Increased Peripheral Blood Eosinophils May Indicate Acute Infection in Neonates

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Abstract

Background

Eosinophils are now being recognized for more varied functions such as antiviral and bactericidal effects. This study aimed to explore the association between increased blood eosinophils and frequent pathogens due to the infections in children.

Methods

A total of 2353 children with acute infections admitted to Guangzhou Women and Children's Medical Center from February 1, 2019 to January 31, 2020 were enrolled in the study. 277 children without infections were comprised the control group. Children's age, peripheral blood parameters including white blood cells, eosinophils, C-reactive protein (CRP) were recorded. In addition, infection stage and departments the patients admitted to were investigated. The study protocol was approved by the institutional ethics committee of the Guangzhou Women and Children's Medical Center (NO.2020110819342581).

Results

Blood eosinophil numbers negatively correlated with the age of children, whereas had no relation to disease stage. The means of eosinophil for neonates (<0.1 year)、infancy (<1year) and children >1year with acute infections were 0.67±0.40, 0.40±0.68, 0.15±0.25 $*10^9$/L compared with control group matched for age(0.44±0.20, 0.45±0.27, 0.24±0.19$*10^9$/L, P <0.001, <0.001, 0.497, respectively). Among them, the mean of eosinophil in the neonates afflicted with acute infections was significantly higher than the others compared to age-matched controls (0.63±0.60 vs 0.44±0.20, P= 0.012). Areas under the curves (AUC) were 0.81 (95% CI 0.75–0.86) for eosinophil combined with CRP and 0.68 (95% CI 0.61–0.75) for CRP alone for acute infections in neonates (P=0.02). Patients admitted in ICU had higher eosinophils than outpatients (0.46±0.60 vs 0.16±0.24, P <0.001) but had no significant difference compared with control group (0.45±0.20, P >0.99).

Conclusion

Increased peripheral blood eosinophils may indicate acute infections among neonates. Eosinophil combined with CRP can contribute to evaluating this population.

Background

Eosinophils are a prominent cell type in particular host responses such as the response to parasites infection and allergic inflammation. Their effector functions have been attributed to their capacity to release the eosinophil granule proteins. Eosinophilia is associated with T helper 2 cell-mediated immune responses, including the production of interleukin5, which enhances eosinophil development, activation and survival. An initial study showed that eosinophil secretory mediators decrease the ability of RSV to infect target host epithelial cells[1–2]. As was expected, viral clearance was significantly attenuated in KO mice compared with WT[3]. In addition, eosinophils express the necessary cellular machinery to mount an efficient bactericidal response. Consistent with this possibility, eosinophils express various pattern recognition receptors enabling them to sense bacterial antigens, to produce pro-inflammatory cytokines, cationic proteins and mitochondrial DNA-containing traps into the extracellular space to kill bacteria[4]. There is evidence to support increased numbers of eosinophils during bacterial infection. For example, eosinophil levels in the peripheral blood and rectum of patients afflicted with the diarrheal-inducing pathogen Shigella are increased[5]. In conjunction with many findings, we focused on the potential functions of eosinophil in viral and bactericidal infections.

RT-PCR and blood cultures are the gold standard for diagnosis of patients with infection[6]. However, it is not universally available and may have limitations in response times[7]. Fast and accurate laboratory diagnosis for pathogeny in routine practice is needed for clinicians optimally manage patients and potentially avoid the unnecessary use of antimicrobial drugs. Although CRP level [8]have been identified as potential predictors of bactericidal infection, none has been determined to have adequate specificity and sensitivity. Eosinopenia, defined as a reduced eosinophil count in peripheral blood, was previously identified as a good diagnostic marker of bactericidal infection [9]. In this study we explore the association between increased blood eosinophils and frequent pathogens due to the infections in pediatric patients treated in the general internal medicine department of our hospital.

Methods

Data source

This retrospective, single-center study included patients in the general internal medicine department, intensive care unit and emergency department, of Guangzhou Women and Children's Medical Center from February 2019 to January 2020, who underwent blood culture testing and either a PCR or an immunoglobulin test indicative of acute viral/MP infection were enrolled in the study, and urine, stool, cerebrospinal or bronchoalveolar lavage fluid cultures were performed according to the relevant symptoms. 11 pathogens as Human Bocavirus (HBoV), influenza A virus (FA), influenza B virus (FB), parainfluenza virus(PIV), rhinovirus(RHV), respiratory syncytial virus (RSV), adenovirus (ADV), Epstein-Barr virus (EBV), enterovirus (EV), herpes simplex virus (HSV), cytomegalovirus (CMV) were detected in PCR or Immunoglobulin test. The study protocol was approved by the institutional ethics committee of the Guangzhou Women and Children's Medical Center (NO.2020110819342581) and the institutional safety procedures were followed. All research was performed in accordance with the Declaration of Helsinki, and informed consent was obtained from all participants' legal guardians. A total of 2353 patients (age ≤17 years) who had been excluded because of cancer, helminth parasite infections and allergic diseases were enrolled in the study. In addition, 277 children in control group were clinically diagnosed with non-infectious and non-inflammatory diseases. No patients in any group developed immunological disorder. We

initially collected demographic data, results of laboratory blood tests from the hospital's electronic records. Additional data were identified in the hospital database or telephone follow up including family history of allergy, comorbidities, and time from early onset to discharge.

Study population and definition

Eosinophilia was defined as an eosinophil count of ≥ 0.6 cells/μL (0.6 *10^9/L), measured using hematologic blood draws obtained during the early onset stage, acute stage and convalescent phase (recovery period). We defined the early onset stage as the time within 5 days after the first day of symptoms appearance, acute stage as the time from 6 days to 2 weeks after onset of infection, recovery period as two weeks later after onset of infection (consult the early study[10]). The tendency of the immune system is toward a Th-2 response which may have effect on eosinophil levels in the first 24 h of life[11], data of complete blood abstracted on neonate with the first 24 h of birth was excluded. Age was divided into 3 categories, <0.1 year (neonates), 0.1-1year(infancy)and >1year (children). The primary study outcomes were peripheral blood parameters, a positive throat swab, bronchoalveolar lavage fluid, blood or secretion culture indicative of infection. We got 12 bacterial species in positive cultures including enterococcus faecium (Efa), Haemophilus influenzae (Hin), Klebsiella pneumoniae (Kpn), Staphylococcus aureus (Sau), Escherichia coli(E. coli), Streptococcus. Peroris (Spn), Salmonella typhimurium (Sty), acinetobacter baumannii (ABA), moraxella catarrhalis (MC), Campylobacter. Jejuni (Cje), Pseudomonas aeruginosa (Pae) and Shigella (Sca). Efa, Spn and Sau belong to G+ organisms, whereas Hin, Kpn, E. coli, Sty, ABA, MC, Cje, Pae, Sca belong to G- organisms. Patients were stratified into 4 groups according to pathogens: 1) virus, 2) G+ organisms, 3) G- organisms, 4) MP. In view of highly prevalent latent CMV infection, we refer to it as active CMV infection which may produce a variety of symptoms including fever, malaise, enlarged lymph nodes, sore throat, muscle aches, loss of appetite, enlarged liver or spleen, and fatigue[12]. Concurrently, we use the detection of CMV DNA in blood or BAL culture, as a measure of active CMV replication (DNA load was >500 copies/ml) [13–14]. The potential markers of infection assessed in this study included the serum CRP concentration and total WBC, platelet, lymphocyte, eosinophil count and CRP. Results from the patients in each group were compared between any two means (Flowchart for patient selection showed as Supplemental Figure 1).

Statistical analysis

We used t-test or the One-Way ANOVA to compare the continuous variables between the groups, whereas the enumeration data were compared by $\chi 2$ test. The association between disease stages with eosinophil count was estimated using repeated measures. ROC was constructed to calculate the best cutoff point and AUC for CRP alone, eosinophil alone, and CRP combined with eosinophil. The sensitivity and specificity were used to show diagnostic accuracy. Significant differences in multiplex variables levels between groups were defined by $p<0.05$. Experimental data were analyzed by SPSS v21.0 statistical software. The measurement data was expressed as the mean ± SD. The 95% confidence intervals (CIs) were used to quantify uncertainty.

Results

Patient characteristics

Patients with a previous diagnosis of cancer, helminth parasite infections and allergic diseases were excluded. A total of 2353 patients met the inclusion criteria, 277 children without infections were comprised the control group. The demographic and baseline characteristics of all subjects are summarized in Table 1. The median age of bacterial group was younger than other groups ($P<0.001$). To eliminate confounding effect age-induced, we analyzed data matched for age.

Peripheral eosinophils count from each group

The mean of eosinophil in the neonates afflicted with bacterial infections particularly G- infections was significantly higher than age-matched controls (0.63±0.60 vs 0.44±0.20, P= 0.015). Blood eosinophil numbers in neonates was very high for virus group compared with the other groups, however, this did not result in an exploration as there were no statistically significant differences among them because of a little sample size of 22 subjects, the same as MP group(n=6) (Table 2). Of the 2353 patients enrolled in the final analysis, 255 patients with eosinophilia defined as the count ≥0.6 *10^9/L, respectively, were identified during the study period. In addition, results from patients with eosinophil levels and 24 pathogens were further compared. The supplemental figure 2A lists the etiological distribution of 255 patients with different eosinophil levels and pathogens. Among them, patients with eosinophils ≥0.6 *10^9/L make up respectively 43.5% and 42.0% of the total patients afflicted with CMV DNA+ and Kpn (Supplemental Figure 2B).

Correlation of peripheral eosinophils count with multiple clinical characteristics

A correlation was found between eosinophils with the age and pathogens by using the Spearman correlation coefficient test, whether 2 of 3 factors were controlled for (R = -0.27, 0.28; $P<0.001$), whereas no significance in eosinophils and diseases stage (R = 0.14; $P = 0.062$). In addition, significant differences were seen among pathogens with different age (R= -0.38; $P<0.001$). Patients admitted in ICU had higher eosinophils than outpatients (0.46±0.60 vs 0.16±0.24, P <0.001), but had no significant difference compared with control group (0.45±0.20, P>0.99). We found that blood eosinophil numbers were high for neonates (<0.1 year) with infection indicative of infectious status in neonates (Figure 1A). The number of subjects with eosinophils ≥ 0.6 cells/μL decreased with ages increased in virus and G- group (Figure 1B-C).

We collected the settings where the patients admitted to, 1631 of the total 2353 children were outpatients, 542 from the general internal medicine department and 180 from the intensive care unit (ICU). The mean eosinophils count at ICU was higher than those of the other two settings. Patients who were bacteria positive possessed a higher number of eosinophils in ICU as well as the general internal medicine department than outpatients (0.46±0.60, 0.35±0.45 vs 0.16±0.24, P <0.001, <0.001), whereas no significant differences were seen with eosinophil count in the patients from ICU and control group (0.46±0.60 vs 0.45±0.20, P>0.99).

The results as shown in the table 2 demonstrated that patients infected with virus, MP and bacteria particularly G- organisms had higher eosinophil numbers compared to age-matched controls in neonates, however, there were no statistically significant differences between patients aged >0.1 year and the controls.

We did not always obtain paired samples for 3 stages of diseases, we lost samples for analyses. For the specific analyses reported here, we only obtained complete data sets from 168 patients (25 on group 1, 47 on group 2, 87 on group 3, and 9 on group 4).Using repeated measures, as is shown in the supplemental table 1, eosinophil numbers of group 1 decreased over hospitalization time, whereas those of group 2-4 reached their peaks on the acute stage, however there were no statistical significant differences in terms of eosinophil count with infection progression (P = 0.101), whether combined with the pathogen. In other words, there was no interaction in terms of eosinophils between stage and pathogen (P = 0.067).

Diagnostic value of eosinophil for infection

Areas under the receiver operating characteristic curve (AUC) was found to have a value of 0.64 (95% CI: 0.56–0.71) for eosinophil alone, which had no significant differences with the value of 0.68 (95% CI 0.61–0.75) for CRP alone in neonates with infections (consist of virus, bacteria and MP). The cutoff value for eosinophil was 0.60 *10^9/L, with a sensitivity and specificity, PPV, and NPV of 52.6%, 82.4%, 71.7% and 64.5%, respectively. When eosinophil was combined with CRP, the AUC was 0.81 (95% CI 0.75–0.86), with sensitivity, specificity, PPV, and NPV values of 60.6%, 99.3%, 97.1% and 67.1%, respectively. There were significant differences when compared with CRP alone in neonates with infections (P = 0.021). (Table 3, Figure 2A). Furthermore, we also analyzed the diagnostic value of eosinophil in neonatal patients with G- bacteria and virus (Figure 2B-C).

Discussion

Since neonatal infection is particularly difficult to diagnose and no dependable predictors exist. Thus, the identification of other predictors for neonatal sepsis is important. History and physical examination do not reliably exclude acute infections in neonates. We show that high blood eosinophil count is a biomarker of acute infection consist of virus, bacteria and MP among neonates, eosinophil combined with CRP can improve the diagnostic efficiency. Persistent eosinophilia after admission correlated with moderate/severe infections and younger age. In the present study, we found that blood eosinophil numbers were negatively correlated with the age of children. Test positivity was higher among neonates compared with elder pediatric patients. Transient eosinophilia is observed relatively frequently in the pediatric population and is generally clinically insignificant[15]. However, eosinophil levels in the peripheral blood vary by age, with higher upper threshold limits seen in infants and toddlers compared to adolescents and adults[16]. To eliminate confounding effect age-induced, we analyzed data matched for age, our study still reveal that increased eosinophils performed better in discriminating acute infections among neonatal age group than older pediatric patients. Elevated blood eosinophils at age 4 weeks may have a predictive value for the onset of atopic dermatitis in infancy and early childhood in children with high risk for atopy[17]. There were no apparent age-related changes in eosinophil or basophil counts in normal[18]. Of note, our findings suggest that increased eosinophils were observed only in neonates but not children with infection which may indicate differences in immune-based control mechanisms of these two subsets. Until now, however, the mechanisms to explain these findings have not been defined. This raises the important question of whether there is a hypothesis linking acute infection with age-related changes in eosinophil counts like age-dependent T-cells and BK polyomavirus-specific cellular immune responses[19–20]. These differences may be a reflection of a developing immune system of newborn[21] that shows features of hyporesponsiveness[22] and age-dependent differences in levels of selectins in children[23].

Eosinophil numbers of all virus decreased over hospitalization time, whereas those of bacteria and MP followed by a delayed increase. Together this suggests that eosinophils exposed to bacteria and virus can drive subsequent inflammatory responses in a specific manner. Furthermore, we found that eosinophils of G+ organisms reached a nadir on the acute stage, whereas G- organisms were in direct contradiction. While peripheral blood eosinophil numbers may rapidly diminish with acute infection, this marked reduction can be accompanied by increased serum levels of the eosinophil granule protein ECP, which suggests eosinophil activation and degranulation.[24] This, however, did not result in an exploration as there were no statistically significant differences between disease progression as is shown in the supplemental table 1.

It has been observed that higher sputum eosinophil counts are associated with lower levels of colonizing bacteria in the airways of COPD patients[25–26]. Eosinophils generate reactive oxygen species, hypohalous acids and lysosomal hydrolases that are toxic for bacteria but also for surrounding tissues[27]. Inducible costimulatory signaling which associated with increased eosinophils recruitment to the airway also contributed to the pathogenesis of the airway pathogens Klebsiella pneumoniae and Pseudomonas aeruginosa[28].

We found a higher percentage of blood eosinophils among young infants who were afflicted with CMV, which was consistent with a view that the immature immune system of young children[29] might not be able to suppress CMV replication after primary infection, which offers a potential explanation for our observations of higher morbidity of CMV infections in the younger age group of children. Previous study demonstrated that CMV reactivation was associated with asthma or recurrent wheeze[30]. The pathophysiology of recurrent wheeze in young children is unclear, more recently it has been reported that the presence of CMV DNA in the blood of adult and elderly patients was associated with an increased risk of asthma, and that CMV DNA copy numbers correlated with certain asthma traits[31]. In addition, among the patients with CMV infection, there was a notable elevation of the mean ECP concentration for the group. This may indicate that CMV pulmonary infection may be associated with eosinophil activation.[32] Nonetheless, we still could not draw the conclusion that high blood eosinophil count is a biomarker of active CMV infection as most immunocompetent individuals with CMV infections experience mild symptoms or are asymptomatic. The limitation that not all patients underwent CMV DNA detection in our study resulted in a selection bias. Prospective studies should further study this important subset of patients at the time of active CMV infection diagnosis.

The findings that patients admitted in ICU had higher eosinophils than outpatients had not led to speculation that high eosinophil level associated with severe infection as there were no significant differences between the ICU and control groups. This contrast with the theory that eosinopenia is frequent and has been

linked to mortality in different settings during critical illness reported by other authors[9, 33–34]. Furthermore, the increase in eosinophil number during the ICU stay in patients who survived is a valuable result given the biological context that relates their presence with the resolution of the inflammatory state[35].

Our findings focused on the patient's history (exclusive of cancer, helminth parasite infections, and allergic diseases), clinical manifestations and comprehensive assessment of peripheral blood parameters as the first step and the level of blood eosinophilia as the second, and this can help the physician to identify patients presenting with an elevated blood eosinophil count that need further laboratory or instrumental investigations. The observed link between increased eosinophils and acute infection in neonates but not older pediatric patients has led to speculation that eosinophils may contribute to the congenital immunodeficiencies. Such detailed analysis deserves further studies.

Limitations

Our study has several limitations. First, its retrospective design involved a limited value in the quality of information. Second, other factors such as effects of blood type and certain medication on eosinophil levels were not evaluated. Thirdly, the children were not systematically tested for all the infections in each group. Finally, the study was conducted at a single center.

Conclusion

Despite these limitations, our findings highlight that increased peripheral blood eosinophils may indicate acute infection in neonates. Eosinophil combined with CRP can contribute to evaluating this population. Elucidation of the precise mechanisms by which especially CMV and G- bacteria are inactivated by eosinophils in this subset of patients awaits further studies.

Abbreviations

CRP C-reactive protein

RT-PCR Reverse Transcription-Polymerase Chain Reaction

HBoV Human Bocavirus

FA influenza A virus

FB influenza B virus

PIV parainfluenza virus(PIV)

RHV rhinovirus

RSV respiratory syncytial virus

ADV adenovirus

EBV Epstein-Barr virus

EV enterovirus

HSV herpes simplex virus

CMV cytomegalovirus

EFa enterococcus faecium

Hin Haemophilus influenzae

Kpn Klebsiella pneumoniae

Sau Staphylococcus aureus

E. coli Escherichia coli

Spn Streptococcus. Peroris

Sty Salmonella typhimurium

ABA acinetobacter baumannii

MC moraxella catarrhalis

Cje Campylobacter. Jejuni

Pae Pseudomonas aeruginosa

Sca Shigella

CIs confidence intervals

AUC Areas under the receiver operating characteristic curve

Declarations

Ethics approval and consent to participate

Ethics committee of Guangzhou Women and Children's Medical Center (NO.2020110819342581). The patients consented to analysis of their medical records was not required. Any further permission from the hospital was not required. This was because of all data was obtained from usual clinical process.

Consent for publication

Not applicable.

Availability of data and materials

Data are available from the corresponding author on reasonable request.

Competing interests

Not applicable.

Funding

Not applicable.

Authors' Contributions

WYW , BFY and YZ contributed to the study concept and design. BFY performed the statistical analyses. WYW contributed to the drafting of the manuscript. BFY and YZ contributed to the critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Tables

Table 1 Patient general characteristics*

Factors	Virus (N=971)	G+ (N=384)	G- (N=611)	MP (N=387)	control (N=277)	F/*X2*	*P*
Gender, n (%)						3.39	0.561
Male	447（56.7）	186（58.4）	331（61.5）	221（57.1）	159（57.1）		
Age, year						106.20	<<0.0011
Mean ± SD	4.47±3.25	2.58±3.16	2.12±2.92	3.97±2.65	2.02±2.09		
Median	4.05	1.17	0.91	3.5	1.48		
95% CI	4.26-4.78	2.23-2.93	1.87-2.37	3.37-4.24	1.78-2.27		
CRP,mg/l						57.59	<<0.0011
Mean ± SD	13.57±22.53	30.87±40.69	28.35±44.12	9.94±16.71	0.86±1.63		
Median	6.1	11.05	7.9	2.8	0.9		
95% CI	12.21-14.94	26.38-35.36	24.62-32.08	8.27-11.61	0.62-1.11		
Eosinophil,*10^9/L						40.56	<0.001
Mean ± SD	0.16±0.34	0.34±0.38	0.33±0.47	0.24±0.24	0.40±0.24		
Median	0.02	0.23	0.16	0.16	0.25		
95% CI	0.01-0.14	0.30-0.38	0.29-0.37	0.21-0.26	0.37-0.43		
LYMPH,*10^9/L						3.88	<0.001
Mean ± SD	3.47±10.50	4.39±2.64	4.26±3.20	3.72±1.87	5.02±1.73		
Median	2.12	4.03	3.69	3.44	5.15		
95% CI	2.82-4.13	4.10-4.68	3.98-4.53	3.54-3.91	4.87-5.28		
PLT,*10^9/L						34.89	<0.001
Mean ± SD	291±118	368±158	342±152	351±152	356±107		
Median	274	347	327	320	334		
95% CI	283-298	351-386	329-355	338-363	344-69		
WBC,*10^9/L						15.06	<0.001
Mean ± SD	9.32±5.38	12.19±6.61	11.73±10.64	9.67±3.42	12.44±6.88		
Median	0.08	10.94	10.11	8.87	9.91		
95% CI	8.96-9.67	11.46-12.92	10.83-12.63	9.33-10.01	9.46-15.42		

* CRP, C-reactive protein; PLT, Platelets; WBC, white blood cells; G+, gram-positive organisms; G-, gram-negative organisms; MP, mycoplasma pneumoniae; CI, confidence interval; SD, standard deviation.

Table 2 Comparison of eosinophils between two groups matched for age(mean±SD)

	<0.1 year			0.1-1year			>1year		
	Positive group	Control group(n=126)	*P*	Positive group	Control group(n=90)	*P*	Positive group	Control group(n=62)	*P*
Infection	0.67±0.40(n=228)	0.45±0.20*	<0.001	0.40±0.68(n=562)	0.44±0.27	<0.001	0.15±0.25(n=1563)	0.24±0.19	0.491
Virus	0.64±0.39(n=22)		0.141	0.48±0.61(n=162)		>0.99	0.09±0.16(n=787)		>0.99
Bacteria(G-&G+)	0.58±0.55(n=200)		0.012	0.35±0.44(n=355)		0.198	0.23±0.32(n=440)		>0.99
G-	0.63±0.60(n=139)		0.017	0.34±0.34(n=226)		0.721	0.22±0.34(n=246)		>0.99
G+	0.47±0.38(n=61)		>0.99	0.37±0.45(n=129)		0.781	0.23±0.30(n=194)		>0.99
MP	0.61±0.16(n=6)		0.211	0.38±0.28(n=45)		0.725	0.22±0.23(n=336)		0.961

* Our results are consistent with earlier study showing the reference range for blood concentration of eosinophils during the first 28 days after birth[38].

Table 3 Diagnostic Accuracy of CRP, eosinophils and CRP with eosinophils in neonates with acute infection*

	Cutoff Value	AUC	95% CI	Sensitivity,%	Specificity,%	PPV,%	NPV,%
Eosinophil, 10^9/L	0.6	0.64	0.56-0.71	52.6	82.4	71.7	64.5
CRP, mg/l	6.5	0.68	0.61-0.75	51.3	98.6	94.9	62.6
CRP and EO	0.7	0.81	0.75-0.86	60.6	99.3	97.1	67.1

* AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value.

Figures

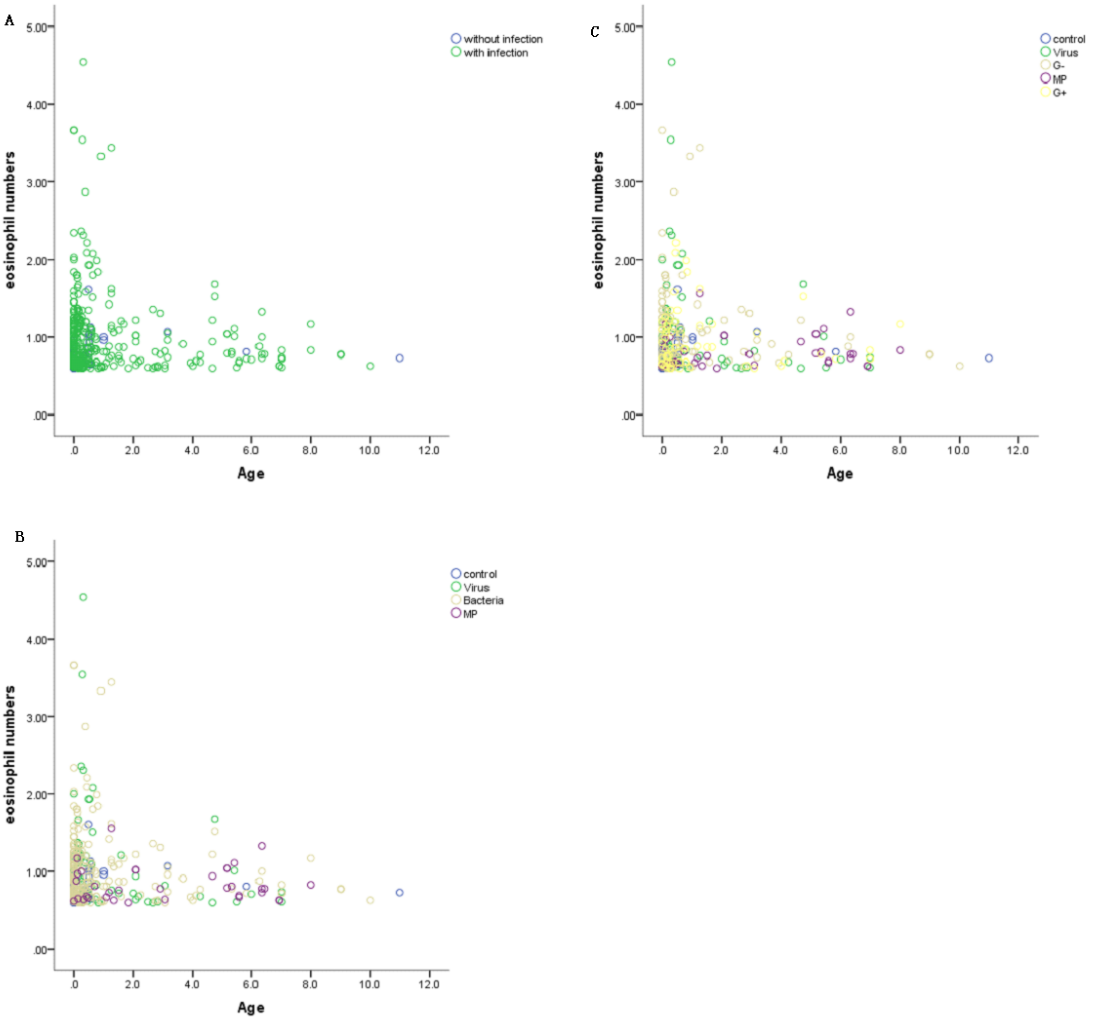

Figure 1

Age distribution of this study population with peripheral eosinophils ≥ 0.6 cells/μL with acute infection. A, Most of neonates with acute infection had eosinophilia. B, In the neonates with acute infection, most of subjects in bacterial group had eosinophils ≥ 0.6 cells/μL. C, Neonates with Gram-negative bacterial infection account for the majority of the population with increased eosinophils.

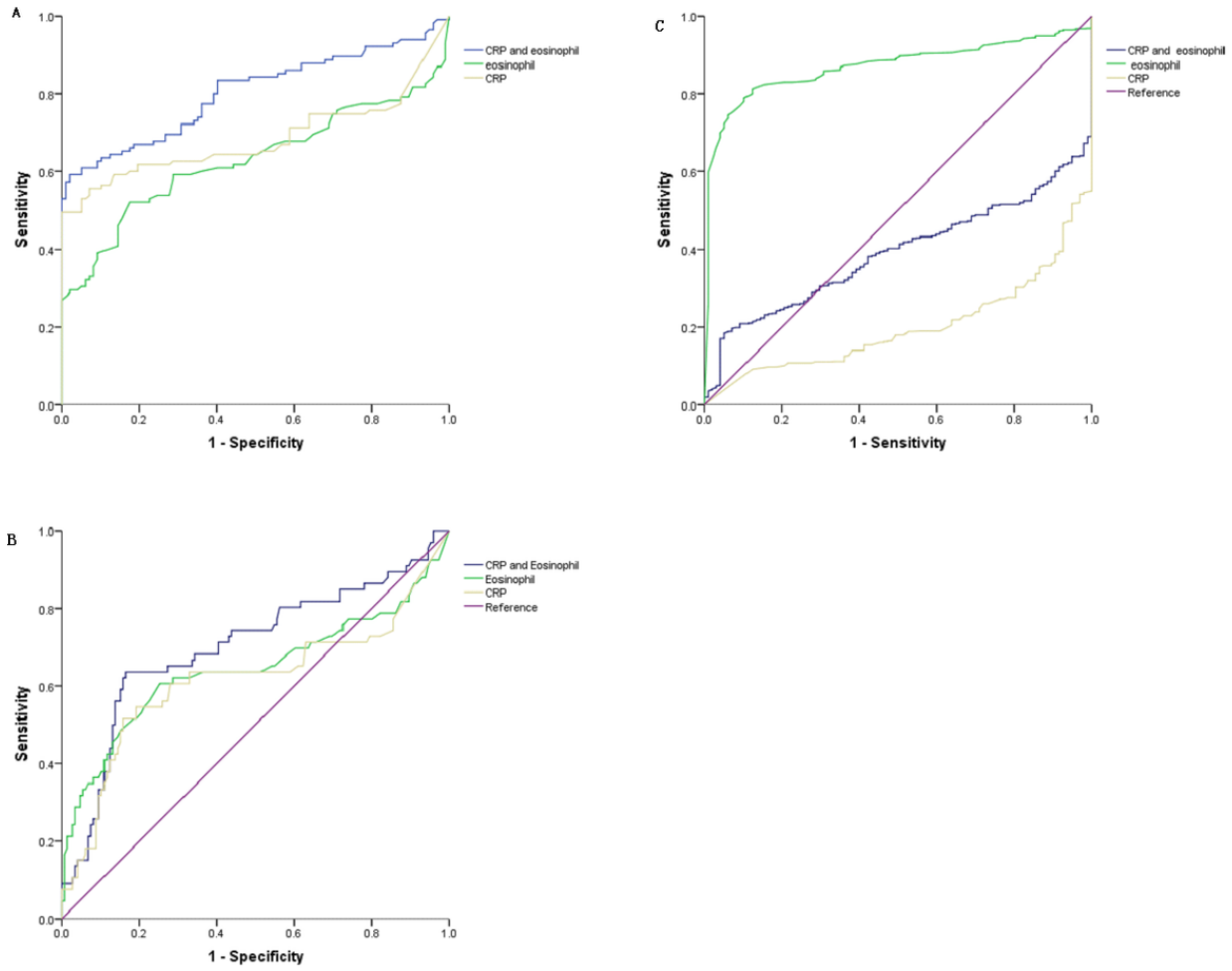

Figure 2

A, AUC of eosinophil alone, CRP alone, and eosinophil combined with CRP to predict acute infection in neonates. B, AUC of eosinophil alone, CRP alone, and eosinophil combined with CRP to predict G- infection in this population. The AUC values were 0.64 (95% CI: 0.55- 0.74), 0.62(0.52-0.71),0.70(0.62-0.79) respectively. C, AUC of peripheral eosinophils to predict viral infection in neonates. The AUC values were 0.87 (95% CI: 0.84- 0.90), 0.20(0.17-0.23),0.39(0.35-0.43)

Supplementary Files

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- SupplementaryFile2.docx