Original article

Hematologic indices of autoimmune hemolytic anemia in adults: a retrospective study

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Abstract:

Background: *Autoimmune hemolytic anemia (AIHA) is a rare condition of hemolytic anemia. Evaluation of hematologic indices such as hemoglobin (Hb), mean corpuscular volume (MCV), reticulocyte count (RC), reticulocyte production index (RPI) and absolute reticulocyte count (ARC) could improve outcomes of treatment.* ***Objective:*** *The study aimed to evaluate hematologic indices such as Hb, MCV, RC, RPI, ARC in a patient with AIHA.* ***Materials and methods:*** *Medical records were reviewed between 2011 and 2015 as a retrospective descriptive study. Data collections included personal demographics, disease characteristics, laboratory results, treatments, and treatment outcomes. Spearman's correlation tests were used to correlate between RPI and factors. All data were analyzed using SPSS Software, Version 20, and p-value less than 0.05 was considered statistically significant.* ***Results:*** *From 88 patients, the mean age was 60.17 (SD 18.92), and males were equal to the females. In all, 45 (51.1%), 18 (20.5%), 10 (11.4), 6 (6.8%), 6 (6.8%), and 3 (3.4%) patients received a diagnosis of idiopathic and infectious etiologies, systemic lupus erythematous (SLE), lymphoma, solid tumor and drug-associated AIHA, respectively. Most patients (92.1%) exhibited positive results of the direct Coombs test. Most (84.1%) patients had warm-type AIHA. The mean Hb, MCV, RPI and ARC at diagnosis were 7.27 g/dL (SD1.99), 83.61 fL (SD12.26), 0.82 (SD 0.68) and 79.11 $x10^9$/L (SD 58.20), respectively. RPI more than 2%, and ARC more than 100 $x10^9$/L was found among 6 (6.8%), and 22 (25%) patients, respectively. RC positive correlated to RPI ($r = 0.85$, 95%CI: 0.75-0.91, $p < 0.001$), and ARC ($r = 0.9$, 95%CI: 0.83-0.94, $p < 0.001$). Moreover, RPI was positively correlated to systemic lupus erythematous ($r = 0.235$, $p = 0.028$, 95%CI: 0.023-0.396).* ***Conclusion:*** *Idiopathic etiology was the common cause of AIHA. RPI and ARC were positive correlated to reticulocyte count among patients with AIHA, while RPI positively correlated to systemic lupus erythematous.*

Keywords : ● *Autoimmune hemolytic anemia (AIHA)* ● *Reticulocyte count* ● *Absolute reticulocyte count* ● *Reticulocyte production index (RPI)*

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นิพนธ์ต้นฉบับ

ดัชนีทางโลหิตวิทยาพบในผู้ใหญ่ที่มีภาวะเม็ดเลือดแดงแตกจากภูมิคุ้มกัน: การศึกษาย้อนหลัง

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นิพนธ์ต้นฉบับ

ความเป็นมา *ภาวะเม็ดเลือดแดงแตกจากภูมิคุ้มกันเป็นภาวะที่พบน้อย การประเมินดัชนีทางโลหิตวิทยา ได้แก่ ระดับฮีโมโกลบิน, Mean corpuscular volume (MCV) reticulocyte (RC), reticulocyte production index (RPI) และ absolute reticulocyte count (ARC) สามารถช่วยในการดูแลผู้ป่วยได้* ***วัตถุประสงค์*** *เพื่อประเมินดัชนีทางโลหิตวิทยา ได้แก่ จำนวนเรติคูโลไซท์, RPI และ ARC ในผู้ป่วยที่มีภาวะเม็ดเลือดแดงแตกจากภูมิคุ้มกัน* ***วัสดุและวิธีการ*** *เป็นการศึกษาย้อนหลังด้วยการทบทวนเวชระเบียนผู้ป่วยที่ได้รับการวินิจฉัยเม็ดเลือดแดงแตก ระหว่างปี 2554-2558 ที่ศูนย์การแพทย์สมเด็จพระเทพรัตนราชสุดาฯ สยามบรมราชกุมารี ข้อมูลที่เก็บได้แก่ อายุ เพศ โรคประจำตัว สาเหตุของภาวะเม็ดเลือดแดงแตก ผลการตรวจทางห้องปฏิบัติการ และการรักษา การหาความสัมพันธ์ระหว่าง RPI กับปัจจัยต่างๆ ใช้สถิติ Spearman's correlation โดยกำหนดค่านัยสำคัญทางสถิติที่น้อยกว่า 0.05* ***ผลการศึกษา*** *จากข้อมูลการศึกษาย้อนหลังจำนวน 88 ราย อายุเฉลี่ยของผู้ป่วยอยู่ที่ 60.17 ปี (SD 18.92) จำนวนผู้ป่วยเพศชายใกล้เคียงกับผู้หญิง พบว่าผู้ป่วย 45 ราย (51.1%), 18 ราย (20.5%), 10 ราย (11.4%), 6 ราย (6.8%) และ 3 ราย (3.4%) เป็นเม็ดเลือดแดงแตกที่ไม่ทราบสาเหตุ การติดเชื้อ เอสแอลอี มะเร็งต่อมน้ำเหลือง มะเร็ง และยาตามลำดับ ส่วนมากร้อยละ 92.1 พบว่ามีผลตรวจ direct Coombs test เป็นบวก ชนิดของเม็ดเลือดแดงแตกเป็น warm type มากที่สุดคิดเป็นร้อยละ 84.1 สำหรับค่าเฉลี่ยระดับฮีโมโกลบินเมื่อวินิจฉัยอยู่ที่ 7.27 g/dL (SD 1.99), ค่า MCV เฉลี่ยอยู่ที่ 83.61 fL (SD 12.26), ค่าเฉลี่ย RPI เมื่อวินิจฉัยอยู่ที่ 0.82 (SD 0.68) และค่าเฉลี่ยของ ARC เมื่อวินิจฉัยอยู่ที่ 79.11 x10^9/L (SD 58.20) จากการศึกษาพบว่ามีผู้ป่วย 6 ราย (68%) มีค่า RPI มากกว่า 2 % และมีผู้ป่วย 22 ราย (25%) มีค่า ARC มากกว่า 100 x10^9/L นอกจากนี้ยังพบมีความสัมพันธ์ทางบวกของจำนวนเรติคูโลไซท์กับ RPI (r = 0.85, 95%CI: 0.75-0.91, $p < 0.001$) และ ARC (r = 0.9, 95%CI: 0.83-0.94, $p < 0.001$) ตามลำดับ* ***สรุป*** *ภาวะเม็ดเลือดแดงแตกที่ไม่ทราบสาเหตุพบได้บ่อยในผู้ป่วยเม็ดเลือดแดงแตกจากภูมิคุ้มกัน พบความสัมพันธ์ทางบวกของค่า RPI และ ARC กับจำนวนเรติคูโลไซท์ในผู้ป่วยที่วินิจฉัยภาวะเม็ดเลือดแดงแตก*

คำสำคัญ : ● *ภาวะเม็ดเลือดแดงแตก* ● *จำนวนเรติคูโลไซท์* ● *Absolute reticulocyte count* ● *Reticulocyte production index (RPI)*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2565;32:45-53.

Introduction

Autoimmune hemolytic anemia (AIHA) is a rare hematologic condition resulting from immune-induced red cell hemolysis. The incidence of AIHA is found at 17 persons per 100,000 populations per year[1]. However, the incidence is under-reported in the Thai population. Several etiologies of AIHA such as idiopathic, autoimmune disease, multiple blood transfusions, infection, hematologic malignancy and solid tumor, where the drug is identified[2]. AIHA has acquired hemolysis caused by the host immune response to red cell antigens[3]. Evidence of red cell breakdown such as spherocytosis, elevated unconjugated bilirubin, raised serum lactate dehydrogenase, reduced plasma haptoglobin, and increased urinary urobilinogen were found among patients with AIHA[4]. Reticulocytosis and erythroid hyperplasia from marrow studies were found due to increased marrow response to anemia[4]. However, a reticulocyte production index (RPI) greater than 2 and an absolute reticulocyte count greater than 100 $x10^9$/L showing the response of marrow to anemia,[5] has been found among most patients with AIHA. However, a single study reported 20% of patients with AIHA had reticulocytopenia[6]. Some related reports have reported reticulocytopenia among children and cases with severe AIHA[6-8]. The mechanism is due to an autoimmune damaged marrow erythroid progenitor[9].

Corticosteroid is the front-line treatment of warm type AIHA. The goal of treatment is becoming symptom-free and maintaining hemoglobin of 10 g/dL. Treatment with prednisolone (1 mg/kg/day)[10-12] requires the response time. About 80 to 90% of patients respond to treatment with prednisolone within three weeks[13]. Among nonresponsive patients, adding another drug is suggested[13]. Complete remission, partial remission, and nonresponders were found in 66%, 21 to 23%, and 10% of patients, respectively, after three months of treatment[14,15].

Our study aimed to determine the hematologic indices such as hemoglobin (Hb), hematocrit (Hct), reticulocyte count (RC), corrected reticulocyte count (CRC), reticulocyte production index (RPI), and absolute reticulocyte count (ARC) among patients with AIHA. The hematologic indices should help to guide diagnosis and treatment of patients.

Materials and Methods

Study Population

Patients with newly diagnosed AIHA from 2011 to 2015 completing medical record reviews and treated at the HRH Princess Maha Chakri Sirindhorn Medical Center, Nakhon Nayok, Thailand were enrolled in a retrospective study. This study received permission from the Srinakharinwirot University Ethics Committee (SWUEC178-58E).

Study participants comprised adults greater than or equal to 18 years of age. Patients with respiratory failure, heart failure, septic shock, acute kidney injury or bleeding were excluded from the study.

Definition

AIHA was defined as hemolytic anemia using the positive direct Coombs test/antiglobulin test[11,12,16]. Coombs negative AIHA was defined as a negative result from the antiglobulin test[17]. The classic clinical signs of hemolysis including jaundice, fatigue, dyspnea, hepatosplenomegaly were identified among our participants[2]. Warm type AIHA was defined as anisocytosis, spherocyte or polychromasia in blood smear, identified using IgG or both IgG and C3d. Cold type AIHA was defined as autoagglutinations on blood smear and identified C3d[3]. Polyspecific antihuman globulin (Coombs) reagent was used in our hospital blood bank.

Calculated reticulocyte count indices are presented in Table 1[5].

The response was defined as Hb greater than or equal to 10 g/dL plus no transfusion and absence of any treatment[11].

The response was evaluated at 2 and 4 weeks of treatment. Patients not receiving medication, stopping treatment for any reason or lost to follow up or death were excluded from response analysis.

Table 1 Definition of calculation of reticulocyte calculation[5]

Reticulocyte	Calculation
Reticulocyte count	=% reticulocytes in RBC population
Corrected reticulocyte count	=% reticulocytes x (patient Hct / 45)
Reticulocyte production index (RPI)	=% reticulocytes x (patient Hct / 45) / maturation time **reticulocyte maturation time in peripheral blood in days 1.0 day for Hct >=40% 1.5 days for Hct 30-40% 2.0 days for Hct 20-30% 2.5 days for Hct < 20%
Absolute reticulocyte count	=% reticulocytes x RBC count $x10^9$/L) (normal 25-75 $x10^9$/L; values <100 $x10^9$/L indicate an inappropriately low erythropoiesis response to anemia)

Baseline Data Collection

Data were extracted from medical records. Baseline characteristics such as age, sex, comorbidities, AIHA etiology, direct Coombs test (DCT), complete blood count (CBC) and reticulocyte count were collected.

Statistical Analysis

Baseline characteristics are expressed as proportions for categorical variables and mean including standard deviation for continuous variables. Spearman's correlation test was used to correlate between RPI and factors. All analyses were performed using SPSS, Version 20.0.

Results

Baseline Characteristics

A total of 88 participants receiving a diagnosis of AIHA were identified. The mean age of patients was 60.17 (SD 18.92) years, and 47 (53.4%) patients were female. The AIHA etiologies included idiopathic (51.5%), infectious (20.5%), systemic lupus erythematous (11.4%), lymphoma (6.8%), solid tumors (6.8%) and drugs (3.4%), consecutively. The patients presented warm (84.1%), cold (10.2%), and mixed (5.7%) types. Only 11.4% of patients with AIHA presented thrombocytopenia. The data are shown in Table 2.

Hematologic Indices

At diagnosis, mean of Hb, RC, CRC, RPI, and ARC were 7.27 (SD 1.99), 2.89 (SD 2.01), 1.45 (SD 1.09), 0.82 (SD 0.68) and 79.11 $x10^9$/L (SD 58.20), respectively. Seventy-four (84.1%) of 88 patients were found to have microspherocyte on peripheral blood smear. Autoagglutinations were found among 10 (11.4%) patients. Only two patients were found to possess alloantibodies. RC greater than 2%, CRC greater than 2%, RPI greater than 2%, and ARC greater than 100 $x10^9$/L were found among 50 (56.8%), 19 (21.6%), 6 (6.8%), and 22 (25%) patients, respectively. (Tables 2, 3 and 4). RC positively correlated to CRC (r = 0.926, 95%CI: 0.87 to 0.96, p < 0.001), RPI (r = 0.85, 95%CI: 0.75-0.91, p < 0.001) and ARC (r = 0.9, 95%CI: 0.83-0.94, p < 0.001).

Treatment Outcomes

Totally, 78 of 88 patients received medications. Thirty-nine (50.0%), 26 (33.33%), 5 (6.41%) and 8 (10.27%) patients were treated with prednisolone, dexamethasone, methylprednisolone, and other drugs, respectively. Red blood cells were transfused in 13 (14.78%) of 88 patients. Azathioprine, cyclophosphamide and intravenous immunoglobulin were administrated among 5, 3, and 1 patient, respectively. After 14 days of treatment, 26

Table 2 Baseline characteristics of patients with AIHA (n = 88)

Characteristic	Number of patients	Rate
Sex		
Male	41	46.6
Female	47	53.4
Diabetes mellitus		
Yes	17	19.3
No	71	80.7
Hypertension		
Yes	34	38.6
No	54	61.4
Dyslipidemia		
Yes	17	19.3
No	71	80.7
Gout		
Yes	1	1.1
No	87	98.9
Chronic kidney disease		
Yes	15	17.1
No	73	82.9
Hematologic malignancy		
Yes	2	2.3
No	86	97.7
Systemic lupus erythematous		
Yes	5	5.7
No	83	94.3
Thalassemia		
Yes	3	3.4
No	85	96.6
Solid tumor		
Yes	7	8.0
No	81	92.1
Cause of AIHA		
- Idiopathic	45	51.1
- Infectious	18	20.5
- SLE	10	11.4
- Lymphoma	6	6.8
- Solid tumor	6	6.8
- Drug	3	3.4
Direct Coombs test		
- Negative	7	8.0
- Weakly positive	10	11.4
- Positive 1+	20	22.7
- Positive 2+	23	26.1
- Positive 3+	15	17.0
- Positive 4+	13	14.8
Indirect Coombs test		
- Negative	44	50
- Weakly positive	9	10.2
- Positive 1+	12	13.6
- Positive 2+	13	14.8
- Positive 3+	5	5.7
- Positive 4+	5	5.7
Identified type of AIHA		
- Mixed	5	5.7
- Warm	74	84.1
- Cold	9	10.2
Evan syndrome		
- Yes	10	11.4
- No	78	88.6

Table 3 Complete blood count at time of diagnosis and treatment

Parameter	Day 1 (n = 88)	Day 14 (n = 77)	Day 21 (n = 64)	Day 28 (n = 62)
Hb (g/dL)				
Mean±SD	7.27±1.99	9.41±2.07	10.07±2.6	10.06±2.51
Median	7.40	9.50	9.30	9.55
Min-Max	2.60-12.3	4.6-14.9	4.60-15.8	5.30-15.60
Hct (%)				
Mean±SD	22.03±2.15	28.25±6.28	30.23±7.72	30.16±7.40
Median	22.15	28.40	28.45	29.10
Min-Max	7.80-37.90	13.50-42.9	13.50-46.90	15.10-15.60
MCV (fl)				
Mean±SD	83.61±12.26	84.25±8.57	84.18±9.20	85.75±10.01
Median	82	84.80	86.20	86.25
Min-Max	54.50-126	62.70-107.30	58.20-109.20	60.60-110.20
MCHC (g/dL)				
Mean±SD	33.06±2.72	33.58±3.92	33.37±2.39	33.63±2.97
Median±	33.40	33.20	33.50	33.60
Min-Max	20.50-39.50	26.20-57.10	27.60-42.30	26.20-48.30
WBC (cell/μL)				
Mean±SD	8,804.08±4,838.61	11,478.31±7,476.16	8,952±4,957.99	8,474.52±5,472.88
Median	7,535	9,590	8,300	7,450
Min-Max	1,900-26,900	1,400-40,900	2,100-25,000	300-29,800
Platelet (cell/μL)				
Mean±SD	235,532.95±135,712.01	220,272.73±135,312.37	227,406.25±147,228.33	242,887.10±162,050.9
Median	213,500	176,000	198,500	211,000
Min-Max	2,000-616,000	12,000-656,000	10,000-686,000	13,000-748,000

Abbreviation: Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cell

Table 4 Reticulocyte count

Parameter	Day 1	Day 14	Day 21	Day 28
RC (%)				
Mean±SD	2.89±2.01	2.47±1.59	2.46±1.20	2.10±1.11
Median	2.42	1.89	2.50	1.77
Min-Max	0.06-11.09	0.11-9.18	0.33-7.09	0.18-5.71
CRC (%)				
Mean±SD	1.45±1.09	1.56±1.06	1.60±0.72	1.36±0.67
Median	1.21	1.23	1.49	1.34
Min-Max	0.01-6.03	0.07-5.79	0.23-3.57	0.11-3.65
RPI (%)				
Mean±SD	0.82±0.68	1.08±0.81	1.13±0.67	1.05±0.66
Median	0.62	0.85	0.97	0.97
Min-Max	0.00-4.02	0.05-3.86	0.07-2.76	0.07-3.65
ARC ($x10^9$/L)				
Mean±SD	79.11±58.20	83.77±54.87	84.52±38.86	74.67±49.83
Median	68.49	72.26	81.99	70.92
Min-Max	1.88-318.40	3.70-283.66	11-238	5.33-370.27

Abbreviation: RC, reticulocyte count; CRC, corrected reticulocyte count; RPI, reticulocyte production index; ARC, absolute reticulocyte count

Table 5 The correlation between reticulocyte production index (RPI) and factors.

Factors	*r*	95%CI	*p*
Gender	0.034	-0.179-0.249	0.756
Age	-0.005	-0.186-0.192	0.961
Diabetes mellitus	-0.096	-0.264-0.078	0.375
Hypertension	-0.072	-0.278-0.169	0.507
Dyslipidemia	0.049	-0.148-0.259	0.648
Gout	-0.036	-0.109-0.000	0.740
Chronic kidney disease	-0.009	-0.196-0.206	0.934
Hematologic malignancy	0.150	0.039-0.285	0.163
Systemic lupus erythematous	0.235	0.023-0.396	0.028
Thalassemia	0.106	-0.091-0.296	0.326
Solid tumors	-0.036	-0.208-0.137	0.737
Idiopathic cause	-0.050	-0.269-0.165	0.646

(38.81%) of 67 patients responded positively. Eleven patients were not evaluated due to loss of follow-up or death. When analyzing after 28 days of diagnosis, 29 (46.77%) of 62 patients exhibited complete response.

Correlation between reticulocyte count indices and their factors

Spearman's correlation test revealed that RPI positively correlated to systemic lupus erythematous (SLE) (r = 0.235, p = 0.028, 95%CI: 0.023-0.396). Data are shown in Table 5.

Discussion

Our study aimed to determine the characteristics and reticulocyte counts in AIHA. The mean age of patients in our study was greater than those in a related study in a foreign country, 51.7 years (SD20.5) in idiopathic and 54.3 years (SD25.7) in nonidiopathic etiologies[18]. We found that our study participants exhibited the same proportion of males and females, differing from that of a related study reporting female predominance (ratio 5:1)[15]. However, a small sample size was collected in our study. The most common AIHA was the warm type, similar to that found in the study of Gently L et al.[15]. The most common cause of AIHA was idiopathic which was greater than that observed in a study among Indian patients[18]. However, the Indian study selected only the warm type, leading to a difference in the frequency [18].

Our study found only 2.3% of patients presented alloantibodies, which was greater than that found in the study of Dara R et al.[19] reporting alloantibodies among 0.3 to 0.5 % of patients. One related study found a higher frequency of alloantibodies in AIHA[20]. The alloantibodies were found in 38.5% and 24.3% of patients using the nonadsorbed method and auto-adsorbed method, respectively[20].

The RPI and ARC values in our study were 0.82 (SD 0.68) and 79.11 x10^9/L (SD 58.20), respectively. Liesveld JL et al.[6] reported various numbers of RPI; the values ranged from less than 2 to greater than or equal to 2. Therefore, reticulocyte count did not indicate the response of marrow, but helped to predict, follow up after treatment and to stop corticosteroids.

In our study, the mean reticulocyte count was quite low. It may be explained by approximately 37% of patients presented secondary causes of AIHA such as infection, drugs or cancer causing marrow suppression. Valent P, et al. reported that laboratory findings in AIHA may not be typical regarding secondary causes[2]. Except for secondary causes from SLE, we found a positive correlation between RPI and SLE. However, a marrow biopsy was not performed among our patients. Moreover, several studies reported reticulocytopenia in severe AIHA[6-8] caused by an auto-immune damaged marrow erythroid progenitor[9].

All patients were treated with prednisolone (1 to 2 mg/kg/day) or dexamethasone (5 to 10 mg/day) followed by prednisolone as the first-line treatment. The patients' response was found at only 29.5 and 33% after 14 and 28 days of treatment. The rate of response in our study was lower than that of the study of Abdulgabar Salama et al.[21], which found 70 to 80% of the patients' responses to prednisolone during 1 to 3 weeks of treatment and 20% in long term treatment. The difference in response in our study may have been due to including all causes and types of AIHA, different populations, and a small sample size.

However, reticulocyte count indices proved helpful in the diagnosis of AIHA. Nevertheless, in our study, mean reticulocyte count indices were not high, possibly including secondary causes among patients with AIHA. The authors suggest that diagnosing AIHA requires a review of the blood smear. Reticulocyte count indices could be used in follow-up after treatment and to decide when to stop treatment.

The limitation of our study was the analysis included all AIHA causes, and hematologic indices were not separated between primary and secondary AIHA. Further studies should separate groups using type and cause of AIHA.

Conclusion

Idiopathic etiology was found as the common cause of AIHA in our study, and mean reticulocyte count was quite low. However, we found a positive correlation between RPI and systemic lupus erythematous.

What is already known on this topic?

Elevated reticulocyte count is found among most patients with AIHA.

What does this study add?

A low frequency of reticulocytosis in AIHA was found in our study.

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Potential conflicts of interest

The authors declare that they have no conflicts of interest.

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