diagnosis		Total	P-value
	F.I	NEC		
	N=92	N=39	N=131	
AMC 1st one on admission	8 (0-20)	10 (4-32)	9 (0-32)	0.090
AMC 2nd one at start of FI	10 (1-20)	10 (4-30)	10 (1-30)	0.242
AMC 3rd one from 3-5 days after development of FI	10 (3-22)	4 (2-21)	10 (2-22)	0.000

All variables were presented as Median (range) and compared utilizing Mann Whitney test.
AMC: absolute monocytic count

Table 3: serial measures of the WCC, ANC, ALC, and AMC in the premature neonates

All Neonate	1st one on admission	2nd one at start of FI	3rd one from 3-5 days after FI	P-value
WCC	12.6 (2.4-66.5)	13.6 (2.3-55.2)	13.4 (3.3-52.5)	0.117
ANC	5.81 (0.92-40.50)	6.61 (0.69-44.00)	6.6 (0.429-28.1)	0.316
ALC	36.0 (4.0-91.0)	35.0 (6.0-86.0)	38 (8-85)	0.725
AMC	9 (0-32)	10 (1-30)	10 (2-22)	0.723

WCC: white cell count, ANC: absolute neutrophil count, ALC: absolute leucocytic count AMC: absolute monocytic count

Table 4: Correlation between the AMC level on admission and other studied parameters in the premature neonates diagnosed with FI and NEC

Item	AMC 1st one on admission			
	Diagnosis			
	F.I		NEC	
	r	P-value	r	P-value
Age	0.033	0.757	-0.230	0.158
Birth weight	0.139	0.186	0.110	0.506
Gestational age,wks	0.033	0.757	0.098	0.552
Apgar scores at 1 minute	0.096	0.363	-0.099	0.547
Apgar scores at and 5 minutes	0.136	0.196	0.031	0.852
Apgar Scores at 10 minutes	0.060	0.572	0.130	0.43
Ballard score, wks	0.176	0.094	0.119	0.47
weight	0.099	0.347	0.037	0.821
length	0.213	0.42	-0.037	0.823
skull circumference	0.195	0.063	-0.064	0.699
abdominal circumference	0.154	0.143	0.221	0.176
chest circumference	0.240	0.021	0.405	0.011
SBP	0.130	0.218	-0.376	0.018
DBP	0.226	0.03	-0.055	0.739
MAP	0.223	0.033	-0.055	0.738
respiratory rate	-0.111	0.291	-0.073	0.659
heart rate	0.067	0.528	0.082	0.618
WCC 1st one on admission	-0.177	0.092	-0.125	0.448
WCC 2nd one at start of FI	-0.053	0.615	-0.149	0.366
WCC 3rd one from 3-5 days after FI	-0.043	0.684	-0.285	0.079
ANC 1st one on admission	-0.214	0.041	-0.191	0.245
ANC 2nd one at start of FI	-0.069	0.511	-0.127	0.44
ANC 3rd one from 3-5 days after FI	-0.167	0.112	-0.200	0.222
ALC 1st one on admission	-0.140	0.182	-0.141	0.39
ALC 2nd one at start of FI	0.050	0.636	-0.080	0.63
ALC 3rd one from 3-5 days after FI	0.140	0.184	0.153	0.354
AMC 2nd one at start of FI	0.091	0.386	0.144	0.383
AMC 3rd one from 3-5 days after f FI	0.094	0.373	0.247	0.13
CRP	-0.052	0.624	0.183	0.264

r = Correlation Coefficient

WCC: white cell count, ANC: absolute neutrophil count, ALC: absolute leucocytic count AMC: absolute monocytic count

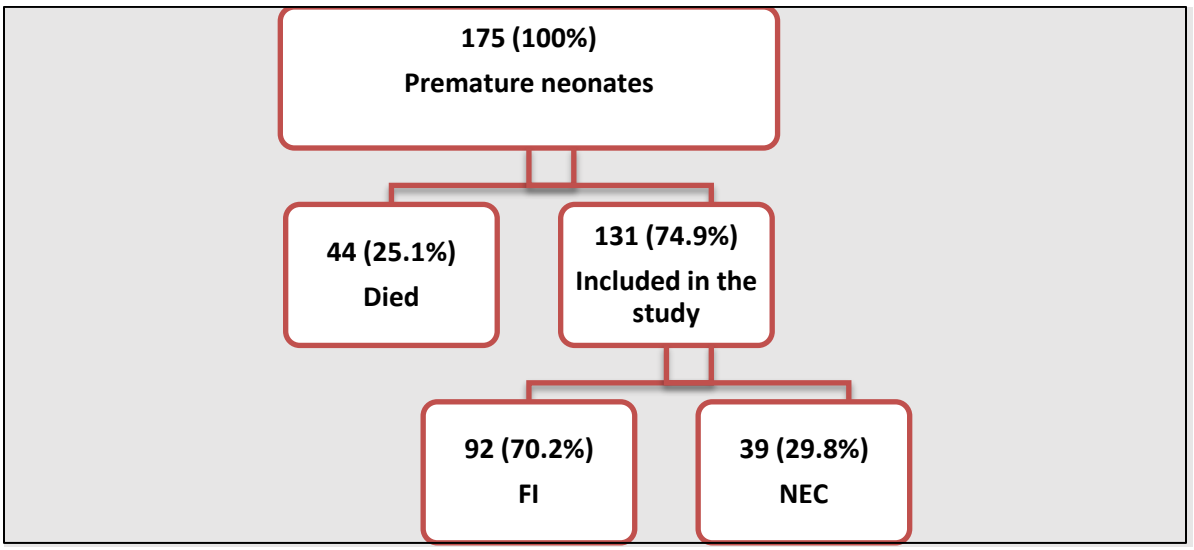

Fig 1: Algorithmic Flow chart of the studied population

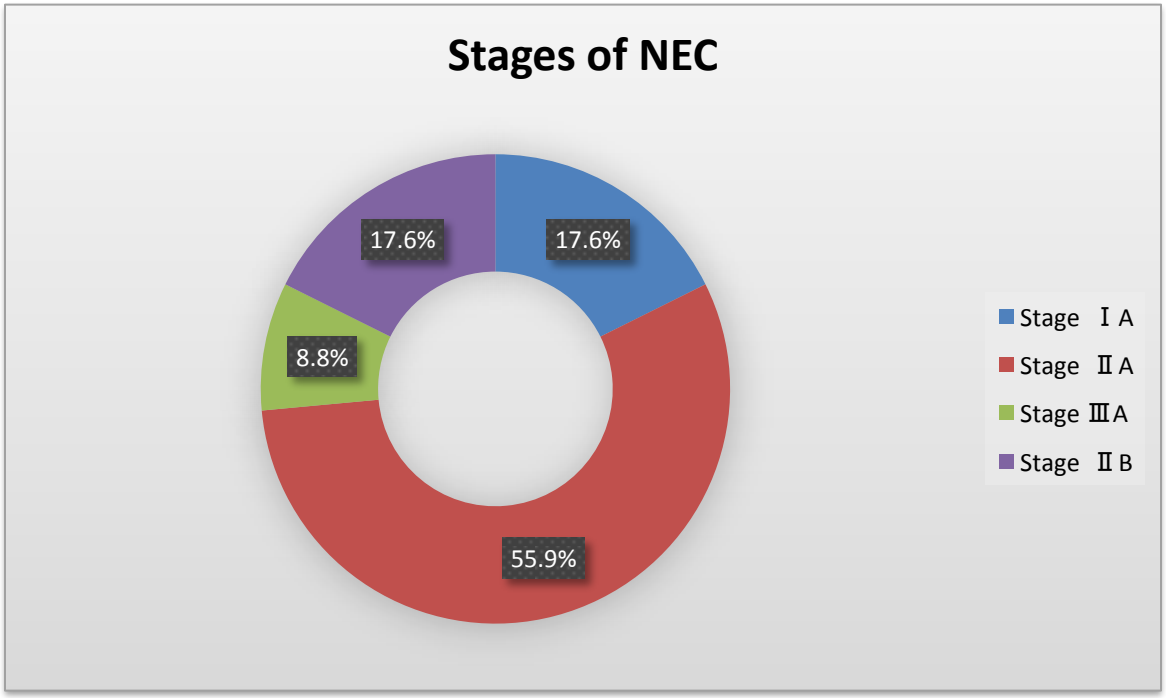

Fig 2: Different stages in the premature neonates diagnosed with NEC

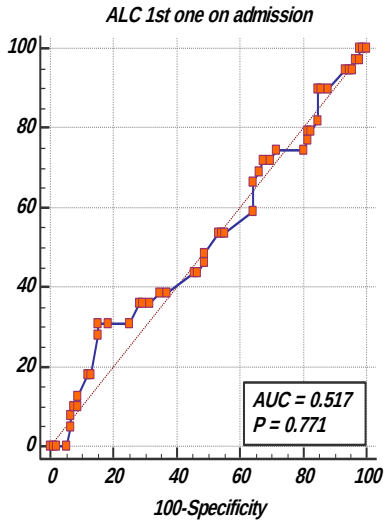

Fig 3: The validity of ALC level on admission with area under the ROC curve (AUC) as a diagnostic marker for NEC in the premature neonate

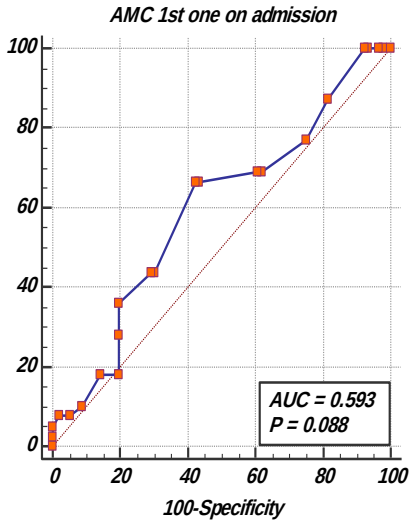

Fig 4: The validity of AMC level on admission with area under the ROC curve (AUC) as a diagnostic marker for NEC in the premature neonate

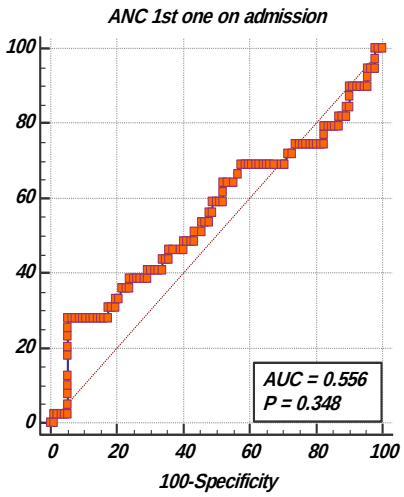

Fig 5: The validity of ANC level on admission with area under the ROC curve (AUC) as a diagnostic marker for NEC in the premature neonate

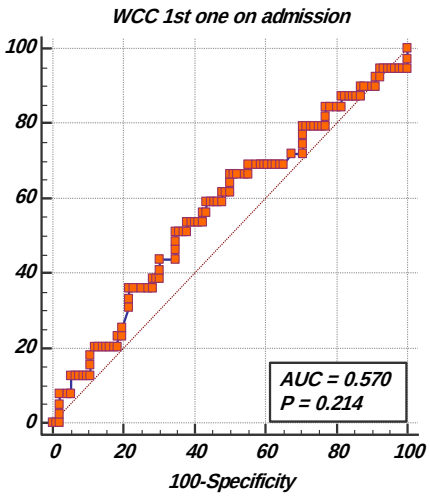

Fig 6: The validity of WCC level on admission with area under the ROC curve (AUC) as a diagnostic marker for NEC in the premature neonate

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