学位論文

Usefulness of parameters of the XN-Series hematology analyzer for patients with suggested bacteremia

（菌血症疑い患者における多項目自動血球分析装置
XNシリーズの有用性の検討）

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Abstract

Objectives: To investigate whether the leukocyte parameters tested using the XN-Series hematology analyzer could be useful in the treatment of bacteremia.

Methods: Patients with suggested bacteremia at Toyama University Hospital during July 1, 2017–January 31, 2018, were included in this study. Patients with suggested bacteremia were divided into the bacteremia and non-bacteremia groups based on blood culture and Tm mapping method results; leukocyte parameters and Tm mapping method results were compared and analyzed between both the groups.

Results: NE-SFL (mean intensity of lateral fluorescence of neutrophils) and NE-WY (variation in lateral fluorescence) were significantly higher in the bacteremia group than in the non-bacteremia group. Concerning the receiver operating characteristic curves of NE-SFL and NE-WY in the diagnosis of bacteremia, the area under the curve of NE-SFL was 0.704 (sensitivity 0.605, specificity 0.800) and that of NE-WY was 0.641 (sensitivity 0.419, specificity 0.840). The correlation coefficients between the number of bacteria per milliliter and NE-SFL and NE-WY were r = 0.197 and r = 0.36, respectively, indicating no strong correlation. The correlation coefficients between IL-6 and NE-SFL and NE-WY were r = 0.484 and r = 0.614, respectively, indicating a relatively strong correlation.

Conclusion: Although it is difficult to specifically diagnose bacteremia using only leukocyte parameters, it may help in the diagnosis of bacteremia owing to their faster turnaround time, simplicity, and cost-effectiveness. To improve the accuracy of bacteremia diagnosis, it is necessary to develop a diagnostic algorithm that combines multiple parameters.

Introduction

In recent years, with the advancement in cancer treatment and organ transplantation, the risk of contracting serious infectious diseases has increased, and the major cause of death among hospitalized patients is reportedly severe infections such as sepsis [1]. Bacteremia causes sepsis and septic shock, which are often severe and have a high mortality rate [2, 3]. Blood culture is generally used to diagnose bacteremia, but owing to the extremely long turnaround time, rapid biomarker testing is desired.

Phagocytic cells involved in innate immunity, such as neutrophils and monocytes, form cytoplasmic vacuoles in a reactive oxygen species production-dependent manner when they recognize pathogens [4–6]. These vacuole-forming neutrophils are known to increase during bacterial infections and have high sensitivity and specificity on testing [7, 8]. However, these vacuoles affect the optics in flow cytometry [9]. Sysmex's automated hematology analyzer is based on the principle of flow cytometry; reportedly, leukocyte parameters of patients with sepsis tested using the analyzer are altered [10–14]. Since leukocyte parameters can be analyzed using the leukocyte fractionation assay and the test is easily available, I considered it possibly useful for the early diagnosis of bacteremia. I also used the melting temperature mapping (Tm mapping) method [15] developed in this study to understand the pathogenesis

of the disease. The Tm mapping method is a new method for the rapid identification of causative microorganisms using 16S rRNA inheritance (16S rDNA). It is based on real-time polymerase chain reaction (PCR) without culturing and can quantitatively measure the detected organisms and capture the microorganisms phagocytosed by leukocytes. In this study, I investigated the usefulness of leukocyte parameters as a novel biomarker for the diagnosis of bacteremia in patients with suggested bacteremia using the Tm mapping method.

This thesis is based on the following paper: Yuki Miyajima, et al. Evaluation of the usefulness of parameters of the automated hematology analyzer XN-Series in patients with suspected bacteremia. *Clinical Chemistry and Laboratory Medicine* (submitted).

Materials and methods

Patients with suggested bacteremia at Toyama University Hospital and 37 healthy individuals were included in this study from July 1, 2017, to January 31, 2018. This study was reviewed and approved by the Ethics Committee of Toyama University Hospital (Approval No.: 29-152) and is in accordance the tenets of the Helsinki Declaration.

Patients with insufficient data, febrile neutropenia, and leukemia were excluded. Patients with suggested bacteremia were classified into two groups: (1) bacteremia, in which microorganisms including fungi were detected in the blood on either blood culture (BacT/ALERT® 3D, bioMérieux) or Tm mapping, and (2) non-bacteremia, in which no microorganisms were detected on any test. Samples that turned positive after more than 48 hours and samples with only one set positive for *Bacillus cereus*, *Staphylococcus epidermidis*, or *Staphylococcus capitis* were excluded on suspicion of contamination by skin commensals [16, 17]. Patients with less than 1–2 bacteria per PCR tube on Tm mapping were also excluded because of suspected contamination.

Considering patient demographics such as age and sex, I then investigated the usefulness of testing the leukocyte parameters in the bacteremia and non-bacteremia groups using an automated hematology analyzer (XN-2000, Sysmex, Kobe, Japan). In addition, the bacterial

load in cases in which microorganisms were detected using the Tm mapping method was also examined.

Statistical analysis was performed using EZR, and leukocyte parameters were compared using the Mann–Whitney U test or Kruskal–Wallis test. Bivariate correlations were evaluated using Spearman's rank correlation coefficient. Based on receiver operating characteristic (ROC) curves, sensitivity, specificity, and the area under the curve (AUC) of the testing method were evaluated. The significance level was set at $P < 0.05$.

Results

A total of 133 patients met the selection criteria; however, 8 with missing data, 1 with febrile neutropenia, and 2 with leukemia were excluded, resulting in 122 suggested cases of bacteremia. Furthermore, blood culture and Tm mapping resulted in 45 cases in the bacteremia group and 77 cases in the non-bacteremia group (Figure 1). Table 1 shows the patient characteristics and laboratory findings, and Table 2 shows the results of the blood culture and Tm mapping. *Escherichia coli* was the most common microorganism detected using both methods, and microorganisms in nine cases could not be identified on Tm mapping, indicating the possibility of multiple bacterial infections.

NE-SFL (mean intensity of lateral fluorescence of neutrophils) and NE-WY (variation in lateral fluorescence) were significantly higher in the bacteremia group than in the non-bacteremia group (Table 3 and Figure 2). There were no significant differences in lymphocyte and monocyte counts between the two groups.

The ROC curve for the diagnosis of bacteremia was constructed and compared (Figure 3). The AUC of NE-SFL was 0.704 (sensitivity 0.605, specificity 0.800, cutoff value 51.600) with high accuracy, whereas that of NE-WY was 0.641 (sensitivity 0.419, specificity 0.840, cutoff value 771.000) with low accuracy.

Thirty-two cases of microorganisms were detected on Tm mapping. The correlation coefficients between the number of bacteria per milliliter and neutrophil parameters were r = 0.197 and r = 0.36, respectively (Figure 4). The correlation coefficients between interleukin-6 (IL-6) and NE-SFL and NE-WY were r = 0.484 and r = 0.614, respectively, indicating a relatively strong correlation (Figure 5).

Discussion

I found that the leukocyte parameters NE-SFL and NE-WY were significantly higher in the bacteremia group than in the non-bacteremia group. NE-SFL indicates lateral fluorescence, reflecting the type and abundance of nucleic acids and organelles in neutrophils, and NE-WY reflects the variability of NE-SFL. Reportedly, the mobilization of juvenile leukocytes with high nucleic acid content in the peripheral blood increases the amount of nucleic acid in the cytoplasm of neutrophils, resulting in high NE-SFL [18]. Neutrophils also respond to innate immunity and undergo cell death through mechanisms such as apoptosis, necrosis, or NETosis [19–21]. Therefore, the presence of both larval leukocytes and dying neutrophils in the peripheral blood causes variation in the amount of nucleic acids in neutrophils, resulting in high NE-WY values, and such changes are considered to be more pronounced in bacteremia.

In this study, in addition to blood culture, a quantitative analysis was performed using Tm mapping. I found that NE-WY but not NE-SFL correlated with the number of bacteria per milliliter. I also examined the influence of cytokines such as IL-6, which may affect leukocyte parameter values, and found a correlation between IL-6 and both NE-SFL and NE-WY, with a stronger correlation with NE-WY. Tm mapping uses plasma containing the buffy

coat, and the number of bacteria indicates the number suspended in plasma and the number phagocytosed by neutrophils. The high value of NE-WY could have been caused by the variation in the number of neutrophils affected by cytokines and neutrophils involved in phagocytosis. Therefore, I suggest that NE-WY, a neutrophil parameter, may more sensitively reflect the pathogenesis of bacteremia. However, the timing of cytokine production and phagocytosis has not been investigated, and further studies including time-series data are needed. Bacteremia often results in severe outcomes, and no specific biomarkers exist. Although detection and identification of microorganisms is via blood culture—the gold standard method—more rapid tests are desirable owing to the extremely long turnaround time. Although it is difficult to specifically diagnose bacteremia using only leukocyte parameters, these can be analyzed during leukocyte fractionation in routine tests. Because results can be obtained quickly, easily, and inexpensively, they may serve as an adjunct test for bacteremia in terms of diagnosability. To further improve the accuracy of diagnosis, it is necessary to consider the combination of multiple parameters in the diagnosis algorithm. Further studies with more cases and time-series data will be necessary in the future.

Declarations

Conflict of interest

I have nothing to declare.

Informed consent

Informed consent was obtained from all individuals included in this study.

Ethical approval

Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013) and has been approved by the authors' Ethics Committee (29-152).

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Tables

Table 1

Patient characteristics and laboratory findings

	Bacteremia (n=45)	Non-bacteremia (n=77)	*p*-value
Age, median (IQR), y	67.5 (61.0-77.0)	70.0 (51.0-79.5)	0.941
Male, n (%)	29.0 (64.4)	49.0 (63.6)	0.865
Female, n (%)	16.0 (35.6)	28.0 (36.4)	0.865
qSOFA,median (IQR)	1.0 (0.0-2.0)	1.0 (0.0-1.0)	0.122
SOFA,median (IQR)	3.0 (1.0-5.0)	1.0 (1.0-3.0)	0.012
BT,median (IQR), ℃	38.1 (37.7-40.7)	37.8 (37.1-38.5)	0.066
WBC,median (IQR), /μL	10480.0 (5620.0-12530.0)	10420.0 (7570.0-14280.0)	0.329
Neutrophils, median (IQR), /μL	8710.0 (4590.0-11600.0)	7990.0 (5400.0-12405.0)	0.540
Lymphocytes, median (IQR), /μL	560.0 (380.0-885.0)	840.0 (565.0-1420.0)	0.003
Monocytes, median (IQR), /μL	330.0 (210.0-615.0)	540.0 (345.0-710.0)	0.004
Platelets, median (IQR), $\times 10^3/\mu L$	175.0 (134.0-217.0)	226.0 (171.0-292.0)	0.002
CRP, median (IQR), mg/dL	6.3 (0.5-11.8)	4.6 (0.9-11.6)	0.669
PCT, median (IQR), ng/mL	0.6 (0.1-4.4)	0.2 (0.07-0.7)	0.003
IL-6, median (IQR), pg/mL	651.8 (48.0-3538.7)	61.0 (26.0-256.5)	< 0.001

qSOFA = quick Sequential Organ Failure Assessment

BT = Body temperature, WBC = White blood cell, CRP = C-reactive protein

PCT = Procalcitonin, IL-6 = Interleukin-6

Table 2

Results of blood culture and Tm mapping

organism	Blood culture, n	Tm mapping method, n
Acinetobacter baumannii		1
Escherichia coli	10	5
Klebsiella pneumoniae	5	4
Listeria monocytogenes	1	
Pseudomonas aeruginosa	1	
Pseudomonas fluorescens		3
Staphylococcus aureus	3	3
Staphylococcus capitis	1	1
Streptococcus dysgalactiae	1	
Staphylococcus epidermidis	1	1
Staphylococcus hominis		1
Streptococcus oralis	1	
Staphylococcus pettenkoferi		1
Streptococcus pneumoniae	1	2
Streptococcus salivarius		1
Candida albicans	2	
Cryptococcus neoformans	1	
Not identified		9

Table 3

Comparison of leukocyte parameters between the bacteremia, non-bacteremia, and control groups

(A) Neutrophil parameter

Leukocyte parameters	Bacteremia (n=45)	Non-bacteremia (n=77)	Healthy control (n=37)	*p*-value*
NE-SSC, median (IQR)	155.1 (151.6-159.7)	153.9 (150.5-157.4)	152.4 (150.6-154.6)	0.259
NE-SFL, median (IQR)	53.0 (48.3-57.2)	48.0 (45.7-50.7)	46.8 (45.3-48.6)	< 0.001
NE-FSC, median (IQR)	88.6 (85.5-92.3)	87.3 (84.2-92.3)	88.5 (86.5-91.5)	0.325
NE-WX, median (IQR)	310.0 (295.0-322.0)	308.0 (301.0-319.0)	292.0 (285.5-304.5)	0.933
NE-WY, median (IQR)	729.0 (676.0-859.0)	690.0 (633.0-750.0)	600.0 (586.5-620.5)	0.011
NE-WZ, median (IQR)	761.0 (702.0-867.0)	737.0 (681.0-805.0)	754.0 (698.5-799.0)	0.148

NE = Neutrophil

*Bacteremia vs. non-bacteremia, *p*-value < 0.05 indicates statistical significance.

(B) Lymphocyte parameter

Leukocyte parameters	Bacteremia (n=45)	Non-bacteremia (n=77)	Healthy control (n=37)	*p*-value*
Ly-SSC, median (IQR)	82.4 (80.7-83.5)	81.8 (80.4-83.5)	80.9 (79.7-82.0)	0.550
Ly-SFL, median (IQR)	67.4 (63.8-69.9)	67.6 (64.6-71.3)	68.4 (65.7-69.5)	0.693
Ly-FSC, median (IQR)	56.3 (54.2-59.2)	56.4 (54.4-59.3)	56.1 (55.1-58.1)	0.869
Ly-WX, median (IQR)	447.0 (426.0-496.0)	486.0 (438.0-539.0)	496.0 (467.5-530.0)	0.085
Ly-WY, median (IQR)	874.0 (736.0-988.0)	870.0 (786.0-983.0)	861.0 (815.0-889.5)	0.675
Ly-WZ, median (IQR)	608.0 (554.0-687.0)	591.0 (556.0-650.0)	636.0 (562.0-686.0)	0.302

LY = Lymphocyte

*Bacteremia vs. non-bacteremia, *p*-value < 0.05 indicates statistical significance.

(C) Monocyte parameter

Leukocyte parameters	Bacteremia (n=45)	Non-bacteremia (n=77)	Healthy control (n=37)	*p*-value*
Mo-SSC, median (IQR)	123.1 (119.6-125.7)	121.8 (119.7-124.1)	120.9 (120.2-121.9)	0.260
Mo-SFL, median (IQR)	112.8 (105.7-119.5)	111.6 (104.9-118.6)	111.2 (109.4-115.1)	0.871
Mo-FSC, median (IQR)	65.9 (63.6-69.5)	66.4 (63.5-68.8)	68.2 (66.8-69.9)	0.924
Mo-WX, median (IQR)	263.0 (242.0-308.0)	263.0 (243.0-282.0)	244.0 (226.0-266.0)	0.340
Mo-WY, median (IQR)	696.0 (550.0-769.0)	697.0 (664.0-781.0)	656.0 (596.5-714.0)	0.178
Mo-WZ, median (IQR)	664.0 (565.0-802.0)	671.0 (617.0-766.0)	690.0 (565.0-802.0)	0.651

Mo = Monocyte

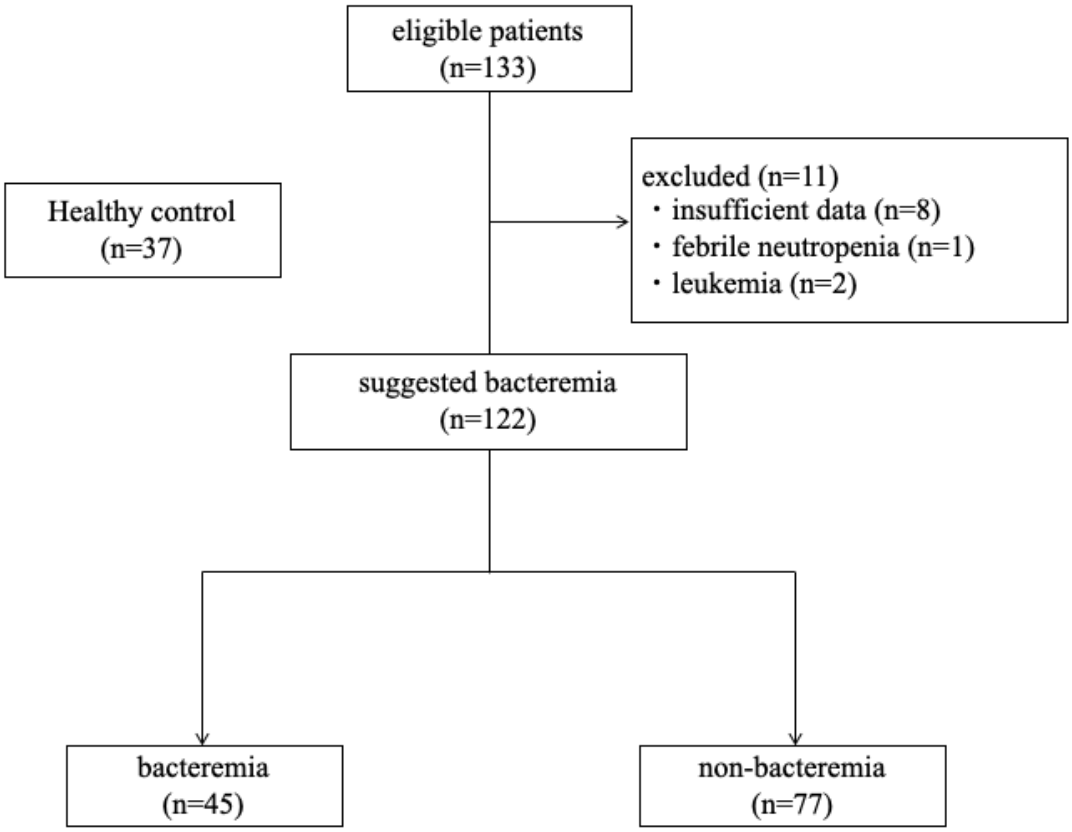
*Bacteremia vs. non-bacteremia, *p*-value < 0.05 indicates statistical significance.

(D) Description and clinical significance of leukocyte parameters

Leukocyte parameters	Analysis method of leukocyte cluster	Meaning
SSC	side scattered light	internal cell structure and granularity
SFL	side fluorescence light	DNA/RNA content
FSC	forward scattered light	cell size
WX	side scattering light distribution width	variation in internal cell structure and granularity
WY	fluorescence distribution width	variation in DNA/RNA content
WZ	forward scattering light distribution width	variation in cell size

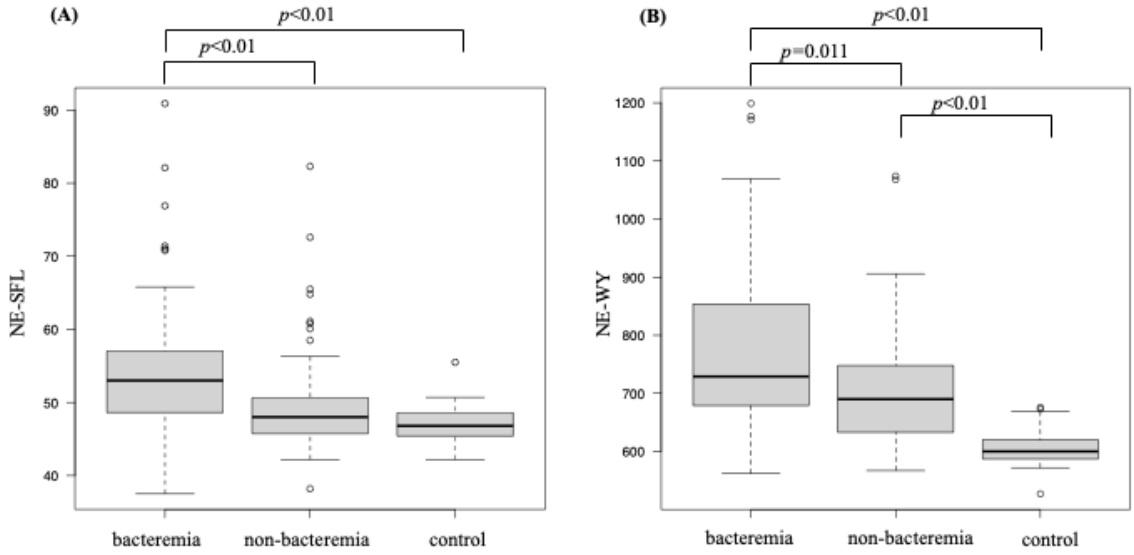
FIGURE LEGEND

Figure 1



Flow chart

Figure 2

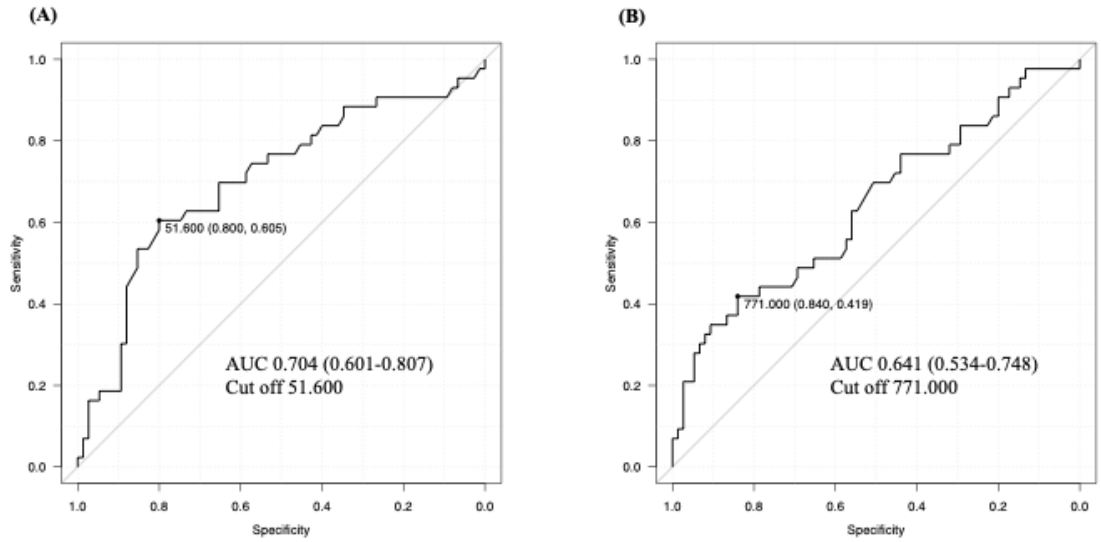


Comparison of the neutrophil parameters (NE-SFL and NE-WY) between the bacteremia, non-bacteremia, and control groups

(A) NE-SFL

(B) NE-WY

Figure 3

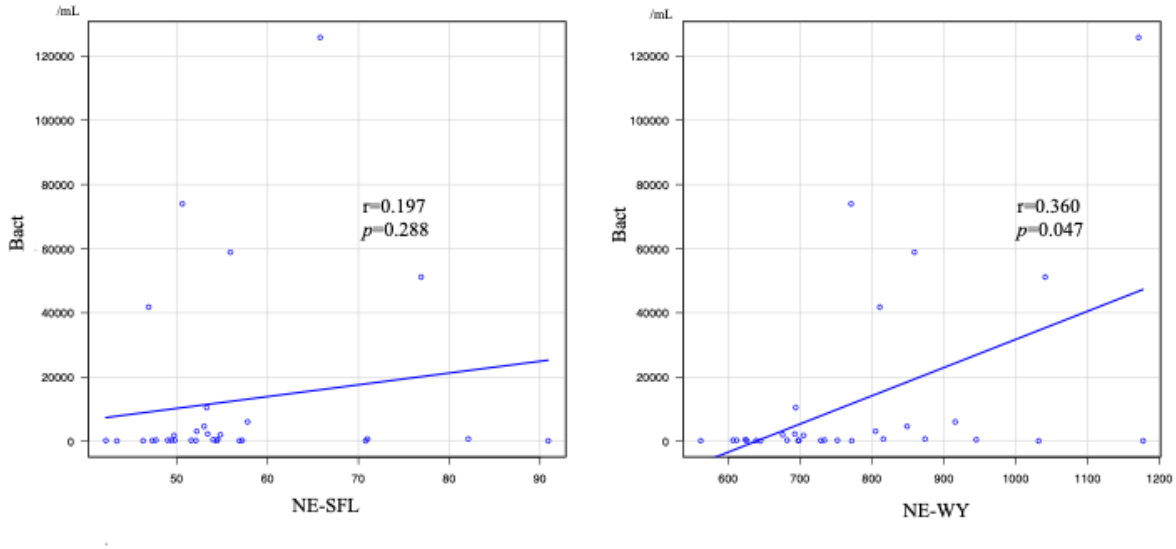


Receiver operating characteristic curves of each neutrophil parameter

(A) NE-SFL

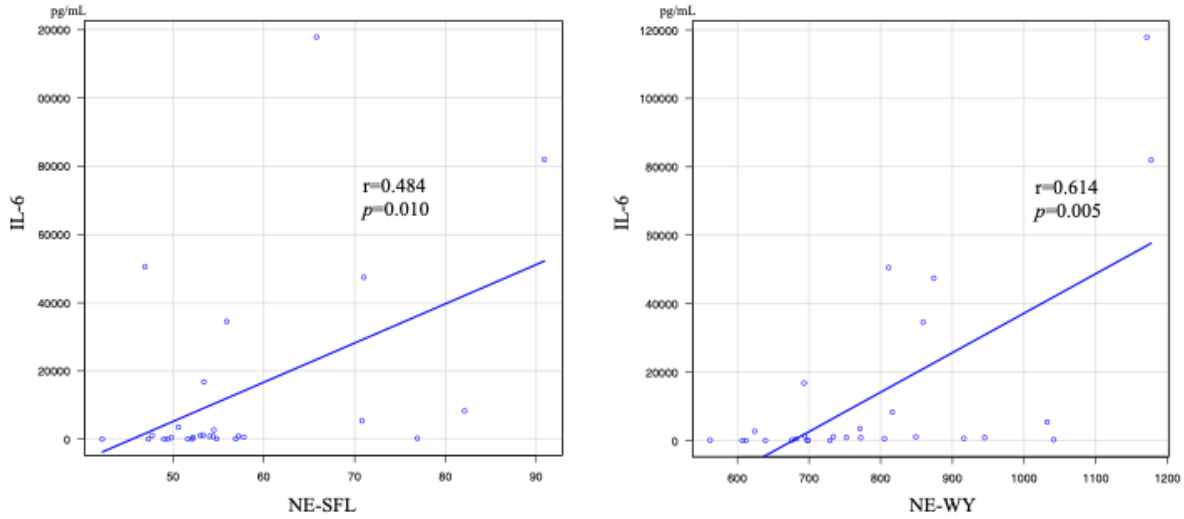
(B) NE-WY

Figure 4



Correlation between the number of bacteria per milliliter and neutrophil parameters

Figure 5



Correlation between interleukin-6 and neutrophil parameters