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Original Article

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Development of a Prediction Model for the Risk of Infection in Patients with Aplastic Anemia: Survival Analysis in Recurrent Events

Pirun Saelue [1] and Thitichaya Penthinapong [2]

[1]Hematology Unit, Division of Internal Medicine, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand
[2]Pharmaceutical Care Training Center, Department of Pharmaceutical Care, Faculty of Pharmacy, Chiang Mai University, Mueang, Chiang Mai, Thailand

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ABSTRACT

Background: In patients with aplastic anemia (AA), infection-related complications are the leading cause of mortality. However, limited knowledge about the predictive factors for infection in these patients exists. Thus, this study aimed to evaluate risk factors for infection and develop a risk prediction model for the occurrence of infection in patients with AA.

Materials and Methods: Between January 2004 and December 2020, 206 patients with AA ≥15 years of age were included in this study. Survival analysis using recurrent event methodologies was conducted to identify predictive factors associated with infection, including the Anderson and Gill model; Prentice, Williams, and Peterson (PWP) Total Time model; PWP Gap Time model; marginal model; and frailty models. The best model was determined using backward stepwise regression, and internal validation was performed using the bootstrapping method with 500 re-samplings.

Results: With a median follow-up of 2.95 years, the incidence rate of infection among patients with AA was 32.8 events per 100 person-years. The PWP Total Time model revealed that cirrhosis comorbidity, lymphocytes ≥80%, and previous infection increased the risk of infection, while bone marrow cellularity ≥20% offered protection. The bone marrow cellularity, lymphocyte percentage, previous infection, cirrhosis, and hematocrit (BLICH) model was generated to predict the risk of infection. The internal validation showed a good calibration of this model.

Conclusion: Cirrhosis, lymphocytes ≥80%, previous infection, and bone marrow cellularity <20% are risk factors for infection in patients with AA. The BLICH model can predict the risk of infection in these patients.

Keywords: Infection; Aplastic anemia; Hematologic disease; Survival

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Corresponding Author: Thitichaya Penthinapong, MPharm, BCP Pharmaceutical Care Training Center, Department of Pharmaceutical Care, Faculty of Pharmacy, Chiang Mai University, Mueang, Chiang Mai, Thailand.
Tel: +66-5394-4391, +66-90318-6788
Email: thitichaya.pen@cmu.ac.th

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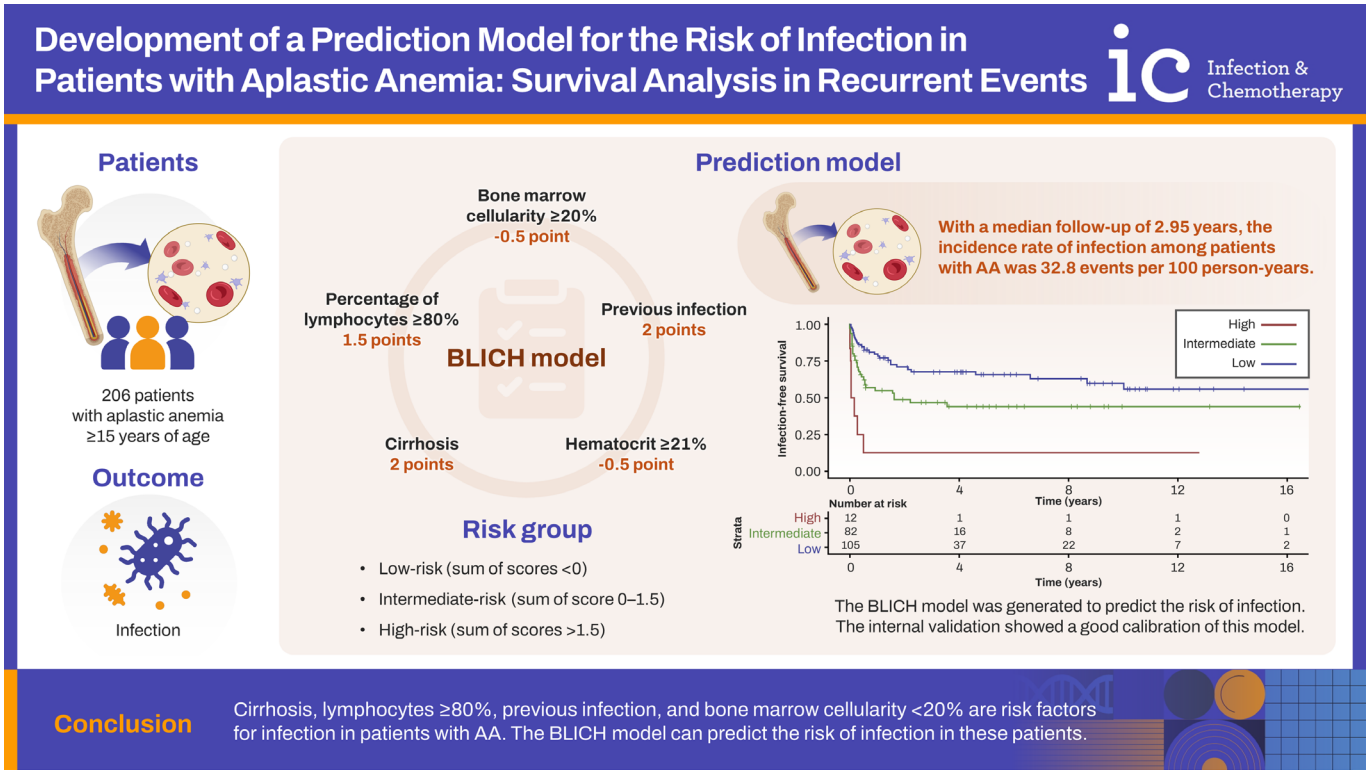
GRAPHICAL ABSTRACT



INTRODUCTION

Aplastic anemia (AA) is a rare hematological disease that can have fatal complications. Severe AA (SAA) has an overall survival rate of 20% with supportive treatment [1]. Effective treatments, including bone marrow transplantation, eltrombopag, and immunosuppressive therapy, have significantly reduced the mortality rate [2, 3]. However, infection remains the leading cause of death, especially in severe or progressive disease [2, 4]. Moreover, infection can lead to delayed treatment, which contributes to higher morbidity and mortality [5, 6].

Previous studies have reported a 5-year overall mortality of approximately 30% in patients with AA with infection [7]. Bacterial infections are the leading cause of complication-related death in patients with AA, with a prevalence of 36–69% [2, 4, 7]. However, invasive fungal infections, particularly with *Aspergillus* spp., are the most lethal, with a 10% prevalence [2, 4, 7]. Various factors, such as regional differences, patient characteristics, and treatment patterns, contribute to the variety of infections observed. Patients previously treated with antithymocyte globulin (ATG), with or without cyclosporine (CsA) have a higher infection rate [7]. Moreover, the duration of neutropenia plays a role in the differences in infection type, as persistent neutropenia increases the likelihood of Gram-negative pathogens or fungal infections [8, 9].

Although the prevalence of infection in patients with AA has been reported, information on the incidence rate, a key metric in infection epidemiology, is limited in patients with AA [10]. Moreover, information regarding infection and its predictive factors in patients with AA is limited, particularly in Asian countries. Therefore, this study aimed to determine the incidence of infection among patients with AA as well as relevant infection risk factors to develop a predictive model for infection in these patients.

MATERIALS AND METHODS

1. Study design and participants

This single-center retrospective cohort study included 206 patients diagnosed with acquired AA between January 2004 and December 2020. The severity of AA

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was defined according to the Camitta Criteria [6]. SAA was defined as bone marrow cellularity <25% (or 25–50% with <30% residual hematopoietic cells), plus satisfaction of at least two of the following criteria: absolute neutrophil count (ANC) <0.5×10^9/L, platelets <20×10^9/L, and reticulocyte count <20×10^9/L. Very SAA (vSAA) was defined the same as SAA but with an ANC <0.2×10^9/L [6, 11]. The patients who did not meet the criteria for SAA or vSAA were classified as non-SAA. Patients <15 years of age, those who underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT), and those with incomplete medical records were excluded.

2. Ethics statement

This study was approved by the relevant hospital's Human Research Ethics Committee (approval number: REC. 63-394-14-1) and was conducted in accordance with the Declaration of Helsinki. The requirement for informed consent was waived owing to the retrospective nature of the study.

3. Data collection and outcomes

Comprehensive demographic and clinical data of patients with AA were collected. Following a literature review, covariates identified as risk factors for infection in AA, including age, neutrophil count, and previous infection were included [2, 4, 7, 8]. Furthermore, other covariates were chosen based on evidence of their association with an increased risk of infection in other groups of patients, particularly immunocompromised hosts, including comorbidities such as chronic kidney diseases (CKD) and cirrhosis, hematocrit, and lymphocytes [12-15]. Cirrhosis was diagnosed using clinical findings, laboratory testing, imaging (ultrasound, computed tomography, magnetic resonance imaging), liver biopsy, and additional tests such as the fibrotest [16]. CKD was defined as either a glomerular filtration rate <60 mL/min/1.73 m^2 or markers of kidney damage, including albuminuria, present for at least 3 months [17]. Previous infection was defined as a bacterial, viral, fungal, or parasitic infection that occurred within 1 month before AA diagnosis. Characteristics of the infection, including the episode, site, pathogens, and treatment, were reported. Both the outpatient and inpatient departments provided this information. The infection types in this study comprised primary bacteremia, oral cavity infections, lower and upper respiratory tract infections, skin and soft tissue infections, gastrointestinal tract infections, urinary tract infections, and other infections. Each type of infection was defined according to the Centers for Disease Control and Prevention/National Healthcare Safety Network surveillance definitions for specific types of infections, 2023 [18]. Additional details are provided in the **Supplementary Methods**.

Infection episodes were defined as events in patients developing signs and symptoms of sepsis with or without organism identification. The source of infection was defined as any infection occurrence that could be identified to a specific location through assessing the patient's clinical condition, imaging data, and microbiological documentation. One infectious episode might have several sources of infection or organisms. The diagnostic cultures used to identify infectious organisms comprised solid culture media consisting of chocolate, blood, MacConkey, and thioglycollate broth agar for identifying general bacteria, Löwenstein–Jensen medium agar for culture and isolation of *Mycobacterium tuberculosis*, and Sabouraud dextrose agar for fungus identification [19-21].

Recurrent infection was defined as re-infection with the presence of new organisms occurring at least 1 week after discontinuing treatment for a prior infection, relapse of infection with same organism after no clinical sepsis for at least 1 week, or a new source of infection during the treatment of another infection. Recurrent infections were counted as new infectious episodes; however, a relapse of infection with clinical sepsis within a week was considered as the same infectious episode.

The primary outcomes were identification of predictive factors associated with infection and development of a predictive model for infections in patients with AA. The secondary outcomes included the incidence of infection and pathogens responsible for the infections.

4. Statistical analysis

Patient demographics were analyzed using descriptive statistics. Continuous variables were tested using the Shapiro-Wilk test to assess data normality and presented as medians with interquartile ranges (IQRs). Categorical variables were summarized as absolute numbers with percentages. Missing data were imputed using the regression imputation method. The maximally selected log-rank statistic was evaluated to determine the cut-off value for the predictive ability of the continuous variables.

In patients with AA, Cox proportional hazards regression is typically used to identify predictive factors associated

with infections. However, because infections are recurrent events that violate the assumptions of the Cox proportional hazards regression due to event correlation, survival analysis using recurrent event methods is more suitable. These methods include the Anderson and Gill model; Prentice, Williams, and Peterson Total Time (PWP-TT) model; Prentice, Williams, and Peterson Gap Time model; marginal model; and frailty models. Variables with *P*-values <0.05 in the univariate analysis were included in the multivariate analysis. A two-sided *P*-value <0.05 was considered statistically significant in the multivariate analysis of all models. The best models for survival analysis of recurrent events were determined based on the lowest log-likelihood, Akaike Information Criterion, and Bayesian Information Criterion. The statistically significant variables in the multivariate analysis of the best model were considered predictive factors for infection in patients with AA.

To generate the prediction model, the variables with a *P*-value <0.05 from the univariate analysis of the best model underwent backward stepwise regression to select the prediction factors. The internal validation of the prediction model was assessed using the bootstrapping method with 500 re-samplings. A calibration plot was constructed to evaluate the model's calibration.

RESULTS

1. Demographic characteristics

In total, 206 eligible patients with AA were included in this study (shown in **Supplementary Fig. 1**). Of those, 51.9% of patients were female, with a median (IQR) age of 53.2 (36.1–68.2) years. Overall, 135 (65.5%) patients had non-SAA, and 189 underwent AA treatment. Most patients (n=142, 68.9%) received oxymetholone, followed by methyltestosterone (31.6%), ATG+CsA (12.6%), and eltrombopag (1.5%). However, 66% of patients did not respond to the treatment. The clinical characteristics of the patients are shown in **Table 1**.

2. Infectious episodes and spectrum of microbiological isolates

Among the 206 patients, 87 (42.2%) had an infection, with 199 total infectious episodes. With a median follow-up of 2.95 years, the incidence rate of infection was 32.8 events per 100 person-years. Of these, 42 patients had more than one episode (**Supplementary Table 1**). Out of 199 infectious episodes, 160 necessitated hospitalization, involving 81 patients. Further, 155 sources of infection were identified in 116 episodes. Multiple infectious sites were found in 34 infectious episodes (29 episodes had 2 sites of infection, and 5 episodes had 3 sites of infection). Primary bacteremia (29.7%) was the most common source of infection, followed by urinary tract infection (24.5%) (**Supplementary Table 2**).

Regarding microbiological evaluation, pathogens were identified in 115 episodes, with bacteria accounting for the majority (92.2%). Notably, Gram-negative bacteria represented 82.1% of all bacteria. Among these, *Escherichia coli* was the most common, with 31 isolates of *E. coli* and 7 isolates of extended spectrum beta-lactamase producing *E. coli*. Gram-positive bacteria were reported in 33 episodes (31.1%), with *Enterococcus* spp. and *Staphylococcus aureus* being the most commonly identified species. *Candida* spp. was the most commonly identified pathogen of fungal infections, and herpesviruses were the most common cause of viral infections (**Table 2**).

Table 1. Baseline characteristics of patients with aplastic anemia

Characteristics	Total (N=206)
Female, n (%)	107 (51.9)
Age, median years (IQR)	53.2 (36.1–68.2)
Underlying diseases	
Diabetes mellitus	19 (9.2)
Chronic kidney disease	16 (7.8)
Cirrhosis	2 (1.0)
Severity of aplastic anemia	
Non severe	135 (65.5)
Severe	34 (16.5)
Very severe	37 (18.0)
Time from symptom to treatment (n=189), median days (IQR)	66.0 (28.0–156.0)
Time from diagnosis to treatment (n=187), median days (IQR)	14.0 (9.0–40.5)
CBC at diagnosis, median (IQR)	
WBC, cells/mm^3 (n=205)	2,700 (1,900–3,520)
Hct, % (n=203)	23.9 (19.6–27.6)
Hb, g/dL (n=195)	8.0 (6.7–9.2)
RBC, cells/mm^3 (n=142)	2.67 (2.18–3.08)
Platelet, cells/mm^3 (n=202)	16,000 (8,000–37,000)
Neutrophil, %, (n=194)	32.0 (18.0–46.5)
ANC, cells/mm^3 (n=194)	869.2 (446.5–1,426.3)
Lymphocyte, % (n=195)	60.0 (41.0–75.0)
ALC, cells/mm^3 (n=195)	1,564.2 (1,007.5–2,014.3)
Reticulocyte, % (n=145)	1.1 (0.5–2.0)
ARC, cells/mm^3 (n=108)	23,940 (9,989.3–50,600.0)
Bone marrow cellularity, median (IQR)	5 (5–10)
Previous infection	20 (9.7)

IQR, interquartile range; CBC, complete blood count; WBC, white blood cell; Hct, hematocrit; Hb, hemoglobin; RBC, red blood cell; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; ARC, absolute reticulocyte count.

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Table 2. Isolated microorganisms

Isolated microorganisms (n=115 episodes)	Number
Bacteria (n=106 episodes, 92.2%)[a]	
Gram-negative bacteria[b] (n=87 episodes, 82.1%)	
Escherichia coli	31
ESBL-producing *E. coli*	7
Klebsiella pneumoniae	21
ESBL-producing *K. pneumoniae*	13
Pseudomonas aeruginosa	15
MDR-*P. aeruginosa*	1
Enterobacter cloacae	10
MDR-*Acinetobacter baumannii*	8
A. baumannii	4
Proteus mirabilis	6
Aeromonas sobria	4
Salmonella spp.	4
Others	22
Gram-positive bacteria[b] (n=33 episodes, 31.1%)	
Enterococcus spp.	7
Staphylococcus aureus	7
S. epidermidis	4
Staphylococcus spp. (other)	4
Streptococcus spp. (other)	4
S. pneumoniae	1
Others	11
Other bacteria[b] (n=12 episodes, 11.3%)	
Mycobacterium tuberculosis	9
Nontuberculous mycobacteria	2
Clostridioides difficile	2
Mycoplasma spp.	1
Fungal (n=9 episodes, 7.8%)[a]	
Candida spp.	5
Mucomycosis	1
Aspergillus	1
Penicillium marneffei	1
Tinea	1
Viral (n=5 episodes, 4.3%)	
Herpesviruses	4
Chikungunya infection	1
Parasite (n=2 episodes, 1.7%)	
Strongyloides stercoralis	1
Trichuris trichiura	1

[a]Seven episodes of bacterial and fungal coinfection.
[b]Polybacterial infection was documented in 43 infectious episodes.
ESBL, extended spectrum beta-lactamase; MDR, multidrug-resistant.

3. Factors influencing infection in patients with acquired AA

Factors associated with infection were evaluated using Cox proportional hazards regression analyses, and survival analysis was performed using the recurrent event method. In the univariate analysis of all models, a low percentage of neutrophils (<15%) and high percentage of lymphocytes (≥80%) were associated with an increased risk of infection (**Supplementary Table 3**). In all the models, multivariate analysis showed that previous infection was associated with an increased risk of infection. Based on the lowest log-likelihood, Akaike Information Criterion, and Bayesian Information Criterion, the PWP-TT model was the best for predicting infection risk. Cirrhosis (hazard ratio [HR], 5.34; 95% confidence interval [CI], 2.45–11.65; *P* <0.001), lymphocytes ≥80% (HR, 3.15; 95% CI, 1.47–6.79; *P*=0.003), and previous infection (HR, 7.36; 95% CI, 3.72–14.57; *P* <0.001) were significant risk factors for the occurrence of infection. In contrast, bone marrow cellularity ≥20% was identified as a protective factor (HR, 0.54; 95% CI, 0.32-0.92; *P*=0.022) (**Table 3**).

4. Prediction model generation by survival analysis in recurrent events

After backward stepwise regression in the PWP-TT model, the prediction model (BLICH model) consisted of bone marrow cellularity (HR, 0.56; 95%CI, 0.33–0.94), percentage of lymphocyte (HR, 4.70; 95% CI, 2.60–8.51), previous infection (HR, 7.40; 95% CI, 3.73–14.68), cirrhosis (HR, 6.22; 95% CI, 3.34–11.59), and hematocrit (HR, 0.63; 95% CI, 0.35–1.14).

For the application of the BLICH model, we assigned risk scores according to the coefficients of each prediction factor (**Supplementary Table 4**): bone marrow cellularity ≥20% (-0.5 point), percentage of lymphocytes ≥80% (1.5 points), previous infection (2 points), cirrhosis (2 points), and hematocrit ≥21% (-0.5 point). Patients were divided into the low-risk (sum of scores <0), intermediate-risk (sum of score 0–1.5), and high-risk (sum of scores >1.5) categories. This model demonstrated a significant difference in infection-free survival among the risk groups (*P*<0.001) (**Fig. 1** and **Supplementary Table 5**). This model was internally validated using the bootstrapping method with 500 re-samplings. The calibration plot at 1 year confirmed good calibration of the model (**Fig. 2**).

DISCUSSION

This study revealed that 87 patients with AA experienced at least one infectious complication. Among 199 infectious episodes, the bloodstream was the most common source of infection. Gram-negative bacteria (82.1%) were the predominant identifiable isolates, with *E. coli* being the most documented organism. On the basis of the PWP-TT model, patients with cirrhosis, lymphocytes ≥80%, and previous infection were associated with an increased risk of infections. The BLICH model performed well in predicting infections in patients with AA.

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Table 3. Multivariate analysis in Cox proportional hazards regression and the five models of survival analysis in recurrent events

Characteristic	Cox			AG model			PWP_TT			PWP_GT			Marginal model			Frailty model		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Underlying disease																		
Diabetes mellitus	-	-	-	-	-	-	-	-	-	1.52	1.04–2.23	0.030	-	-	-	-	-	-
Cirrhosis	-	-	-	2.97	1.33–6.61	0.008	5.34	2.45–11.65	<0.001	-	-	-	-	-	-	-	-	-
Bone marrow cellularity (≥20%)	-	-	-	0.52	0.30–0.91	0.022	0.54	0.32–0.92	0.022	-	-	-	0.43	0.26–0.70	<0.001	0.39	0.16–0.93	0.034
CBC at diagnosis																		
WBC (≥3,000/mm^3)	-	-	-	0.77	0.42–1.42	0.400	0.99	0.52–1.85	0.965	-	-	-	0.64	0.33–1.24	0.189	0.82	0.38–1.75	0.600
Hct (≥21%)	-	-	-	-	-	-	0.66	0.36–1.20	0.171	-	-	-	0.90	0.50–1.62	0.737	0.41	0.13–1.28	0.120
RBC (≥2.0×10^9/mm^3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.75	0.23–2.46	0.640
Platelet (≥40,000/mm^3)	0.47	0.23–0.94	0.032	-	-	-	-	-	-	-	-	-	-	-	-	0.62	0.24–1.66	0.340
PMN (≥15%)	0.47	0.20–1.14	0.095	0.69	0.40–1.20	0.192	0.73	0.34–1.58	0.428	0.75	0.50–1.13	0.169	0.66	0.27–1.63	0.374	0.47	0.03–7.56	0.590
Lymphocyte (≥80%)	1.37	0.57–3.31	0.480	3.26	1.74–6.08	<0.001	3.15	1.47–6.79	0.003	2.08	1.23–3.50	0.006	3.04	1.46–6.35	0.003	2.67	0.17–42.58	0.490
Reticulocyte (≥1%)	-	-	-	-	-	-	0.82	0.45–1.48	0.506	-	-	-	1.05	0.58–1.91	0.870	-	-	-
Previous infection	3.08	1.71–5.55	<0.001	9.54	5.92–15.39	<0.001	7.36	3.72–14.57	<0.001	4.40	2.94–6.58	<0.001	5.89	3.94–11.83	<0.001	5.41	2.93–10.00	<0.001
Log Likelihood		−334.53			−509.69			−285.34			−538.14			−335.29			−374.96	
AIC		677.05			1,031.37			586.67			1,084.27			684.58			918.34	
BIC		686.27			1,050.08			611.61			1,096.75			706.41			1,180.92	

AG, Anderson and Gill; PWP-TT, Prentice, Williams, and Peterson Total Time; PWP-GT, Prentice, Williams, and Peterson Gap Time; HR, hazard ratio; CI, confidence interval; CBC, complete blood count; WBC, white blood cell; Hct, hematocrit; RBC, red blood cell; PMN, polymorphonuclear; AIC, Akaike Information Criterion; BIC, Bayesian Information Criterion.

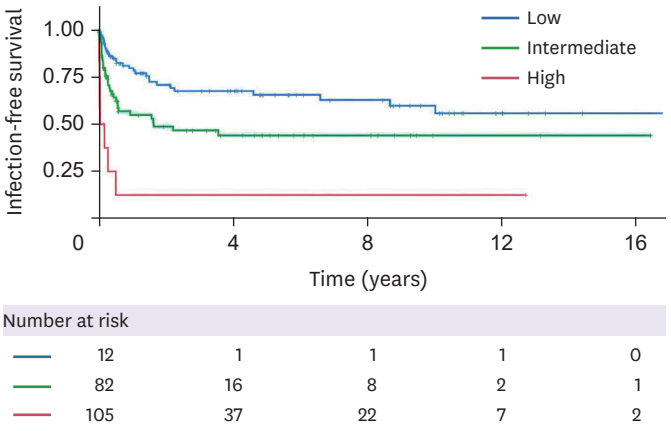

Figure 1. **Infection-free survival in each risk group of the BLICH model.**
BLICH, bone marrow cellularity, percentage of lymphocyte, previous infection, cirrhosis, and hematocrit level.

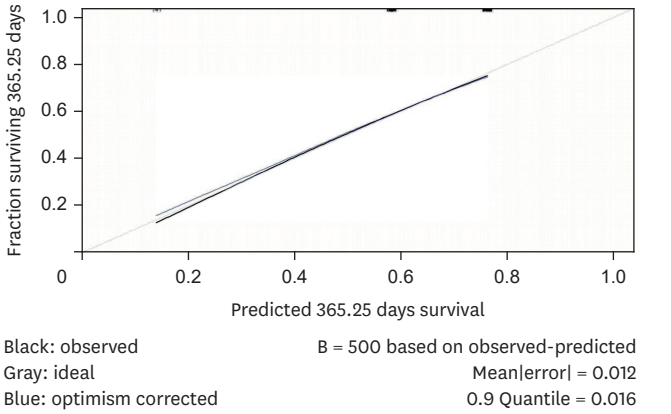

Figure 2. **Calibration plot of the BLICH model to predict the risk of infection in patients with aplastic anemia at 1 year.**
BLICH, bone marrow cellularity, percentage of lymphocyte, previous infection, cirrhosis, and hematocrit level.

Although effective treatments for AA have been developed, infection remains the major cause of morbidity. To our knowledge, this was the first study to report the incidence of infection in patients with AA, which was 32.8 events per 100 person-years, whereas previous studies have presented the results in terms of prevalence, ranging from 32.8% to 86.6% [4, 7, 9, 22]. The variation in prevalence can be attributed to differences in the proportion of patients with non-SAA, SAA, and vSAA. Lower prevalences might be caused by the inclusion of fewer patients with severe conditions. Furthermore, the treatment regimen might have an impact on this finding. Infection has been related to adverse outcomes following allo-HSCT [23]. However, because this treatment is not available at our hospital, the occurrence of infection in patients undergoing allo-HSCT was not evaluated in the current study. In previous

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studies, most patients received immunosuppressive therapy with antithymocyte globulin and cyclosporine, which increase the risk of infection. In contrast, in our study, most patients received oxymetholone, which is less immunosuppressive [22, 24], resulting in a lower infection rate than previous studies. The most common source of infection in patients with AA was primary bacteremia (29.7%), followed by urinary tract infections (24.5%) and lower respiratory tract infections (19.4%). Our finding was consistent with those of previous studies, which showed that primary bacteremia was the common source of infection (22–38%) [4, 7, 9].

The current study revealed that Gram-negative pathogens were the predominant causative organisms. Our findings were consistent with those of a previous study [7]; however, they differed from previous studies that reported Gram-positive organisms, notably coagulase-negative Staphylococci, as the most common pathogens [2, 4]. The difference in our study's results could be attributed to the lower rate of central venous catheter use, which could alter the spectrum of microorganisms involved in the infection. *E. coli* was documented as the predominant Gram-negative pathogen in the present study, whereas *Stenotrophomonas maltophilia* [4] and *Acinetobacter baumannii* [7] have been previously reported. This inconsistency in infection patterns may be related to the variations in routine antibiotic use and antimicrobial susceptibility patterns in each region. *Aspergillus* spp. and *Candida* spp. were the main fungal species identified [2, 4, 7]. Similarly, we found that *Candida* spp. were the most common pathogens in invasive fungal infections. Moreover, herpesvirus infections were the most frequent viral infections in our study.

Several risk factors for infection in patients with AA have been reported, including reduced immunological function, which contributes to lower white blood cell and neutrophil counts, and immunosuppressive therapy [2, 5]. Low leukocyte counts and persistent neutropenia have been documented as important risk factors for infection development [2, 4, 7, 8]. The proportion of lymphocytes was significantly elevated, likely attributable to the reduced absolute number of neutrophils [15], and results in increased risk of infection. Using the PWP-TT model, we found that the lymphocyte percentage was associated with infection occurrence. Patients with AA with ≥80% lymphocytes had an increased infection risk.

Previous studies found that patients with liver cirrhosis or underlying gastrointestinal disease were more likely to experience recurrent infection [25, 26]. Similarly, our study revealed that cirrhosis was associated with an increased risk of infection in patients with AA. To our knowledge, patients with cirrhosis are at an increased risk of infection owing to their compromised immune status [27, 28]. Moreover, because these patients are more likely to undergo diagnostic or therapeutic procedures that can impair their immune defenses, they are at a higher risk of nosocomial infections [29]. Bacterial infections are the most commonly diagnosed infection in hospitalized patients, ranging from 25–47% [30]. Several factors predispose these patients to the occurrence of infections, including a compromised immune system, intestinal bacterial overgrowth, and increased intestinal permeability [30, 31].

Previous infection posed a risk of reinfection or recurrent infections [12, 14, 32-34]. A previous study found that recurrent *Klebsiella pneumoniae* bacteremia could develop in patients with underlying hematological malignancies or primary bacteremia [33]. Furthermore, patients who had previously been diagnosed with invasive aspergillosis were more likely to experience relapsed infection [14]. Consistent with prior studies, previous infection was associated with infection occurrence in our current study.

Anemia is a risk factor for infection [13, 35], and it was specifically found to be a significant risk factor in patients with myelodysplastic syndromes with infection [13]. Low hematocrit levels were associated with an increased infection risk, hospital stay duration, and mortality [35]. Although the association between anemia and infection is not fully understood, increasing blood transfusion rates due to anemia might increase the risk of infection [35]. Moreover, low hematocrit levels were associated with poor prognosis and high mortality in patients with sepsis. This could be explained by the inflammatory response and oxidative stress, both of which are related to oxygen transport and metabolism [36].

This is the first study to present the incidence rate of infection and analyze it using the appropriate method for infection, which was the occurrence of recurrent events. However, this study has some limitations. First, it was conducted at a single site and, hence, may not be representative of other locations. Second, this retrospective study relied on routinely collected data. Although this represented real-world practice conditions, it resulted in missing data. Third, five predictive factors were included using a backward stepwise regression.

This number of variables may not be enough to predict infection in patients with AA, and no other potentially essential predictive variables were identified. Fourth, the BLICH model included patients with cirrhosis, which was found in only 2 patients who developed infections in 1 and 3 episodes, as a predictor of infection. This limited number of patients may have resulted in an inflated HR. Therefore, external validation is required to confirm this finding. Finally, the prediction model was generated using a single-center population and was internally validated to evaluate overfitting. However, the generalizability of this model could not be confirmed. External validation from other studies should be performed to test this hypothesis.

In conclusion, infection remains a major complication in patients with AA, particularly bacterial infections. Patients with cirrhosis, lymphocytes ≥80%, previous infection, and bone marrow cellularity <20% were associated with an increased risk of infection. The BLICH model could predict the risk of infection in patients with AA. However, further study with external validation is required to strengthen the evidence supporting these findings.

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SUPPLEMENTARY MATERIALS

Supplementary Methods
Definitions of infection according to each site

Supplementary Table 1
Frequency of each infectious episode in patients with aplastic anemia (N=87)

Supplementary Table 2
Sources of infection in patients with aplastic anemia (N=155)

Supplementary Table 3
Univariate analysis in Cox proportional hazards regression and the five models of survival analysis in recurrent events

Supplementary Table 4
Prediction factors for infections in 206 patients with aplastic anemia and associated prognostic scores

Supplementary Table 5
Infection-free survival of each risk group in the BLICH model

Supplementary Figure 1
Study flow.

ORCID iDs
Pirun Saelue
https://orcid.org/0000-0003-3900-460X
Thitichaya Penthinapong
https://orcid.org/0000-0001-8046-5999

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Conceptualization: PS, TP. Data curation: PS, TP. Formal analysis: PS. Investigation: PS, TP. Methodology: PS, TP. Project administration: PS. Validation: PS, TP. Visualization: PS, TP. Writing - original draft: PS, TP. Writing - review & editing: PS, TP.

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