นิพนธ์ต้นฉบับ • Original Article

ลักษณะทางคลินิก, เชื้อก่อโรคสาเหตุ, บทบาทของซีรัม Galactomannan และผลการรักษาผู้ป่วยมะเร็งเม็ดเลือดขาวแบบเฉียบพลันที่มีภาวะไข้ร่วมกับเม็ดเลือดขาวชนิดนิวโตรฟิวต่ำ

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Clinical Characteristics, Causative Organisms, Role of Serum Galactomannan and Treatment Outcomes of Acute Leukemia Patients with Febrile Neutropenia

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หลักการและวัตถุประสงค์: ภาวะไข้ร่วมกับเม็ดเลือดขาวชนิดนิวโตรฟิวต่ำเป็นภาวะแทรกซ้อนที่สำคัญในผู้ป่วยมะเร็งเม็ดเลือดขาวแบบเฉียบพลัน เป็นเหตุให้เสียชีวิต ข้อมูลเชื้อก่อโรคสาเหตุจะช่วยแพทย์ตัดสินใจเลือกใช้ยาต้านจุลชีพที่เหมาะสมและลดอัตราการเสียชีวิตดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อศึกษาลักษณะทางคลินิก, เชื้อก่อโรคสาเหตุและผลการรักษาของผู้ป่วย

วิธีการศึกษา: เป็นการศึกษาย้อนหลังเชิงวิเคราะห์และพรรณนา เก็บข้อมูลย้อนหลังมกราคม 2556 ถึงธันวาคม 2558

ผลการศึกษา: เก็บข้อมูลได้ 203 ครั้ง ตำแหน่งการติดเชื้อที่พบบ่อยคือการติดเชื้อในกระแสเลือด, ปอด และทางเดินอาหาร เป็นร้อยละ 23.9, 19.8 และ 12.1 ตามลำดับ โดยเชื้อก่อโรคที่พบบ่อยแตกต่างกันตามตำแหน่งการติดเชื้อ โดยพบการติดเชื้อแบคทีเรียกรัมลบได้บ่อย เชื้อราลุกลามที่พบบ่อยคือ Aspergillus spp. และ Candida spp. ระดับซีรัม galactomanan ในการวินิจฉัยการติดเชื้อราลุกลามทั้งหมดมีความไว ความจำเพาะ ค่าทำนายเมื่อผลเป็นบวกผลเป็นลบและความถูกต้อง ร้อยละ 42.9, 97.9, 88.9, 81.7 และ 82.7 ตามลำดับ ค่ามัธยฐานระยะเวลานอนโรงพยาบาลคือ 28 วัน และอัตราการเสียชีวิตคิดเป็นร้อยละ 6.9

สรุป: ตำแหน่งการติดเชื้อสามารถช่วยบอกเชื้อก่อโรคที่เป็นสาเหตุ และช่วยตัดสินใจเลือกการตรวจทางห้องปฏิบัติการและยาต้านจุลชีพที่เหมาะสม การติดเชื้อราลุกลามโดยเฉพาะ

Background and Objective: Febrile Neutropenia (FN) is considered to be an important complication in Acute Leukemia (AL) patients leading to death. Information about common causative organisms will guide physicians to use empirical antibiotic properly and reduce infectious mortality consequently. This study aimed to determine clinical characteristics, causative organisms and treatment outcomes of AL with FN.

Materials and Methods: Retrospective descriptive and analytical study. Any episodes of FN have been collected from January 2013 to December 2015.

Results: A total of 203 FN episodes were recorded. Blood stream infection (23.9%), pneumonia (19.8%) and gastrointestinal (GI) tract infection (12.1%) were frequently notified more than other sources of infection. Common pathogens were differentiated by sources of infection. The most common infection was gram negative bacteria. Common Invasive Fungal Infections (IFIs) were Aspergillus spp. and Candida spp. Serum galactomanan was a practical useful diagnostic tool for all IFIs with sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 42.9%, 97.9%, 88.9%, 81.7% and 82.7% respectively in IFIs. A median length of hospitalization was 28 days. Mortality rate of FN was 6.9%.

Conclusions: Source of infection can guide causative pathogens, appropriate investigations and empirical

Aspergillus spp. ยังเป็นสาเหตุของการเสียชีวิตที่สำคัญ ระดับซีรัม galactomannan เป็นการตรวจที่มีความจำเพาะและค่าทำนายเมื่อผลเป็นบวกสูงในวินิจฉัยการติดเชื้อราลุกลามโดยเฉพาะอย่างยิ่งเชื้อรา Aspergillus spp.

treatment during FN. IFIs were still an important problem leading death especially invasive aspergillosis. Serum galactomannan is one of a practical tool for diagnosis IFIs especially Aspergillosis with high specificity and positive predictive value.

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Introduction

Febrile Neutropenia (FN) is commonly found in Acute Leukemia (AL) patients[1-3]. Neutropenia is a result of an impair bone marrow (BM) production by invasion of leukemic cell in BM and/or BM injury after chemotherapy. Due to the host immune function is severely compromised, infections are invasive and rapidly progressed leading dead. Early and appropriate of antimicrobial agents is the most important factor for survive[4, 5]. Causative pathogens have been gradually changed overtime by antibiotic using behavior[6, 7]. Information about common causative organisms will assist physicians to use empirical antimicrobial agent suitably and reduce infectious mortality consequently. Routine treatment strategy and its outcome will guide developing of treatment guideline in the future.

This study aimed to determine clinical characteristics, causative organisms and treatment outcomes of AL with FN

Material and Methods

Operational definitions

Febrile neutropenia (FN) is defined by Fever with either Absolute Neutrophil Count (ANC)< 500 cells/mm^3 or ANC < 1,000 cells /mm^3 with expected to fall below 500 cells/mm^3[3, 8-10].

ANC is calculated by (% Band + %Neutrophil) X WBC / 100

The diagnosis of invasive fungal infection completed by using the revised definition of invasive fungal disease from the European Organization for Research and Treatment of Cancer (EORTC)[11].

Study Design

We designed a retrospective descriptive and analytical study of all adult AL with FN in Srinagarind hospital, Thailand. Data has been collected from medical records for 3 years (January 1st 2013 to December 31st 2015). Any episodes of FN among AL patients were included and explored.

Statistical Analysis

Patients'data were analysed by statistical software STATA version 10.0. Patients' information that included categorical and continuous data, was summarized differently. In case of categorical data, the information was presented in form of percentage, while two sets of statistical tools, median with range and mean with standard deviation (SD) were applied to interpret continuous data. Magnitude of difference in prevalence was presented as percentage, considering p-value that is less than 0.05 as statistically significant.

Ethical consideration

This study protocol including with the study information and case record form was approved and accepted by the Ethic committee for Research in Human Subjected at Srinagarind hospital.

Results

A total of 203 FN episodes were recorded. Clinical characteristics were as followed: female sex 52.2%, median age 40 years (range 18-71 years). A half of participants were female. The majority of cases were acute myeloid leukemia (AML) (77.9%), while 22.1% were acute lymphoid leukemia (ALL). The median of peripheral blood blast and bone marrow blast at diagnosis were 54% (0-99) and 80% (20-100), respectively. Patients were mainly treated by intensive standard chemotherapy and palliative care. FN usually occurred after the 1st week of chemotherapy. Median time of FN was 8 days. The lowest absolute neutrophil

count (ANC) was generally less than 100 cells / mm^3. Persisting fever after the 4^{th} day of FN and prolonged broad spectrum antibiotics more than 10 days were found in 55.7%, and 62.6% respectively.

Sources of infection were revealed in **Figure 1**. Definite source of infection could not be specified in 54 episodes (26.7%) of FN. Blood stream infection, pneumonia and gastrointestinal (GI) tract infection were frequently notified more than other sources of infection.

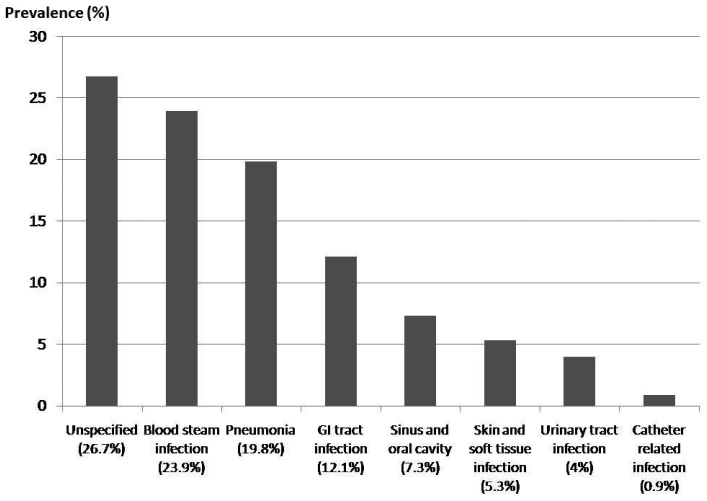


Figure 1 Sources of infection

The causative pathogens were specified only in 101 episodes (49.5%). Causative group of pathogens were demonstrated in **Figure 2**. Bacterial infection was 72.2 % (Gram negative 48.5%, Gram positive 17.8% and anaerobic bacteria 5.9%), fungal infection was 22.7%, viral infection was 2.9%, and tuberculosis was 2.2% respectively. Reactivated Tuberculosis was still found. Hemoculture, Chest X-ray and urine examination should be done routinely.

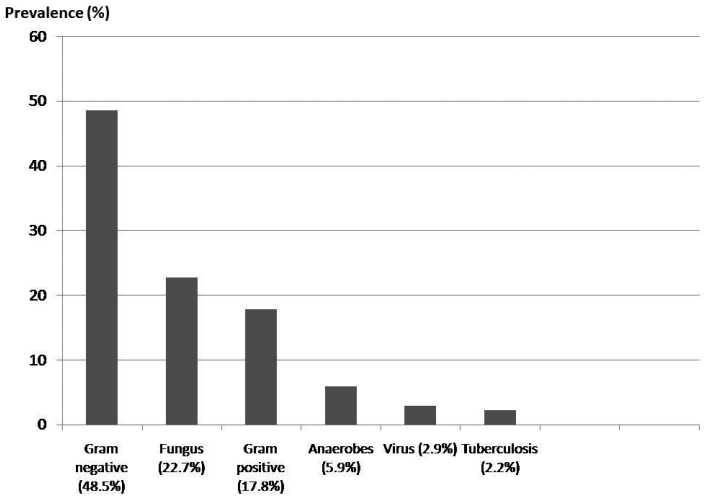
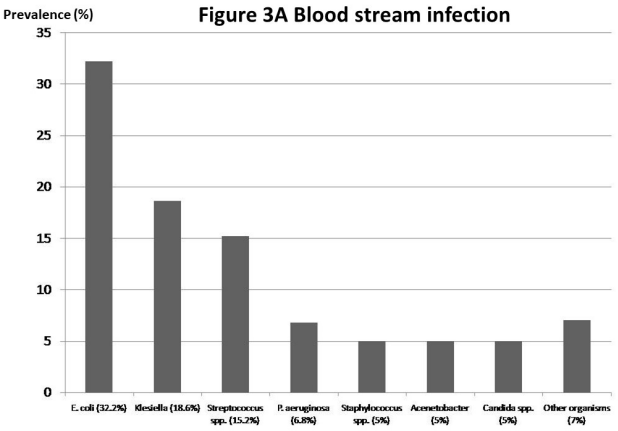
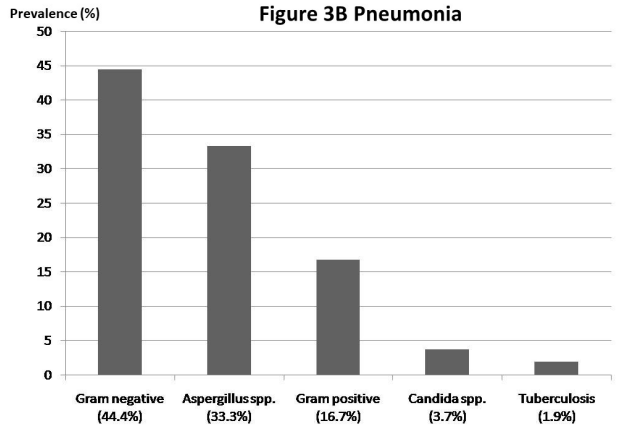


Figure 2 Causative pathogens





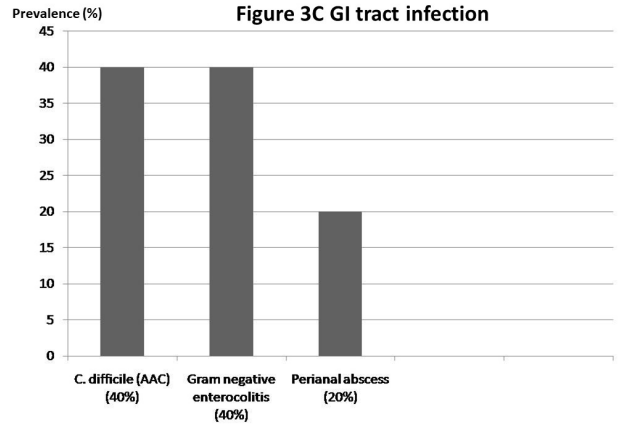


Figure 3 A-C demonstrated causative organisms in each common site of infection.

Sources of infection can indicate specific causative organisms, guide the appropriate investigations and point out empirical treatment as shown in Figure 3 A-C.

Empirical antibiotic was depended on the source of infection. The spectrum of initial antibiotic should be covered at least gram negative, gram positive and anaerobic bacteria. In the case of pneumonia and/or sinusitis, Invasive pulmonary Aspergillosis should be ruled out firstly. If patients presented with gastrointestinal tract symptoms, Diagnostic test for *Clostridium difficile* should be tested and excluded. Enterococcus species were commonly found in urinary tract infections

In this study, patients were immediately received broad spectrum antibiotic after diagnosed FN. Antibiotic was adjusted according to clinical signs and specified pathogens. Among group of unspecified organisms, patients who had persisting fever after the 4th day of antibiotic were routinely received Amphotericin-B. Overall response rate was 93.1% (responded with an empirical antibiotic 61.6%, combination of antibacterial and antifungal agent 24.1%, antifungal agent 5.4%, antiviral agent 1% and anti-tuberculosis 1%).

Invasive fungal infections (IFIs) had been diagnosed in 57 episodes of FN. Definite (Proven) fungal infections were found in 37 episodes, probable fungal infections were 2 episodes and possible fungal infections were 18 episodes. Risk factors of IFIs were explored as in **Table 1**.

Performance of serum galactomannan in diagnosis of IFIs was determined in 202 episodes of FN (1 data missing) and revealed in **Table 2**. Serum galactomannan more than 0.5 was highly predicted invasive fungal infection ($p<0.01$) with sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 42.9%, 97.9%, 88.9%, 81.7% and 82.7% respectively.

The role of serum galactomannan in subgroup of proven IFIs was demonstrated in **Table 3** (mostly Invasive aspegillosis).

Table 1 Risk factors of IFIs

Risk factors		Invasive Fungal Infections		p-value
		Present	Absent	
Previous FN	Present	20	95	< 0.01
	Absent	26	45	
Previous IFIs	Present	8	39	0.197
	Absent	45	102	
Persisting fever after the 4th day of FN	Present	52	61	<0.01
	Absent	5	85	
Prolonged antibiotic ≥ 10 days	Present	49	78	<0.01
	Absent	8	68	
The lowest ANC < 100 mm3	Present	54	126	0.289
	Absent	3	20	
Serum Galactomannan (≥0.5)	Present	17	3	<0.01
	Absent	32	141	

Table 2 Serum galactomannan in all IFIs

Diagnostic Test		Invasive Fungal Infections		Total
		Present	Absent	
Serum Galactomannan	Positive (≥0.5)	24	3	27
	Negative (<0.5)	32	143	175
	Total	56	146	202
Sensitivity (%)		42.9		
Specificity (%)		97.9		
Positive Predictive Value (PPV) (%)		88.9		
Negative Predictive Value (NPV) (%)		81.7		
Accuracy(%)		82.7		

Table 3 Serum galactomannan in proven IFIs

Diagnostic Test		Proven IFIs		Total
		Present	Absent	
Serum Galactomannan	Positive (≥0.5)	23	7	30
	Negative (<0.5)	14	158	172
	Total	37	165	202
Sensitivity (%)		62.1		
Specificity (%)		95.7		
Positive Predictive Value (PPV) (%)		76.7		
Negative Predictive Value (NPV) (%)		81.6		
Accuracy(%)		89.6		

Sensitivity of serum galactomannan for detection of invasive Aspergillosis (70.4%) was higher than invasive Candida infection (3.7%). Specificity and negative predictive value of serum galactomannan were high in both IFIs.

A median length of hospitalization was 28 days (range 2-102 days). Previous FN, and persisting fever were associated with prolonged hospitalization. Fourteen patients (6.9%) were died due to uncontrolled infection (71.4% developed severe invasive fungal infection).Patients who had persisting fever after the 4th day of FN and developed proven IFIs had increased mortality (p-value <0.01)) as in **Table 4**.

Table 4 Risk factors related mortality

Risk factors		Death Status		p-value
		Death	Alive	
Previous FN	Present	7	111	0.611
	Absent	6	71	
Previous IFIs	Present	3	44	0.920
	Absent	11	137	
Persisting fever after the 4th day of FN	Present	13	1	<0.01
	Absent	0	189	
Prolonged antibiotic ≥ 10 days	Present	11	116	0.200
	Absent	3	73	
Bacterial infections	Present	6	64	0.494
	Absent	8	125	
Proven IFIs	Present	9	26	<0.01
	Absent	5	163	

Discussion

FN should always be considered in AL patients. Infection during FN can occur in any part of body. Complete history taking and exhaustive physical examination should not be ignored. Hidden area should be aware and investigated such as sinus, oral cavity and unexposed skin including peri-anal area. The appropriate investigation and the empirical antimicrobial agents were guided by suspected source of infection.

Causative pathogens have been changed from the previous study. Gram negative bacterial infection was the most common infection during FN as in conducted studies[12-17]. However, IFIs has been increasing since 2005. In 2010, the prevalence of IFIs was only 6.2%[13]. In our study the prevalence of IFIs was 28% (proven IFIs 18.2%). High prevalence of IFIs can be explained by lacking of routine antifungal prophylaxis in our hospital. The prevalence of drug resistance bacteria has been increased overtime especially in previous hospitalized FN patients. It is activated by using broad spectrum antibiotic popularly. Organisms have to improve their defensive mechanisms for survival.

Streptococcal infection in particularly *Streptococcus viridans* was found more frequently than Staphylococcal infection in our study and catheter related infection was only 0.9%. This finding may imply improving of routine catheter care and adequate skin hygiene. Chemotherapy related mucositis and oral hygiene should be explored and secured.

This study emphasized that AL patients with FN have high risk for developing IFIs[18-20]. Early antifungal therapy is recommended to reduce mortality[8-10, 12, 21-23] especially in patients who had persisting fever after the 4th day[8-10, 12, 21-23].

IFIs were the major cause of death in FN patients. Common IFIs were similar to previous studies[18-23]. We use 0.5 as a standard cutoff point of serum galactomannan for diagnosis IFIs. Serum galactomannan demonstrated sensitivity and specificity of 70% and 92% respectively in previous study[24-29] correlate with our study that showed sensitivity and specificity of 42.9-62.1% and 95.7-97.9%.Negative predictive value (NPV) of serum galactomannan should be confirmed in the future.Based on high PPV (88.9%), FN patients with positive serum galactomannan should routinely receive antifungal therapy. If NPV is constantly high, we will use this test for excluding IFIs and reducing overuse of antifungal agent.

This study clearly provided causative pathogens in each source of infection and demonstrated good outcome of empirical antibiotic and/or in combination with antifungal agent. Mortality rate in this study was in acceptable range.

Conclusions

Source of infection can guide causative pathogens, suitable investigations and empirical treatment during FN. The gram negative bacterial infection and the invasive fungal infection were yet common in AL patients with FN especially *Escherichia coli* and *Aspigillus species*. In case of unspecified source and undetermined pathogens, empirical treatment with antibiotic in combination with antifungal agent produced high response rate particularly in patients who had persisting fever after the 4th day. IFIs were still an important problem leading death especially invasive aspergillosis. Serum galactomannan is one of a practical tool for diagnosis IFIs with high specificity (97.9%) and positive predictive value (88.9%). Its sensitivity was low in candida infection (3.7%) but high in invasive aspergillosis (70.4%).

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