

Original Article

Study of hematologic change among patients with sepsis at internal medicine wards

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Abstract:

Background: Sepsis is a serious medical condition caused by an overwhelming immune response to infection. The hematological changes are related to clinical sepsis but the evidence is limited in Thailand. **Objective:** The study aimed to identify the incidence of hematologic changes and correlation with other organs among patients with sepsis. **Method:** This was a retrospective review of patients with sepsis admitted to medicine wards from January 2012 to July 2012. Data on medical records were reviewed at the first day (day 0) and the seventh day of sepsis (day 7). **Results:** The study involved a total of 196 patients with 37 cases of septic shock (18.9%). The median age was 70 years (range 16-105). Eighty-seven patients (44.4%) were male and 109 patients (55.6%) were female. The primary sources of sepsis were predominantly UIT (58 cases, 29.6%), followed by septicemia (39 cases, 19.39%), pneumonia (33 cases, 16.8%), diarrhea (22 cases, 11.2%), and cellulitis (18 cases, 9.2%). Identified pathogen were 21 cases (10.7%) with Gram positive cocci, 95 cases (48.5%) with Gram negative bacilli, 13 cases (6.6%) with other (virus, fungus, mycobacterium, spirochete, parasite), and 67 cases (34.2%) unknown. Most cases had a duration of sepsis ranging from 1-3 days (120 cases, 61.2%), the second frequent ranged from 4-7 days (57 cases, 29%), and 19 patients (9.7%) had clinical sepsis more than 7 days. The hematologic changes found were anemia with 80 cases (40.8%) having hemoglobin (HB) less than 10 g/dL and 77 patients (39.3%) had decreased hematocrit (HCT) more than 3% at day 7. In all, 40 (20.4%) patients had white blood cell (WBC) count below $4 \times 10^9/L$ or more than $12 \times 10^9/L$ day 7. The decreased HB, HCT and neutrophil counts (PMN) while increased platelets significantly correlated with long duration of sepsis ($p = 0.014$) but only decreased PMN related to a long duration of shock ($p = 0.009$). Decreased albumin up to 1 g/dL was associated with increased prothrombin day 7 ($p = 0.047$). Increased creatinine was correlated with increased partial thromboplastin time day 7 ($p = 0.027$). **Conclusion:** The incidence of hematologic changes in sepsis was high. Hematologic changes were related to factors including long duration of sepsis more than 7 days and long duration of shock, however without significance.

Keywords : ● Hematological change ● Sepsis

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นิพนธ์ต้นฉบับ

การศึกษาการเปลี่ยนแปลงทางโลหิตวิทยาในผู้ป่วยที่มีภาวะพิษเหตุติดเชื้อในหอผู้ป่วยอายุรกรรม

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บทคัดย่อ

ที่มา การติดเชื้อในกระแสเลือดหรือภาวะพิษเหตุติดเชื้อเป็นภาวะวิกฤตซึ่งอันตรายถึงชีวิตมีลักษณะการอักเสบทั่วร่างกายการเปลี่ยนแปลงทางโลหิตวิทยาพบได้ในผู้ป่วยภาวะพิษเหตุติดเชื้อนี้แต่ข้อมูลในประเทศไทยยังน้อย **วัตถุประสงค์** ศึกษาอุบัติการณ์ของการเปลี่ยนแปลงระบบโลหิตของผู้ป่วยที่มีภาวะติดเชื้อในกระแสเลือดในหอผู้ป่วยใน แผนกอายุรกรรม **วิธีการ** รวบรวมข้อมูลผู้ป่วยที่มีภาวะติดเชื้อในกระแสเลือดที่หอผู้ป่วยในแผนกอายุรกรรม ช่วงเวลา 1 มกราคม 2556 ถึง 3 กรกฎาคม 2556 บันทึกข้อมูล ความสมบูรณ์ของเม็ดเลือด (complete blood count) ค่าการทำงานของไต (creatinine, Cr) ค่าการแข็งตัวของเลือด (PT/INR, aPTT) ค่าอัลบูมิน (albumin) ค่าบิลิรูบิน (bilirubin) ในวันแรกและวันที่เจ็ดของการมีภาวะติดเชื้อ **ผลการศึกษา** ผู้ป่วย 196 ราย อายุเฉลี่ย 70 ปี เพศชาย 87 ราย (44.4%) เพศหญิง 109 ราย (55.6%) ผู้ป่วย 37 ราย มีภาวะความดันโลหิตต่ำจากการติดเชื้อในกระแสเลือด (septic shock) พบการติดเชื้อทางเดินปัสสาวะ 58 ราย (29.6%) ติดเชื้อในกระแสเลือด 39 ราย (19.39%) ปอดอักเสบ 33 ราย (16.8%) อูจจาระร่วง 22 ราย (11.2%) เนื้อเยื่ออักเสบ (cellulitis) 18 ราย (9.2%) เชื้อจุลินทรีย์ที่ก่อโรคพบแกรมบวก 21 ราย (10.7%) แกรมลบแบบเส้น 95 ราย (48.5%) 13 รายเป็นจุลินทรีย์ชนิดอื่นๆ (6.6%) และ 67 รายไม่พบเชื้อก่อโรค (34.2%) ระยะเวลาที่มีภาวะติดเชื้อในกระแสเลือด 1-3 วันจำนวน 120 ราย (61.2%) 4-7 วันจำนวน 57 ราย (29%) และ 19 ราย (9.7%) มีภาวะติดเชื้อในกระแสเลือดมากกว่า 7 วัน ผู้ป่วยมีภาวะโลหิตจาง (ฮีโมโกลบินน้อยกว่า 10 กรัม/เดซิลิตร) 80 ราย (40.8%) และ 77 ราย (39.3%) มีฮีมาโตคริตลดลง 3% ในวันที่ 7 หลังจากติดเชื้อผู้ป่วยที่มีภาวะติดเชื้อในกระแสเลือดมีเพียง 40 ราย (20.4%) ที่มีเม็ดเลือดขาวน้อยกว่า 4,000 เซลล์ต่อมิลลิลิตร หรือมากกว่า 12,000 เซลล์ต่อมิลลิลิตร การลดลงของ Hb/HCT จำนวนเม็ดเลือดขาวชนิดนิวโทรฟิลและการเพิ่มขึ้นของเกล็ดเลือดมีความสัมพันธ์กับจำนวนวันที่ติดเชื้อในกระแสเลือด ($p = 0.014$) แต่การลดลงของนิวโทรฟิล สัมพันธ์กับจำนวนวันที่มีภาวะความดันโลหิตต่ำจากการติดเชื้อในกระแสเลือด ($p = 0.009$) การลดลงของอัลบูมินมากกว่า 1 กรัม/เดซิลิตร สัมพันธ์ผกผันกับการเพิ่มขึ้นของ PT ในวันที่ 7 ($p = 0.047$) การเพิ่มขึ้น Cr สัมพันธ์กับการเพิ่มขึ้นของ PTT ในวันที่ 7 ($p = 0.027$) **สรุป** การเปลี่ยนแปลงระบบโลหิตในผู้ป่วยติดเชื้อในกระแสเลือดพบได้บ่อยปัจจัยสำคัญในการเปลี่ยนแปลงทางระบบโลหิตคือระยะเวลาการติดเชื้อในกระแสเลือดที่นานกว่า 7 วันและการมีความดันโลหิตต่ำ แต่จากการศึกษาไม่พบนัยสำคัญทางสถิติ

คำสำคัญ : ● Hematologic change ● Sepsis

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2561;28:449-57.

Introduction

Sepsis or SIRS (Systemic Inflammatory Response Syndrome) is a medical crisis presenting an inflammatory response throughout the body.¹ Severe sepsis involves an inflammatory process with organ dysfunction. The risk for mortality does not depend on the type of organism, but the inflammatory response of the host or MODS (multiple organ dysfunction syndrome), which is related to a high mortality rate. Treatment should consider which signs lead to organ failure or complications.

In the United States, the prevalence of sepsis was 0.3% with 51.1% of this in the ICU. The mortality rate was as high as 28.6% and increased with patient. This problem costs around 16.7 billion USD yearly and tends to increase by 1.5% yearly.²

Anemia is the most common hematologic change during infection, especially chronic infection.³ Incidence of anemia ranges from 18-95% depending on the etiology of infection. The hematologic system is often affected by sepsis and changes in the hematologic system in sepsis increases mortality rate, so early detection and appropriate treatment of the hematologic abnormalities would decrease the mortality rate.

SIRS is the condition response to the inflammatory process in severe infection or sepsis. Definition is positive for at least 2 of 4 of the criteria described below.

- 1) Body temperature above 38.0°C or below 36.0°C
- 2) Heart rate above 90 /min.
- 3) Respiratory rate above 20 /min. or PaCO₂ below 32 mmHg.
- 4) White blood cell count more than 12 x10⁹/L, below 4 x10⁹/L or an increase band form.

Severe sepsis is the condition leading to multi-organ failures followed by septic shock.⁵ Sepsis or septic shock will then cause multiple organ dysfunction syndrome (MODS), disseminated intravascular coagulation (DIC) and death.

Common red blood cell alteration during infection is one type of anemia which is define by hemoglobin (Hb) less than 13 g/dL among male and less than 12 g/

dL among female.⁶ Seventy-seven percent of patients in intensive care units have anemia, and 29% have hemoglobin less than 10 g/dL.⁷ The multifactorial causes of anemia among ICU patients include hemodilution, anemia of inflammation, hemolysis from disseminated intravascular coagulopathy (DIC) and blood loss or iatrogenic blood loss. Smoller and Kruskall reported estimated iatrogenic blood loss at an average of 944 mL or around 20% of a 70-kg weight man.⁸ Another red cell change is hemoconcentration caused by leakage of intravascular fluid.

The alteration of WBC can be the increased up to 50 x10⁹/L, called leukemoid reaction, or decreased WBC (leukopenia).⁹

Thrombocytopenia is another common hematologic change among ICU patients. From 20-40% of ICU patients have platelet counts lower than 100 x10⁹/L. Thrombocytopenia is caused by DIC, infection and platelet consumptions or hemophagocytosis. Infection also interferes with anticoagulant and procoagulant systems by stimulating the coagulation system forming thrombin and leading to consumptive coagulopathy and DIC.

Objective

The study aimed to identify the incidence of hematologic change and correlate findings with other organs dysfunctions among patients with sepsis.

Patients and methods

This is a retrospective review of patients with sepsis admitted to medicine wards in Thammasat University Hospital from January 2012 to July 2012. Inclusion criteria included all inpatients, age 15 years or higher, and admitted in the internal medicine with a diagnosis of sepsis or SIRS. All patients who did not have complete data were excluded from this study and patients with any hematologic disease were excluded. Data on medical records were reviewed including patients' demographic data and laboratory results on the first day (Day 0) and seventh day of sepsis (Day 7).

Data analysis

Using SPSS, Version 13.0 statistical analysis, basic information or baseline characteristics of patients were shown by percent, minimum, maximum, mean \pm standard deviation or median. The relationship between different factors using Pearson's correlation with p-values ≤ 0.05 were considered significant. Comparisons between groups using t-test were made for continuous data and chi-square or Fisher's exact test was used to enumerate data type.

Results

From January 2012 to July 2012, 196 patients with sepsis were admitted to internal medicine wards in Thammasat University Hospital. Forty-four percent were male while 66% were aged over 60; the median age was 70 years. Baseline characteristics of the patients are shown in Table 1.

Ninety-three patients (47.4 %) had hypertension, 70 patients (35.7%) had diabetes mellitus, 68 patients (34.7%) had dyslipidemia, and 57 patients (29.3%) had stroke. The primary infections were 58 urinary tract infections (29.6%), 39 bacteremia (19.9%), 33 pneumonia (16.8%), 22 gastrointestinal tract infections (11.2%) and 18 skin and soft tissue infections (9.2%). The most common organism was gram negative bacteria among 95 patients (48.5%). Others organisms included 21 patients (10.7%) with gram positive, and 13 patients (6.6%) with other organisms such as mycobacterium, viruses (Herpes, CMV, Flu), fungi or parasites. In all, 67 patients (34.2%) had an unidentified organism.

One-hundred twenty patients (61.2%) had sepsis duration from 1-3 days. Fifty-seven (29.1%) had a duration of 4 to 7 days, and 19 patients (9.7%) had a duration longer than 7 days (29.1%). Thirty-seven patients (18.9%) presented septic shock with a duration of 1 to 2 days as shown in Table 1.

Of 196 patients, 80 patients (40.8%) had hemoglobin lower than 10 g/dL the first day of admission. At the 7th day (D7) of admission, 56 patients (30.4%) presented

increase hematocrit (Hct) and 128 patients (69.5%) presented decreased Hct, probably due to receiving blood transfusion. Of 128 patients, 51 (27.7%) had decreased Hct less than 3% and 77 patients (41.8%) have decreased Hct more than 3%.

Forty patients (20.4%) had a diagnosis of SIRS on the day of admission. One-hundred fifty-six (79.6%) had the white blood cell counts between 4-12 $\times 10^9$ /L. Six percent of patients (12 of 196) had septicemia with platelet level lower than 100 $\times 10^9$ /L the first day of admission while the rest (184 patients, 93.9%) had more than 100 $\times 10^9$ /L. (Table 2)

Regarding the change of coagulogram, 47 patients (35.9%) revealed INR > 1.3 . Thirty-one patients (23.7%) had PT more than 15 seconds and thirteen patients (10%) have aPTT more than 37.4 seconds (Table 3).

Using Pearson's Correlation, no correlation of age or others factors was found. However, this study found the duration of sepsis more than four days significantly correlated with longer duration of hypotension, change of hemoglobin, increased or decreased WBC count and neutrophils ($R = 0.722$, $p < 0.05$; $p = 0.007$; $p = 0.019$; $p = 0.014$, respectively).

We found the relationship between duration of hypotension longer than two days and the increased number of WBC at D7 ($R = 0.389$, $p = 0.019$), but the opposite relation for the number of WBC in the 1st day and PMN changes ($R = -0.326$ and -0.43 , $p = 0.046$ and 0.009 , respectively).

Concerning the changes of Hb/Hct, a relationship was found between increased or decreased Hb/Hct levels at D1 ($R = 0.615$ and 0.601 , $p < 0.05$), but the opposite relation was observed for Hb/Hct level at D7 ($R = 0.259$, $p < 0.05$).

The change of white blood cell count on D7 correlated with the number of white blood cell on D1 and the PMN count in D1 ($R = 0.846$, $p < 0.05$) ($R = 0.41$, $p < 0.05$). However no correlation was found concerning the number of WBC on D7 and the change of WBC ($R = -0.171$, $p = 0.02$).

Table 1 Baseline characteristics of patients

Characteristic	Total (n = 196)	%
Age, median	70 yrs. (16-105)	
Age, > 60 yr.	130	66.33
Sex: male/female	87/109	44.39/55.61
Underlying disease		
- Hypertension	93	47.4
- Diabetes mellitus	70	35.7
- Dyslipidemia	68	34.7
- Cerebrovascular disease	57	29.3
- Renal disease	37	18.9
- Heart disease	29	14.8
- Chronic lung disease	18	9.2
- Other	97	49.5
- Pneumonia	33	16.8
- Infectious diarrhea	22	11.2
- Cellulitis	18	9.2
- Other sources	38	19.4
Albumin < 3 g/dL	94 (158)	59.5
Albumin ≥ 3 g/dL	64 (158)	40.5
Albumin decrease > 1 g/dL	8 (108)	7.4
Creatinine increase	57	31.5
- Cr decrease 0.01-0.29 mg/dL	47	26.0
- Cr decrease ≥ 0.30 mg/dL	77	42.5
Pathogen		
- Gram negative bacilli	95	48.5
- Gram positive cocci	21	10.7
- Other	13	6.6
- Unknown	67	34.2
Duration of Sepsis		
- 1-3 days	120	61.2
- 4-7 days	57	29.1
- > 7 days	19	9.7
Shock; median age (yrs.)	76 yrs. (67.04-75.72)	
- 1-2 days	34	91.9
- > 2 days	3	8.1

Table 2 Red blood cell, white blood cell, and platelets changes among patients with sepsis

Characteristic	Total (n = 196)	%
HB < 10 g/dL	80	40.8
Hct decrease <3%	51	27.7
Hct decrease ≥ 3%	77	41.8
Hct increase	56	30.4
WBC < 4 x10 ⁹ /L or > 12 x10 ⁹ /L	40	20.4
WBC 4-12 x10 ⁹ /L	156	79.6
Band form increased	18	9.2
Platelet < 100 x10 ⁹ /L	12	6.1
Platelet ≥ 100 x10 ⁹ /L	184	93.9

Table 3 Coagulogram changes among patients with sepsis

Characteristic	Total (N = 131)	%
INR < 1.3	84	64.1
INR ≥ 1.3	47	35.9
PT < 15 sec	100	76.3
PT ≥ 15 sec	31	23.7
PTT < 37.4 sec	116	89.9
PTT ≥ 37.4 sec	13	10.0

Table 4 Correlation between clinical parameters and the duration of sepsis more than 4 days

Factors	Duration of sepsis > 4 days		
	R	p-value	N
Duration of shock	0.722	0.00*	38
Hemoglobin level change	- 0.197	0.007	183
WBC level change	- 0.171	0.019	186
PMN level change	- 0.181	0.014	

*Statistically significant at $p \leq 0.05$

Table 5 Relationships between shock more than 2 days and WBC

Factors	Shock more than 2 days		
	R	p-value	N
Number of WBC in the 1 st day	- 0.326	0.046	38
Number of WBC in the 7 th day	0.389	0.019	36
PMN changes	- 0.43	0.009	36

The study showed a correlation between the change of platelet and duration, D1 and D7 (0.251, $p = 0.001$). Moreover, a correlation was found between the platelet level change and PTT result, D7 with a low correlation coefficient (0.24, $p = 0.047$). However, regarding the number of platelets D7, the correlation is the opposite. (0.558, $p < 0.05$)

In a subgroup study comparing patients whose hematocrit decreased more than 3% and those whose did not found that the first group was elderly, median age 73 and 68 years with significance ($p = 0.027$). The first group also had patients with underlying chronic lung disease more than in the second group ($p = 0.045$).

Table 6 Relationships between a change of hematocrit level and other white blood cell or platelet level

Factors	The change of Hb/Hct		
	R	p-value	N
Hb level at D1	0.615	0.00*	183
Hb at D7	- 0.259	0.00*	183
Number of WBC in the 1 st day	0.846	0.00*	186
Number of PMN in the 1 st day	0.41	0.00*	186
Number of WBC in the 7 th day	- 0.171	0.02	186
PMN changes	0.446	0.00*	185
D1 platelet count	0.251	0.001	186
D7 platelet count	- 0.558	0.00*	186
PTT on D7	0.24	0.047	69

*Statistically significant at $p \leq 0.05$

Table 7 Factors affecting hematocrit level to decrease more than or not more than 3%

Characteristic	Total	Hct drop $\geq 3\%$	Hct drop $< 3\%$	95%CI	p-value
	184	77 (39.3)	107 (54.6)		0.347
Sex; male	81	31 (40.3)	50 (46.7)	0.453-0.473	0.383
Age; median	184	73 yr. (52-84)	68 yr. (56-79)	0.518-0.538	0.027
date of sepsis ≥ 4 days	184	77	107	0.669-0.687	0.095
Shock	35	16 (45.7)	19 (54.2)	0.706-0.723	0.61
date of shock ≥ 3 days	35	15 (42.8)	20 (57.1)	0.627-0.626	0.56
Acute kidney injury	72	36 (50)	36 (50)	0.118-0.131	0.24

Table 8 Factors affecting white blood cell count changes

Characteristic	Total	WBC decrease	WBC increase	95%CI	p-value
	186	144 (77.4%)	42 (22.6%)		0.215
Sex; male	82	67 (81.7%)	15 (18.3%)	0.143-0.221	0.221
Sex; female	104	77 (74%)	27 (26%)	0.143-0.289	0.213
Age; median	186	69.5 (55-81)	71 (52.75-72)	0.432-0.452	0.009
date of sepsis ≥ 4 days	178	138	40	0.243-0.299	0.421
Shock	35	28 (80%)	7 (20%)	0.792-0.890	0.685
date of shock ≥ 3 days	36	28	8	0.173-0.223	0.04
Acute kidney injury	73	54 (74%)	19 (26%)	0.553-0.615	0.665

Regarding WBC changes, the patients whose WBC decreased after treatment had significantly lower average ages than whose WBC increased after treatment (69.5 vs. 71-year-old)($p = 0.009$). Patients with pneumonia, diarrhea and shock more than three days mostly had significantly decreased WBC count levels more than increased levels ($p = 0.037$, 0.036 and 0.04 , respectively).

(Table 8). However, no significant effect of systemic disease to PLT level was found in our study (Table 9).

Discussion

The majority of our patients were elderly (aged ≥ 60 years) and predominantly female with underlying illness. Most patients (93 patients; 47%) had known underlying

Table 9 Factors affecting platelet level changes

Characteristic	Total	Plt increase	Plt decrease	95%CI	p-value
	145	103 (55.38)	83 (44.6)		0.176
Sex; male	67	43 (64)	24 (35.8)	0.524-0.586	0.474
Sex; female	78	45 (57.7)	33 (42.3)	0.285-0.553	0.413
Age; median	145	69 (55-79)	70.5 (44-81)	0.720-0.774	0.053
date of sepsis \geq 4 days	178	98	80	0.016-0.036	0.061
Shock	35	14 (40)	21 (60)	0.043-0.071	0.042
date of shock \geq 3 days	36	15	21	0.549-0.611	0.310
Acute kidney injury	73	41 (56.2)	32 (43.8)	0.306-0.364	0.024

*Statistically significant at $p \leq 0.05$; Plt, platelet

diseases, i.e., diabetes, dyslipidemia, chronic kidney disease and cerebrovascular disease. The most common infections in this study were UTI, sepsis, pneumonia and gastrointestinal infection. No differences were observed among immunocompromised patients or involving other hospital acquired infections; gram negative rod bacteria were the most common organisms.

Sixty-one percent of the patients experienced a duration of infection between 1-3 days, with a small population (18.9%) presenting shock. No patient died in this study, which may have been because severe case was transferred to the ICU. This might imply that patients in this study has less severity and lower mortality rate than patients in the study of Angus DC, et al. involving 0.3% severe septicemia, 5.1% ICU patients and a 28.6% mortality rate that increased with age.²

In our study, the prevalence of anemia (Hb < 10 g/dL) among patients with septicemia was 40.8% (in total 196 patients) as high as the study by Means et al. in 2000.³ The revealed prevalence of anemia in the study was 18-95% depending on infection type. However, this study showed anemia in acute infection for the majority of patients (41.8%) had decreased Hct more than 3% without bleeding or hemolysis. One reason was the patients presented dehydration at the first day and anemia after receiving fluid resuscitation. Another reason was that anemia was associated with old anemia inflammation and chronic disease (AI/ACD) of the patients on top of dilutional anemia. Weiss G and

Goodnough LT described AI/ACD as the body responses to infection and inflammation by secreting inhibitory cytokines to inhibit erythropoietin function, which could be demonstrated in both acute and chronic infections.¹¹

This study found only 40 patients with septicemia (20.4%) with SIRS. Also, a few patients with platelets count lower than $100 \times 10^9/L$ were admitted the first day (6.1%). Due to mild disease in this whole group, the prevalence of DIC was only 5-12%.¹² However, our study demonstrated 42.5% of kidney impairment (Cr above normal more than 0.3 mg/dL) and 10-35% of hepatic function abnormalities leading to a high prevalence of abnormal coagulogram.

Pearson's Correlation, revealed no correlation between age and other factors. Correlations were found between duration of septicemia more than four days and hypotension ($R = 0.722$, $p < 0.05$). The opposite correlation was found between duration of septicemia more than 4 days and Hb ($p = 0.007$), abnormal WBC ($p = 0.019$) and abnormal PMN ($p = 0.014$), but no relationship was found between duration of septicemia more than four days and abnormal platelet count. The reasons included dehydration during infections and return to normal after treatment, red blood cell changes depending on the duration of infections and WBC count. In addition, duration of infection more than two days correlated with WBC and PMN changes. Duration of infections was not correlated with platelet number or platelet changes.

The changes of Hb/Hct and WBC correlated with the initial number including platelet count, however platelet count abnormality correlated with partial thromboplastin time (PTT) D7 may have been due to DIC causing fibrin formation. One subgroup study found that elderly patients and patients with underlying chronic lung diseases had more severe anemia. Matthias John also showed anemia involving chronic disease could be found among around 13% of patients with chronic lung disease/COPD.¹³ Our study confirmed some systemic conditions (pneumonia and diarrhea, shock, AKI, diabetes and hypertension) could affect hematologic parameters.

One limitation of this study was its retrospective design which depended recorded and incomplete of the data. Another limitation was the variety of diseases which was difficult to evaluate.

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