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ORIGINAL RESEARCH

Genetic Risk Surveillance for Invasive Aspergillosis in Hematology Patients: A Prospective Observational Study

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ABSTRACT

Introduction: The association between genetic background and the risk of invasive aspergillosis (IA) has not been addressed in Thailand. We conducted genetic risk surveillance for IA among Thai hematologic patients.

Methods: We conducted a prospective observational cohort study including moderate- to high-risk hematology patients at Ramathibodi Hospital. IA occurrence, relevant clinical data, and genetic analyses were assessed. Odds ratios (ORs) of IA were assessed for the presence of the selected single nucleotide polymorphism genotype using logistic regression.

Results: A total of 357 patients were enrolled. The most common hematologic disease was non-Hodgkin lymphoma (45.1%). IA was diagnosed in 36 patients (10.10%). The C allele of $IL10_{rs1800896}$ was associated with an increased risk of IA (adjusted OR 5.297; 95% confidence interval [CI] 2.032–13.809, $p = 0.001$). In multivariate Cox regression analysis, prolonged neutropenia and the C allele of $IL10_{rs1800896}$ were associated with IA (hazard ratio [HR] 12.585; 95% CI 3.866–40.967, $p < 0.001$ and HR 2.449; 95% CI 1.097–5.468, $p = 0.042$, respectively).

Conclusions: Carrying the C allele of $IL10_{rs1800896}$ was associated with an increased risk of IA among moderate- to high-risk Thai patients with hematologic diseases. This finding can potentially lead to a novel risk stratification scheme to further prevent IA in resource-limited settings.

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Keywords: Antifungal prophylaxis; Genetic susceptibility; Hematology patients; Invasive aspergillosis

Key Summary Points

Why carry out this study?

Hematology patients are at high risk for invasive aspergillosis.

Universal antifungal prophylaxis is not feasible in a resource-limited setting even in high-risk patients.

Genetic risk surveillance may assist in risk stratification among this population.

What was learned from the study?

Carrying the C allele of $IL10_{rs1800896}$ was associated with an increased risk of IA among Thai patients with moderate- to high-risk hematologic diseases.

This finding potentially leads to a novel risk stratification scheme to further prevent IA in a resource-limited setting.

DIGITAL FEATURES

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INTRODUCTION

Aspergillus is a ubiquitous fungus with a wide range of clinical features, depending on the degree of host immunity [1]. Invasive aspergillosis (IA) is a major cause of morbidity and mortality in immunosuppressed patients. IA has been estimated to occur in 5–40% of hematology patients, especially in acute myeloid leukemia (AML) and allogeneic hematopoietic stem cell transplant (HSCT) [2–6], with a mortality rate attributable to IA of 25–50% [2, 3, 7, 8]. Additionally, patients with IA usually require high-cost treatment, either antifungal therapy or radiologic investigations [7, 9].

Although exposure to *Aspergillus* conidia through inhalation is common, only a minority of those exposed will develop lung disease [10]. Several factors have been shown to affect the risk of IA development [5, 11–13]; however, the factors determining IA susceptibility have not been completely discovered. Innate immunity and genetic susceptibility have been proposed among several other important factors determining disease occurrence [14–16].

The physical barrier of the respiratory tract is the first line of resistance against inhaled conidia of *Aspergillus* [17]. If passed successfully through the ciliated epithelium, conidia are then challenged by alveolar macrophages, dendritic cells, and polymorphonuclear cells [15]. In the early stages of infection, conidia are destroyed by local alveolar macrophages, and extracellular killing of germinating hyphae is mediated by recruited polymorphonuclear cells. Conidia and hyphae are recognized via soluble and membrane-bound pattern recognition receptors [18]. Pattern recognition receptors (PRRs) sense pathogen-associated molecular patterns (PAMPs) and drive the secretion of proinflammatory cytokines and chemokines [18–20].

Genetic variation within key innate immune response genes could influence disease susceptibility and the outcome of this infection [21–23]. Defects in Toll-like receptors (TLR)-2, TLR-3, TLR-4, TLR-9, dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN; also known as CD209 antigen), dendritic cell-associated C-type lectin 1 (DECTIN-1), pentraxin-3 (PTX-3), and interleukin (IL)-10 have been proposed to affect IA pathogenesis [15]. However, only certain single nucleotide polymorphisms have been documented in Asian populations, according to national and global databases. Of these, a single polymorphism on *DECTIN-1* is the most frequent genotype identified in Asia, followed by *PTX-3*. Although positive associations between

genetic variants in cytokine genes and vulnerability to IA have been reported [24–36], the lack of functional validation and underpowered design of most studies preclude definite conclusions [15]. Furthermore, most previous studies were conducted among white participants with HSCT status only; therefore, studies among Asian populations are needed.

Genetic surveillance for risk stratification of IA is a logical step to identify patients at risk and potentially assist clinicians to design more intensive monitoring and to more specifically select anti-mold prophylaxis [11, 23]. We analyzed the association of selected single nucleotide polymorphisms (SNPs) and the risk of IA among moderate- to high-risk hematology patients.

The primary objective of this study was to analyze the potential genetic predisposition to IA among moderate- to high-risk hematology patients. The secondary objectives were to study the epidemiology of IA and the potential impact of genetic predisposition on clinical outcomes related to IA.

METHODS

Definitions

- Confirmed and probable IA were specified according to the revised criteria of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group in the year of 2008 [37].
- Moderate-risk hematologic diseases were defined as any of the following conditions: acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), non-Hodgkin lymphoma (NHL), myelodysplastic syndromes (MDS), severe aplastic anemia (AA), multiple myeloma, and graft-versus-host disease (GVHD) after HSCT [13, 14].
- High-risk hematologic diseases were defined as diagnoses of AML and allogeneic HSCT [13, 14].
- Prior history of proven or probable invasive mold disease was defined as evidence of IA within 1 year at study entry [11].
- High-dose corticosteroid treatment was defined as receipt of corticosteroids, at least 0.3 mg/kg/day of prednisolone equivalent for longer than 3 weeks [37].
- Hospital admission for high-risk chemotherapy was defined as receipt of chemotherapy for AML and conditioning chemotherapy for allogeneic HSCT [38].
- Prolonged neutropenia was defined as absolute neutrophil count of less than 500 cells/mm^3 for longer than 10 days within 30 days before admission or following chemotherapy [11].
- Lymphocytopenia and probable impaired lymphocyte function at the time of admission were defined as an absolute lymphocyte count of less than 1000 cells/mm^3 or CD4 count of less than 50 cells/mm^3, or any allogeneic HSCT patient receiving cyclosporine, tacrolimus, or anti-thymocyte globulin [11].
- Positive serum galactomannan was defined as an optical density ratio of at least 0.5 in two consecutive positive samples or at least 0.7 in a serum sample [39].
- Positive bronchoalveolar lavage (BAL) fluid galactomannan was defined as an optical density ratio at least 0.8 in a fluid sample [40].

Patients

We conducted this prospective observational study at Ramathibodi Hospital, Mahidol University, an academic tertiary care medical center in Bangkok, Thailand between January 2015 and December 2016. All Thai patient of all ages with moderate- to high-risk hematologic disease and no known diagnosis of ongoing IA were prospectively screened, and 357 patients were enrolled. Blood samples were obtained for genetic analyses. Patients were excluded if palliative care had been administered and the chemotherapy plan was discontinued. This study was conducted in accordance with the Declaration of Helsinki of 1964 and its later

amendments and was approved by the Institutional Review Board of Ramathibodi Hospital (approval no. MURA 2015/18). The committee approved the use of patient samples and data for the publication of this study. Written consent was obtained from all participants.

Data Collection

We retrieved information of patient demographics including age, sex, smoking history, medical condition, prior diagnosis of IA, diagnosis of hematologic disease, type, and intensity of immunosuppressive agents received, relevant clinical data during hospitalization, detection of cytomegalovirus (CMV) DNAemia or infection, medical ward assignment (high-efficiency particulate air filter equipped or non-high-efficiency particulate air filter equipped), type of antifungal prophylaxis if one was to be received, site of infection in individuals with IA. Galactomannan assay and radiological findings were recorded. Treatment outcome was also collected, to compare patients.

DNA Extraction and SNP Genotyping

A 5- to 10-mL sample of whole blood was obtained from each patient. Genomic DNA was extracted using the FavorPrep^{TM} Blood Genomic DNA extraction kit (Favorgen Biotech Corp., Taiwan) and stored at 4 °C. Samples were subsequently stored at − 20 °C for possible future analyses. In individuals who underwent stem cell transplantation, donor DNA was isolated from post-engraftment blood samples. The genotyping process was performed using the TaqMan SNP genotyping assay (Thermo Fisher Scientific, USA) according to the manufacturer's instructions in a ViiA^{TM} 7 system (Applied Biosystems, California, USA). Primer and probe sequences used in this study are shown in Table 1 in the supplementary material. Laboratory personnel were blinded to patients' history and conditions.

Serum and BAL fluid galactomannan were tested using a Platelia galactomannan assay (Bio-Rad Laboratories, California, USA), according to the manufacturer's instructions. Factors potentially associated with false positive galactomannan status were recorded and were carefully reviewed, namely concomitant use of piperacillin–tazobactam and amoxicillin–clavulanic acid, diagnoses of other fungal infection such as histoplasmosis, and certain types of dietary consumption [41–43].

Study Endpoint

The study endpoint was documentation of proven or probable IA, the need for second stem cell transplantation, death, or at 12 months after diagnosis of the hematologic disease. For patients with IA, the clinical outcome at 90 days after IA diagnosis was indicated.

Selection of Genetic Polymorphisms

A total of five SNPs in five genes of interest were selected for genotyping analyses in this study, namely rs4804800 in *DC-SIGN* [32], rs1800896 in *IL10* [24, 25], rs7309123 in *DECTIN-1* [32], rs4986790 in *TLR4* [26, 31, 33], and rs2305619 in *PTX3* [34], on the basis of previous clinical studies identifying important SNPs and the frequency of gene identification among Asian populations. The analysis was performed in all allele types for every gene of interest. According to the global Single Nucleotide Polymorphism Database (https://www.ncbi.nlm.nih.gov/SNP/) and accessed national data on ThaiSNP2 (https://www4a.biotec.or.th/thaisnp2/), the frequency of the five high-risk genotypes in Asian populations was 37.2%, 4.3%, 46.5%, 1.3%, and 44.2%, respectively. There was only one study from Asia by Seo et al. that reported an association of *IL10* polymorphisms with a higher risk of IA among Korean patients who underwent allogeneic HSCT [24].

Statistical Analysis

Genotypes and allele frequencies were calculated and compared between patients with and without IA using Pearson–chi-square and Fisher's exact tests. Odds ratios (ORs) and 95% confidence intervals (CIs) were assessed for the presence (homozygous or heterozygous) or

absence (homozygous wild-type allele) of the selected SNPs using logistic regression analysis. All polymorphisms were tested for the Hardy–Weinberg equilibrium to evaluate genetic variation and distribution patterns. Demographics in patients with and without IA were compared using a two-tailed *t* test or Mann–Whitney test for continuous data, and a chi-square test or Fisher's exact test for categorical data. Significant variables ($p < 0.05$) were included in multivariate Cox regression analysis. The log-rank test was used to compare the time to IA and survival time according to different baseline characteristics and polymorphisms of interest.

RESULTS

Between January 2015 and December 2016, a total of 410 patients were screened. Fifty-three patients were excluded from the study because they did not meet the criteria for moderate- to high-risk hematologic malignancy or had not received any treatment for such hematologic conditions at the time. The patients' characteristics are shown in Table 1. Among 357 patients, 188 were male patients (52.7%). The median age was 57 years old (interquartile range [IQR] 44.0–64.0). The most common hematologic disease was NHL, documented in 161/357 patients (45.1%), followed by AML and multiple myeloma (60/357, 16.8% each). The median age of patients with AML and allogeneic HSCT was 37 years (IQR 31.0–57.5 years). Twenty-eight patients among the total had diabetes mellitus, with median glycated hemoglobin (HbA1C) of 7.0% (IQR 6.0–7.5%). Five patients had poorly controlled diabetes with HbA1C at enrollment over 8.0%. Eight patients with NHL were co-infected with HIV. Only 25/357 patients (7.0%) received antifungal prophylaxis; among these, 3 patients were given itraconazole, 21 patients received fluconazole, and 1 patient received micafungin. No patients received more than one agent during their clinical course. However, complete data on prophylaxis were missing in 45 patients.

IA was diagnosed in 36/357 patients (10.1%), characterized as proven IA in 6 patients; the remainder were probable cases. The median time from enrollment to IA diagnosis was 56.5 days (IQR 26.0–150.5) days. IA was mostly diagnosed in patients with AML, followed by those with NHL receiving salvage therapy. No patients with poorly controlled diabetes were found to have developed IA (Table 1). The median age of patients with IA was 43.5 years (IQR 32.5–58.0 years). Thirty-three of 36 patients had single organ involvement; 30 patients had pulmonary involvement, and three patients had paranasal sinus infections. Three patients had multiple organ infections involving the respiratory and central nervous systems. *Aspergillus* species were identified in five patients, among which three patients were found to have *A. flavus,* one patient had combined *A. flavus* and *A. fumigatus* infection, and one patient had *A. terreus* infection. The 90-day IA-related mortality rate was 36.1% (13/36 patients). The median hospital length of stay among patients with IA was 34.0 days (IQR 25.5–58.5 days) compared with 16.0 days (IQR 7–27 days) in patients without IA ($p < 0.001$). There was no significant difference between probable and proven IA in terms of length of stay, duration of treatment, and survival. One patient each developed IA while taking fluconazole and itraconazole prophylaxis, but therapeutic drug monitoring was not performed in either patient when IA was diagnosed.

The genotypes of interest, $DC\text{-}SIGN_{rs4804800}$, $IL10_{rs1800896}$, $DECTIN1_{rs7309123}$, $TLR4_{rs4986790}$, and $PTX3_{rs2305619}$, were identified in 47 patients (14.0%), 3 patients (0.9%), 230 patients (68.2%), 0 (0%), and 141 patients (42.0%), respectively; Table 2 displays the alleles of interest according to proportion. Individuals carrying the C/C and T/C genotypes of the $IL10_{rs1800896}$ SNP had a higher incidence of IA diagnosis (adjusted OR 5.297; 95% CI 2.032–13.809, $p = 0.001$). The associated risk factors of IA are listed in Table 3. In the multivariate Cox proportional hazards model, only prolonged neutropenia and the C allele of $IL10_{rs1800896}$ SNP were independent factors contributing to IA development (HR 12.585; 95% CI 3.866–40.967, $p < 0.001$ and HR 2.449; 95% CI 1.097–5.468, $p = 0.029$, respectively). Figure 1 shows the cumulative risk of IA in

Table 1 Characteristics of patients

Characteristics	Patients, n = 357 (%)	No IA, n = 321 (%)	IA, n = 36 (%)	IA rate, %	P value
Median age (IQR)	57.0 (44.0–64.0)	57.0 (46.0–65.0)	43.5 (32.5–58.0)	–	0.001
Age, years					< 0.001
< 40	77 (21.6)	60 (18.7)	17 (47.2)	22.1	
41–60	147 (41.2)	135 (42.1)	12 (33.3)	8.1	
> 60	133 (37.3)	126 (39.3)	7 (19.4)	5.3	
Male gender	188 (52.7)	172 (53.6)	16 (44.4)	8.5 (male), 11.8 (female)	0.298
Underlying hematologic disease					< 0.001
Allogeneic HSCT	21 (5.9)	19 (5.9)	2 (5.6)	9.5	
Acute myeloid leukemia	60 (16.8)	40 (12.5)	20 (55.6)	33.3	
Acute lymphoblastic leukemia	28 (7.8)	24 (7.5)	4 (11.1)	14.3	
Chronic lymphocytic leukemia	9 (2.5)	8 (2.5)	1 (2.8)	11.1	
Non-Hodgkin's lymphoma	161 (45.1)	156 (48.6)	5 (13.9)	3.1	
Myelodysplastic syndrome	13 (3.6)	12 (3.7)	1 (2.8)	7.7	
Severe aplastic anemia	4 (1.1)	3 (0.9)	1 (2.8)	25.0	
Multiple myeloma	60 (16.8)	59 (18.4)	1 (2.8)	1.7	
Severe GVHD after HSCT	1 (0.3)	0 (0)	1 (2.8)	100.0	
Other underlying disease					
Diabetes mellitus	28 (14.8)	26 (17.0)	2 (5.6)	7.1	0.082
HIV	8 (2.3)	8 (2.5)	0 (0)	0	1.000

IA invasive aspergillosis, *IQR* interquartile range, *HSCT* hematopoietic stem cell transplant, *GVHD* graft versus host disease

association with individuals carrying C/C and T/C genotypes of $IL10_{rs1800896}$ SNP, who tended to develop IA earlier in their clinical course compared with other patients. A combination of the SNPs of interest did not increase the risk of IA development in this cohort. There was no significant association between the tested polymorphisms and galactomannan levels in either serum or BAL fluid.

All-cause 1-year mortality in this cohort was 14.8% (53/357 patients). Age over 60 years old, prolonged neutropenia, and uncontrolled malignancy were associated with increased all-cause mortality (HR 2.313, 95% CI 1.209–4.426,

Table 2 Association of invasive aspergillosis and selected polymorphisms

SNP	Genotype	No IA, *n* (%)	IA, *n* (%)	Adjusted OR (95% CI)[a]	*P* value
DC-SIGN$_{rs4804800}$	A/A	123 (41.1)	10 (30.3)	1.000	–
	A/G	135 (45.2)	17 (51.5)	1.499 (0.622–3.616)	0.367
	G/G	41 (13.7)	6 (18.2)	1.568 (0.485–5.069)	0.453
	G/G + A/G	176 (58.9)	23 (69.7)	1.516 (0.656–3.501)	0.330
IL10$_{rs1800896}$	T/T	263 (88.9)	24 (70.6)	1.000	–
	T/C	31 (10.5)	9 (26.5)	**5.517 (2.043–14.896)**	**0.001**
	C/C	2 (0.7)	1 (2.9)	3.538 (0.194–64.546)	0.394
	C/C + T/C	33 (11.1)	10 (29.4)	**5.297 (2.032–13.809)**	**0.001**
DECTIN1$_{rs7309123}$	C/C	8 (2.7)	2 (6.1)	1.000	–
	C/G	83 (28.0)	8 (24.2)	0.311 (0.046–2.098)	0.230
	G/G	205 (69.3)	23 (69.7)	0.449 (0.074–2.725)	0.384
	G/G + C/G	288 (97.3)	31 (93.9)	0.404 (0.068–2.404)	0.319
TLR4$_{rs4986790}$	A/A	284 (96.3)	33 (100.0)	1.000	–
	A/G	11 (3.7)	0 (0.0)	–	–
	G/G	0 (0.0)	0 (0.0)	–	–
PTX3$_{rs2305619}$	A/A	34 (11.4)	2 (5.9)	1.000	–
	A/G	140 (47.0)	15 (44.1)	1.130 (0.228–5.598)	0.881
	G/G	124 (41.6)	17 (50.0)	1.398 (0.283–6.909)	0.681
	G/G + A/G	264 (88.6)	32 (94.1)	1.253 (0.267–5.893)	0.775

SNP single nucleotide polymorphism, *IA* invasive aspergillosis, *OR* odds ratio, *DC-SIGN* dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin, *IL10* interleukin-10, *DECTIN1* dendritic cell-associated c-type lectin 1, *TLR4* toll like receptor 4, *PTX3* pentraxin-3

[a] Logistic regression analysis with model adjusted for age group and high-risk hematologic disease

$p = 0.003$; HR 3.240, 95% CI 1.663–6.312, $p = 0.001$; HR 2.661, 95% CI 1.039–6.813, $p = 0.041$, respectively). Individuals carrying the C allele on *IL10*$_{rs1800896}$ tended to have increased all-cause 1-year mortality (HR 2.275; 95% CI 1.166–4.441, $p = 0.016$) in the univariate Cox regression model, but this did not retain statistical significance in the multivariate Cox proportional hazards model. The presence of the other tested SNPs did not influence hospital length of stay and overall mortality, regardless of IA diagnosis. A complete list of associated risk factors affecting all-cause 1-year mortality is given in Table 4.

DISCUSSION

A variety of associated risk factors for IA have been identified, e.g., prolonged neutropenia with absolute neutrophil count of less than 500/mm^3 for longer than 10 days, uncontrolled hematologic malignancy, poorly controlled diabetes mellitus particularly among Asians, and the influence of genetic components in

Table 3 Associated risk factors of invasive aspergillosis in this cohort

Risk factors	No IA, *n* = 380 admission episodes (%)	IA, *n* = 36 admission episodes (%)	Univariate Cox regression HR (95% CI), *P* value	Multivariate Cox regression HR (95% CI), *P* value
Female	163 (42.9)	20 (55.6)	1.681 (0.871–3.245), 0.121	
Age > 40 years	272 (71.6)	19 (52.8)	**0.504 (0.262–0.970), 0.040**	0.956 (0.443–2.063), 0.909
At-risk occupation[a]	27 (8.8)	1 (2.8)	0.281 (0.038–2.053), 0.252	
Current smoking	12 (3.9)	4 (11.1)	2.309 (0.816–6.534), 0.115	
Prior invasive mold disease	7 (2.3)	1 (2.8)	1.177 (0.161–8.594), 0.873	
Diabetes mellitus	40 (12.7)	2 (5.6)	0.388 (0.093–1.615), 0.193	
Corticosteroids	72 (23.8)	2 (5.6)	**0.215 (0.052–0.894), 0.035**	0.424 (0.089–2.021), 0.282
Lymphocytopenia or anti-T cell use	52 (17.1)	5 (13.9)	0.813 (0.316–2.094), 0.668	
Prolonged neutropenia	37 (12.1)	26 (74.3)	**12.672 (5.930–27.081), < 0.001**	**12.585 (3.866–40.967), < 0.001**
Mucositis, grade 3–4	9 (3.0)	4 (11.1)	**3.068 (1.084–8.681), 0.035**	1.672 (0.460–6.086), 0.435
CMV DNAemia	13 (4.3)	3 (8.3)	1.675 (0.512–5.471), 0.393	
Admitted in non-HEPA room	168 (62.9)	25 (75.8)	1.951 (0.877–4.338), 0.101	
High-risk malignancy[b]	87 (23.0)	23 (65.7)	**5.001 (2.488–10.052), < 0.001**	1.074 (0.420–2.743), 0.882
Uncontrolled malignancy[c]	213 (69.2)	31 (86.1)	**2.986 (1.160–7.688), 0.023**	2.835 (0.813–9.892), 0.102
High-risk chemotherapy[d]	156 (49.4)	27 (75.0)	**2.453 (1.153–5.219), 0.020**	0.609 (0.237–1.569), 0.305
Antifungal prophylaxis	34 (11.2)	2 (5.6)	0.474 (0.114–1.973), 0.305	
DC-SIGN$_{rs4804800}$ G/G genotype	48 (13.4)	6 (18.2)	1.445 (0.596–3.500), 0.415	
DC-SIGN$_{rs4804800}$ G carriage	195 (54.5)	23 (69.7)	1.761 (0.838–3.699), 0.135	
IL10$_{rs1800896}$ C/C genotype	0 (0)	1 (2.9)	–	

Table 3 continued

Risk factors	No IA, *n* = 380 admission episodes (%)	IA, *n* = 36 admission episodes (%)	Univariate Cox regression HR (95% CI), *P* value	Multivariate Cox regression HR (95% CI), *P* value
IL10$_{rs1800896}$ C carriage	36 (10.1)	10 (29.4)	**3.436 (1.641–7.196), 0.001**	**2.449 (1.097–5.468), 0.029**

IA invasive aspergillosis, *HR* hazard ratio, *CMV* cytomegalovirus, *HEPA* high efficiency particulate air filter, *DC-SIGN* dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin, *IL10* interleukin 10, *DECTIN1* dendritic cell-associated c-type lectin 1, *TLR4* Toll-like receptor 4, *PTX3* pentraxin-3

[a] Patient works as a farmer, mason, carpenter/construction, or has outdoor work with likely spore exposures

[b] High-risk malignancy was defined as diagnosis of acute myeloid leukemia or allogeneic HSCT

[c] Uncontrolled malignancy was defined as malignancy status that was not in partial or complete remission

[d] High-risk chemotherapy was defined as chemotherapy for AML and conditioning chemotherapy for allogeneic HSCT

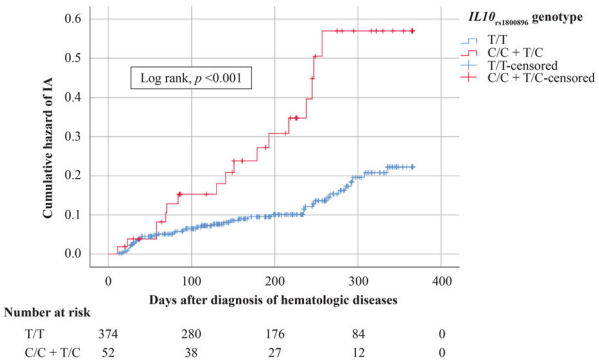

Fig. 1 Kaplan–Meier analysis shows association of *IL10*$_{rs1800896}$ genotype and cumulative risk of IA

patients at risk, according to various clinical studies [44–46]. In this study, most patients at risk of having IA were individuals with AML because they tended to have prolonged neutropenia following chemotherapy, with a prevalence rate of IA 33.3%. Most of these patients were diagnosed with AML and IA at a young age. Despite the high prevalence rates of IA among patients with ALL, CLL, and severe AA, these were not considered to represent a large proportion of our study population.

Prior history of invasive mold infection and lymphopenia have been reported to be independent risk factors of IA according to Stanzani et al. [11]. However, neither factors were good predictors for IA development in this study. This was potentially owing to a low prevalence of previous IA diagnosis in three patients. Uncontrolled diabetes mellitus has been reported more frequently as a comorbid condition in IA; more recently, a study in five Asian countries emphasized this unique associated risk factor [46]. The precise mechanism is still unknown, but one possibility is impaired neutrophil function causing compromised oxidative and non-oxidative killing mechanisms, which are crucial for hyphal and germinating conidia control [14, 47]. However, diabetes mellitus was not a significant contributing factor to IA in this cohort. Garcia-Vidal et al. described that CMV infection has a strong relationship with invasive fungal infection

Table 4 Risk factors of all-cause one-year mortality

Risk factors	Survived, n = 363 admission episodes (%)	Dead, n = 53 admission episodes (%)	Univariate Cox regression HR (95% CI), P value	Multivariate Cox regression HR (95% CI), P value
Female	159 (43.8)	24 (45.3)	1.110 (0.647–1.907), 0.704	
> 60 years old	100 (27.5)	24 (45.3)	**1.972 (1.148–3.388), 0.014**	**2.313 (1.209–4.426), 0.011**
At risk occupation[a]	25 (8.5)	3 (6.4)	0.667 (0.207–2.151), 0.498	
Current smoking	11 (3.7)	5 (10.4)	2.153 (0.852–5.437), 0.105	
Prior invasive mold disease	7 (2.3)	1 (2.1)	0.933 (0.129–6.768), 0.945	
Diabetes	34 (11.2)	8 (16.3)	1.264 (0.592–2.701), 0.545	
Corticosteroids	66 (22.5)	8 (17.4)	0.763 (0.356–1.636), 0.487	
Lymphocytopenia or anti-T cell use	44 (15.1)	13 (27.1)	1.859 (0.982–3.520), 0.057	
Prolonged neutropenia	44 (15.1)	19 (39.6)	**2.904 (1.626–5.187), < 0.001**	**3.240 (1.663–6.312), 0.001**
Mucositis, grade 3–4	9 (3.1)	4 (8.3)	2.335 (0.839–6.501), 0.105	
CMV infection	10 (3.4)	6 (12.5)	**2.624 (1.113–6.187), 0.028**	2.077 (0.777–5.552), 0.145
Admitted in non-HEPA room	164 (64.3)	29 (64.4)	1.125 (0.609–2.076), 0.707	
High-risk malignancy[b]	91 (25.2)	19 (35.8)	1.460 (0.833–2.561), 0.186	
Uncontrolled malignancy[c]	202 (68.2)	42 (87.5)	**3.374 (1.433–7.944), 0.005**	**2.661 (1.039–6.813), 0.041**
High-risk chemotherapy[d]	63 (21.1)	14 (29.2)	0.879 (0.499–1.550), 0.656	
Antifungal prophylaxis	35 (11.9)	1 (2.1)	0.178 (0.025–1.290), 0.088	
DC-SIGN$_{rs4804800}$ G/G genotype	49 (14.4)	5 (9.8)	0.718 (0.285–1.808), 0.482	

Table 4 continued

Risk factors	Survived, *n* = 363 admission episodes (%)	Dead, *n* = 53 admission episodes (%)	Univariate Cox regression HR (95% CI), *P* value	Multivariate Cox regression HR (95% CI), *P* value
DC-SIGN$_{rs4804800}$ G carriage	186 (54.7)	32 (62.7)	1.277 (0.724–2.253), 0.399	
IL10$_{rs1800896}$ C/C genotype	0 (0)	1 (2.0)	–	–
IL10$_{rs1800896}$ C carriage	35 (10.4)	11 (21.6)	**2.275 (1.166–4.441), 0.016**	1.770 (0.881–3.554), 0.109

HR hazard ratio, *CMV* cytomegalovirus, *HEPA* high efficiency particulate air filter, *DC-SIGN* dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin, *IL10* interleukin-10, *DECTIN1* dendritic cell-associated c-type lectin 1, *TLR4* Toll-like receptor 4, *PTX3* pentraxin-3

[a] Patient works as a farmer, mason, carpenter/construction, or has outdoor work with likely spore exposures

[b] High-risk malignancy defined as diagnosis of acute myeloid leukemia or allogeneic HSCT

[c] Uncontrolled malignancy defined as malignancy status that is not in partial or complete remission

[d] High-risk chemotherapy was defined as chemotherapy for AML and conditioning chemotherapy for allogeneic HSCT

diagnosis, especially in individuals with lymphoproliferative malignancies [48]. The exact mechanism to illustrate this finding is not fully understood but is potentially owing to the immunomodulatory effects of CMV itself [49]. However, CMV DNAemia did not predict an increased IA risk in this cohort, likely secondary to the low prevalence of CMV DNAemia (14 patients). Therefore, an impact of CMV was not noted in this study. A higher-risk trend of IA development was observed among individuals with high-risk malignancy, uncontrolled malignancy, and receipt of high-risk chemotherapy. Nevertheless, none of these showed significant effects in multivariate Cox regression. Among all well-established risk factors mentioned, the only two associated factors that remained significant regarding increased IA risk were prolonged neutropenia and *IL10*$_{rs1800896}$ C carriage (Table 3).

Genetic predisposition to IA has been increasingly studied over the past decade, mostly in North America and Europe. Some studies have demonstrated an increased risk of IA with the presence of polymorphisms of *DC-SIGN*, *DECTIN1*, *IL10*, *TLR4*, and *PTX3* [24–26, 32, 34], given that the corresponding proteins have been discovered to be involved in IA pathogenesis. Moreover, some SNPs have been identified to be associated with elevated serum galactomannan levels, without any significant correlation to IA development [32]. IA has long been documented to be a fatal infectious complication among immunocompromised patients, particularly hematology patients [50]. Data on the epidemiology of IA in Thailand are limited, with only a few retrospective studies conducted but none in hematology patients [8, 48]. In the modern medicine era, antifungal prophylaxis has been prescribed widely in at-risk patients, to minimize invasive fungal infection occurrence. This schematic approach is considered a high-cost treatment and generally applies in high-income countries only. Therefore, applying a personalized medicine strategy by screening genetic predisposition to IA may be worthwhile, to identify patients truly in need of antifungal prophylaxis.

Among our study population, the prevalence rate of IA was highest in individuals with AML, followed by those with severe AA, ALL, CLL, and allogeneic HSCT recipients. Patients with

AML accounted for 16.8% of all patients, second only to those with NHL. This reflects that patients with AML undergoing chemotherapy may be an appropriate group in which to consider antifungal prophylaxis when the cost of the antifungal agent is a concern. However, prescribing universal antifungal prophylaxis to all patients with AML undergoing chemotherapy may not be a feasible approach in low-income countries.

Regarding human immune response, macrophages and dendritic cells are responsible for the initial step in pathogen recognition, particularly fungi including *Aspergillus* spp., via various types of receptors on their surface, e.g., DC-SIGN, TLR-4, PTX-3, and so on. A complex interplay between immune cells is then triggered to control the invading microorganisms. Hence, any change in these genes could impair normal host immune response, leading to devastating fungal infection [21, 51]. Several genetic polymorphisms have been found to be associated with increased risk for IA, e.g., *DECTIN- 1*, *DC-SIGN*, *TLR4*, *IL10*, *S100B*, *PTX3*, *PLG*, *IFNG*, and *TNFR1* [15, 35, 52]. However, these proteins have mainly been identified in North America and Europe, except for a single study in South Korea [24]. On the basis of national data of ThaiSNP2 (https://www.4a.biotec.or.th/thaisnp2/) and the global Single Nucleotide Polymorphism Database (https://www.ncbi.nlm.nih.gov/SNP/), only five SNPs of interest in this study have been identified in the Thai population.

In this study, we identified a strong association between the C allele of $IL10_{rs1800896}$ and increased risk of IA, regardless of a diagnosis of hematologic disease; the same was not true for the other tested SNPs. The influence of this finding is second only to a well-known risk factor, namely prolonged neutropenia affecting IA development in moderate- to high-risk patients, despite the fact that there was no trend of increased mortality among individuals carrying such an SNP. Carrying the $IL10_{rs1800896}$ C/C genotype, however, showed less effect on IA diagnosis in comparison with other genotypes. This finding may be explained by the relatively small number of individuals with a homozygous C genotype. The overall mortality of IA in our study was 14.8%; this was relatively lower than in previous studies and had no significant correlation with any tested SNPs [2, 3, 6].

Among 21 cases of HSCT in our study, only two patients developed IA. This prevalence rate was much lower than AML and severe AA [53]. One reason that could explain this relatively low prevalence could be the stringent pre-transplant scheme; complete remission of patients' hematologic condition at transplant may reduce the risk of IA development at our institution.

The limitations of this study are as follows. (1) This was a single-center study, and therefore the data may not represent the characteristics of the entire region; (2) missing data in some patients made a complete genetic analysis impossible; (3) there was a lack of data on environmental exposure after hospitalization, which may affect IA development. The strength of this study is that we included a relatively large number of participants and gained new insight into IA development among hematology patients in Thailand. Genetic surveillance of patients at risk, particularly those with AML, and considering antifungal prophylaxis with $IL10_{rs1800896}$ C carriage is recommended, especially when universal antifungal prophylaxis is not an option. Finally, a multicenter study is encouraged, to further clarify the effect of genetics on IA susceptibility.

CONCLUSION

Apart from traditional clinical risk factors, polymorphisms of immunoregulatory gene $IL10_{rs1800896}$ may be an important risk factor to consider in risk stratification of IA development, particularly in patients with AML. This could potentially be a new strategy in hematology patients to lessen the risk of IA, especially in a resource-limited setting where universal antifungal prophylaxis is not possible in real-life practice.

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Compliance with Ethics Guidelines. This study was conducted in accordance with the Declaration of Helsinki of 1964 and its later amendments and was approved by the Institutional Review Board of Ramathibodi Hospital (approval no. MURA 2015/18). The committee approved the use of patient samples and data for the publication of this study. Written consent was obtained from all participants.

Data Availability. The datasets generated during and/or analyzed in the current study are not publicly available but are available from the corresponding author on reasonable request.

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