Mediterranean Journal of Hematology and Infectious Diseases

Original Article

T-lymphocytes Expression of Toll-like Receptors 2 and 4 in Acute Myeloid Leukemia Patients with Invasive Fungal Infections

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Competing interests: The authors declare no conflict of Interest.

Abstract. ***Background:*** **Invasive fungal infections (IFIs) are important cause of mortality in acute myeloid leukemia (AML) patients on treatment with intensive induction chemotherapy. Toll-like receptors, mainly Toll-like receptors 2 and 4 (TLR2 and TLR4), play a considerable role in the host defense against microorganisms. The involvement of TLR signaling in modulation of innate immune response is extensively discussed, but the TLR expressions profiling on adaptive immune cells are not specified. Also, the expressions of TLRs and their association with the occurrence of IFIs in patients with AML are not studied. So, the novel aim of this study was to investigate the associations between the T-lymphocyte expression of TLR2 and TLR4 and the occurrence of IFIs in AML patients treated with intensive induction chemotherapy.**
Materials and Methods: **One hundred twenty two newly diagnosed AML patients were evaluated. The laboratory diagnostic techniques for IFIs include culture, microscopic examination, histopathology, galactomannan assay and PCR. The expressions of TLR2 and TLR4 were analyzed by flow cytometry. The Control group included 20 age and sex-matched individuals.**
Results: **There was a significant increase in the expression of TLR4 in AML patients with IFI compared to healthy controls (p = 0.001). TLR2 and TLR4 expressions increased significantly in AML patients with mixed fungal and bacterial infection compared to healthy controls (p= 0.002 and p=0.001, respectively).**
Conclusion: TLRs expressions could be important biological markers for the occurrence of IFI in non-M3 AML patients after intensive induction chemotherapy.

Keywords: Invasive Fungal Infection, TLR2, TLR4, Acute Myeloid Leukemia.

Citation: Abdel Hammed M.R., Elgendy S.G., El-Mokhtar M.A., Sayed D., Mansour S.M., Darwish A.M. T-lymphocytes expression of Toll-like receptors 2 and 4 in acute myeloid leukemia patients with invasive fungal infections. Mediterr J Hematol Infect Dis 2022, 14(1): e2022022, DOI: http://dx.doi.org/10.4084/MJHID.2022.022

Published: March 1, 2022 **Received: October 29, 2021** **Accepted: February 8, 2022**

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Introduction. Acute myeloid leukemia (AML) represents the hematologic malignancy with the highest risk of invasive fungal infections (IFIs). The overall mortality rate in AML patients due to fungal infections was improved in recent years to 20%-30%.[1] IFIs represent a considerable clinical problem due to the high costs of the prophylaxis and treatment of fungal infections in limited resource localities.[2]

Multiple risk factors can predispose AML patients to develop fungal infections including old ages, pulmonary comorbidities, duration of neutropenia, relapse/refractory disease, intense chemotherapy, and a high dose of steroids.[3]

The Infectious Diseases Working Party of the German Society of Hematology and Medical Oncology (AGIHO) postulates that prolonged severe neutropenia in AML patients (<500 cells/μL of at least 8 days) post-induction/consolidation chemotherapy or allogeneic stem cell transplantation are considered as individuals at high-risk for IFI.[4]

Diagnosis of IFI is challenging, particularly in AML patients as symptoms can be absent or subtle. Fever may be the only sign. Thrombocytopenia and coagulopathy due to the underlying cause and chemotherapy may impair the ability to tissue biopsy which is the preferred method for diagnosis establishment.[5] The European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC) and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG) defining IFI as proven, probable, and possible infections.[5,6]

Recognition of fungi by immune cells is mediated through pattern recognition receptors (PRRs); like Toll-like receptors (TLRs) and C-type lectins (CLRs). Binding of fungal pathogen-associated molecular patterns (PAMPs) to PRRs triggers phagocytes to the infection site, microbial killing, and dendritic cells (DCs) activation.[7,8]

Toll-like receptors are widely expressed on myeloid cells of innate immune system, such as macrophages, DCs.[9] TLR signaling in DCs triggers a maturation program that increases their ability to prime naïve T cells through up-regulation of MHC and co-stimulatory molecules and expression of pro-inflammatory cytokines, such as TNF-α, IL-1, and IL-6.[10]

TLRs have been considered traditionally to play an important role in the innate immune system. However, other few studies have found that TLRs are also expressed on various adaptive immune cells, such as B cells,[11] CD4+ and CD8+ T cells,[12] and the CD4+CD25+ regulatory T cell population.[13] Two studies, sorted CD4+CD45high T cells from C57/BL6 (B6) mice were found to express TLR1, 2, 3, 6, 7 and 8, but low levels of TLR 4, 5 and 9 mRNA.[14] Naïve CD8+ T cells from B6 mice were reported to express mRNA for TLR1, 2, 6 and 9 but not TLR4.[15] Naïve CD4+ T cells from BALB/c mice express mRNA for TLR3, 4, 5 and 9.[16] Thus, the involvement of TLR signaling in modulation of immune response is not limited to innate immune cells, but also modulate cellular and humoral adaptive immunity. TLR2 and TLR4 are two of the most studied TLRs to have an important role in the recognition of both bacterial and fungal pathogens.[17] So, we have focused on the associations between T-lymphocyte expression of TLR2 and TLR4 and the occurrence of IFIs in AML patients which remains unclear.

Materials and Methods.

Ethics Statement. This study was approved by the Regional Ethical Committee in South Egypt Cancer Institute (SECI), Assiut University, in accordance with the provisions of the Declaration of Helsinki. Informed written consent obtained from all participants before enrolment.

Study Design and Setting. This study was performed at Clinical Hematology Unit, Internal Medicine Department, Assiut University Hospital, and South Egypt Cancer Institute (SECI), Assiut University, Egypt. All newly diagnosed AML patients (aged more than 18 years old), admitted in the duration from October 2017 to July 2020 were enroll in this study. The diagnosis was performed according to the WHO criteria for AML.[18] The intensive induction chemotherapy was (idarubicin 12 mg/m^2 per day for 2–3 days, and cytarabine 100 mg/m^2/day for 5–7 days). Patients received prophylactic treatment during the period of neutropenia following chemotherapy (sulfamethoxazole 400 mg/ trimethoprim 80 mg once or twice daily). Patients receiving antifungal prophylaxis or preexisting antifungal treatment were excluded. Also, AML with antecedent hematologic malignancies like Myelodysplastic syndrome, and Myeloproliferative neoplasms, AML M3, relapsed AML patients and chemotherapy courses with low-intensity regimen were excluded. Baseline demographic and clinical data, type of AML, chemotherapy courses, duration of febrile neutropenia, complete blood cell count, cytogenetic risks, radiological examination" high-resolution chest computed tomography (CT)", IFI incidence, site of fungal infection, and patients outcome were recorded. Twenty age and sex-matched healthy individuals were the control group in this study.

Diagnosis of Invasive Fungal Infection. Diagnosis of IFI was applied according to 2008 consensus criteria of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC) and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG), which classified IFI into possible, probable, or proven IFI.[5] Proven IFI requires that a fungus be detected by

culture or pathohistological blood analysis in a sterile site sample. Probable IFI requires lesions on imaging indicative of fungal infection and mycological evidence, not only culture and pathohistological analysis of a sample but also indirect tests, such as galactomannan. Possible IFI only requires imaging lesions indicating fungal infection without presence mycological evidence.

Neutropenia was defined as a neutrophil count <500 cells/ μL.[19] The duration of neutropenia in each course of chemotherapy was collected. When patients remain febrile neutropenic >72 hrs after antibacterial agent, a thorough history and physical examination were recorded, along with culture for blood and other potentially infectious focuses including oral mucositis grade III or IV, or lower respiratory tract infection (LRTI). For patients with no identified focuses, high resolution computed tomography (CT) was performed, together with galactomannan (GM) assay and PCR. Broncho-alveolar lavage (BAL) was not performed routinely. Fluconazole 400 mg IV/day was given if IFI were suspected with the CT findings, positive galactomannan or PCR assays, or other clinical evidence.

Sample Collection and Processing. Blood, oral swabs, and sputum samples were collected from the patients according to their clinical presentation and different localizing signs and symptoms before the initiation of antifungal therapy.

Identification of Candida spp. Blood cultures were done by adding 5-10 mL blood to 50-100 mL Sabouraud dextrose broth (Himedia, India) and incubated at 37ºC for 10 days with subculture every other day.[20] Oral swabs and sputum samples were cultivated on Sabouraud dextrose agar (Himedia, India) with chloramphenicol (16 mg/mL). The isolates were further identified by colony morphology on CHROMagar® Candida medium (CHROMagar, Paris, France), germ tube test, chlamydospores on Tween 80 cornmeal agar (Difco) and growth at 45°C.[21]

Identification of Mold. Direct microscopic examination is performed on a fresh sample between a glass slide and coverslip. The morphological characteristics of *Aspergillus spp* are the presence of hyphae (hyaline and septate) with dichotomous branches at 45°angles and with uniform width (3–6 μm). However, it is hard to distinguish the species of *Aspergillus* because of the difficulty in distinguishing the morphology of the different fungi species. *Aspergillus spp* were cultivated on Sabouraud's dextrose-agar at 37 °C for 2 to 5 days. Fungi that grew in culture were identified according to morphological and microscopic criteria and Roth's flag technique.[22,23] In addition, Patient sera were tested for galactomannan (GM) by Galactomannan ELISA kits according to the manufacturer instructions[24] (Bio-Rad, Hercules, CA). The presence of bacterial infections was tested by VITEK® 2 system.

DNA Extraction and PCR Amplification. DNA extraction was performed using a commercial kit (QIAamp DNA Mini Kit (Qiagen, Germany)) according to the manufacturer's instructions. PCR was performed utilizing the fungus specific, universal primer pair ITS1 (′5TCCGTAGGTGAACCTGCGG3′) which hybridizes at the 3′ end of 18S rDNA and ITS4 (′5TCC TCC GCTTATGATAT GC3′) which hybridizes at the 5′end of 28S rDNA (Sigma, USA).[25] The concentration was measured by a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). The PCR reaction mix contained 0.5 μM of each primer, 10 μM deoxynucleotides, 1.5 mM MgCl2 and 1 x buffer (Promega). One unit of the Taq Polymerase (Promega) was added to each tube. DNA amplification was carried out in a Gene Amp9600 thermal cycler under the following conditions: 35 cycles of denaturing at 94°C for 1 min; annealing at 55.5°C for 2 min and extension at 72°C for 2 min; and final extension at 72°for 10 min.[25,26] PCR products were visualized by electrophoresis on a 1% agarose gel stained with ethidium bromide.

Flow Cytometry. Whole blood samples (anticoagulated with EDTA) were collected from enrolled individuals and stained with the following antibodies (all from BD bioscience, USA); Alexa fluor 488-conjugated anti-CD282 for detection of TLR2, PE-conjugated anti-CD284|MD-2 complex for detection of TLR4, PerCP-conjugated anti-HLA-DR, and APC-conjugated anti-CD3. RBCs were lysed with the lysis buffer, then at least 10.000 events were acquired and analyzed by FACS Caliber flow cytometer (BD bioscience, USA). Appropriate isotype-matched controls were included in the experiments to identify positive populations.[27] Data was analyzed with cell Quest software (BD bioscience, USA), (**Figure 1**).

Statistical Data. Descriptive results of continuous variables were expressed as mean±SE for non-parametric variables and as mean±SD for parametric variables. Comparison of the demographic characteristics between cases and control was calculated using the chi-square test for categorical data and independent sample t-test for numerical variables. Qualitative variables were expressed as the number of positive cases (%). Differences in mean values of TLR2 and TLR4 level of expression between different groups were calculated using the Mann-Whitney test. *P*-value was considered significant at < 0.05. Statistical calculation was performed with the statistical package for social science software (SPSS version 16.0 Inc, Chicago, III).

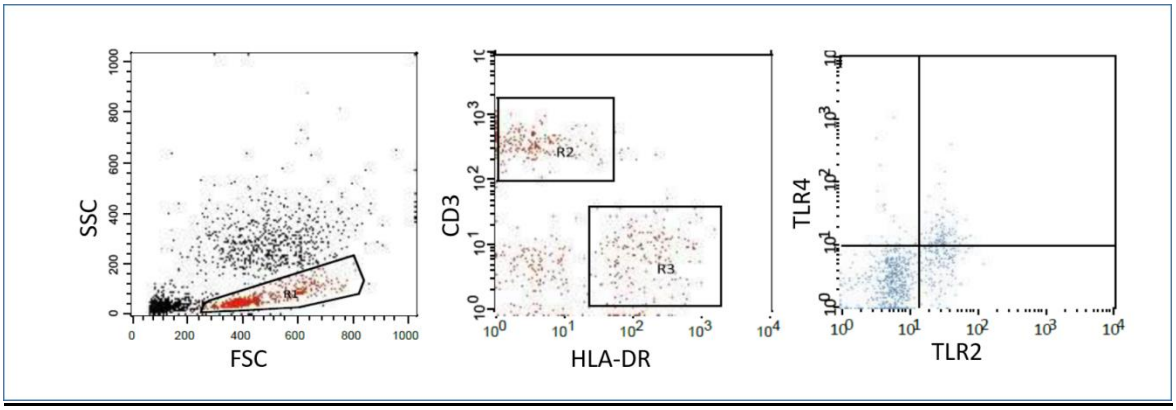

Figure 1. Representative gating strategy used to detect the TLR2+ and TLR4+ T cells. Blood samples were stained with PerCP-conjugated anti-HLA-DR, APC-conjugated anti-CD3, Alexa fluor 488-conjugated anti-CD282 (TLR2), and PE-conjugated anti CD284 (TLR4). Lymphocytes were selected (R1) and then gated on CD3+ cells (R2) and HLA-DR+ cells (R3). R2 was further analyzed for TLR2 and TLR4 expression.

Results. From 2017 to 2020, 122 newly diagnosed non-M3 AML patients (aged more than 18) who received induction chemotherapy were admitted to Clinical Hematology Unit, Internal Medicine Department, Assiut University Hospital, South Egypt Cancer Institute (SECI). Forty patients (32.78%) developed IFIs. The demographic and clinical characteristics of these 40 non-M3 AML patients with IFIs were presented in Table 1. The median age was 38.8 years (range, 18–65 years); male patients were 27 (67.5%). The diagnosis was applied according to the WHO criteria for AML. There were mainly AML4, AML1 and AML2 (30 %, 25% and 22.5% respectively). Eleven (27.5%) patients were favorable-cytogenetic group, 9 (22.5%) poor group, and 20 (50%) the intermediate- risk group. No significant differences were found between TLR4 or TLR2 expressions and age, sex, Type of AML, cytogenetic risk and Patient's outcome ($P > 0.05$).

The most common sites of infection were the lower respiratory tract 47.5% (19/40), and oral mucosa (mucositis grade II or IV) 37.5% (15/40). Mixed infection sites (bloodstream, oral, and LRTI) were detected only in 15% (6/40), **Table 1**.

The fungal pathogens among the 40 AML patients was identified as 2 (5%) proven, 28 (70%) probable, and 10 (25%) possible IFIs. The pure fungal growth was observed in 24 patients, whereas mixed bacterial and fungal growth was encountered in 16 patients. *Candida* species was the most encountered fungi. It was present in 21 specimens (2 specimens were mixed *candida* and mold pathogen) followed by *Aspargillus* in 19 specimens then *penicillum* in 2 specimen, **Table 2**.

TLR2 expression in AML patients with IFIs in comparison to healthy controls showed no significant difference (p = 0.659), while there was a significant increase in the expression of TLR4 in AML patients with IFI compared to healthy controls (p = 0.001). TLR2 and TLR4 expression in AML patients with no IFI in comparison to healthy controls had no significant difference (p = 0.72, 0.69 respectively), **Table 3**.

Moreover, we observed that TLR2 expression increased significantly in AML patients with mixed fungal and bacterial infections compared to healthy controls (p= 0.002). Also, TLR4 expression in AML patients with mixed fungal and bacterial infection was significantly increased (p=0.001), **Table 4**.

Discussion. This is the first study about T-lymphocytes expressions of TLRs and the development of IFIs in AML patients receiving induction chemotherapy in Assiut University Hospitals, and up to our knowledge in Egypt. We reported that the overall incidence of IFIs in AML patients is 40/122 (32.78%), this incidence is considered high in comparison with other reports from different countries.[28-30] In these reports, the incidences of IFIs in AML patients varied from 4.0% to 48.4%. This variation is due to differences in patient populations, chemotherapy regimens, antifungal prophylaxis, and geographic variation. Recent study reported that (29%) of AML patients developed an IFI. Patients with AML remain at risk for IFI despite the use of several different antifungal agents for prophylaxis.[31]

The high incidence in our study can be explained by many factors, including limited health resources, the lack of routinely administered anti-fungal prophylaxis, and environmental factors such as high temperature, which facilitates fungal growth. This high incidence of IFIs should start a new cost-effectiveness consideration about the requirement of anti-fungal prophylaxis in AML patients with induction chemotherapy. Hagiwara et al.[32] reported that AML in developing countries with limited health resources, favors the health authorities to use their low budget preferentially in another illness that has a higher incidence and a better chance for achievement of higher social impact.

The fungal pathogens among the 40 AML patients was identified as 2 (5%) proven, 28 (70%) probable, and 10 (25%) possible IFIs. Tang et al.[33] reported that the incidence of all-category IFIs was 34.6% (5.7% proven IFIs, 5.0% probable IFIs and 23.8% possible IFIs). Nucci

Table 1. Demographic and clinical characterization of the AML patients with IFI

Characters	Category	Number (%)
All Patients		40 (100%)
Age in years		
Mean ± SD	38.8 ± 14.4	
Range	18-65	
Gender	Female	13 (32.5%)
	Male	27 (67.5%)
Type of AML		
	AML0	4 (10%)
	AML1	10 (25.0)
	AML2	9 (22.5%)
	AML4	12 (30.0%)
	AML5	2 (5.0%)
	AML6	2 (5.0%)
	AML7	1 (2.5%)
Cytogenetic risk	Favorable	11 (27.5%)
	Intermediate	20 (50%)
	Poor	9 (22.5%)
Clinical infection site		
	Oral mucositis grade II or IV	15 (37.5%)
	LRTI	19 (47.5%)
	Mixed infection site (Bloodstream, oral & LRTI)	6 (15%)
High resolution computed tomography (C.T.)*		
	Positive CT chest finding	16/37 (43.2%)
	Negative CT chest finding	21/37 (56.7%)
Neutrophil count		
	500 -100 neutrophil	23 (57.5%)
	< 100 neutrophil	17 (42.5%)
Patients Outcome		
	Alive	26 (65%)
	Died	14 (35%)
EROTEC/MSG Classification	Proven	2 (5 %)
	Probable	28 (70%)
	Possible	10 (25%)

* 37 patients of the studied cases underwent chest C.T. scan. LRTI; lower respiratory tract infection Qualitative data were expressed as (%).

Table 2. Identified fungal pathogens* among the 40 AML patients.

Fungal species No (%)
Overall 42 (100 %)
Yeast 21 (50 %)
Candida albicans 13
Candida tropicalis 5
Candida parapsilosis 3
Mold 21 (50 %)
Aspergillus spp. 19
Penicillium 2

*Two cases had more than one species of fungal infection

et al.[34] report a Brazilian incidence of (18.7%) for proven/probable IFIs in AML patients after diagnosis. Kim et al.[19] reported (9.6%) with 20 IFI diagnosed following HMA (three proven, four probable, 13 possible).

In our study the pure fungal growth was observed in 24 (60%) patients, whereas mixed bacterial and fungal growth was encountered in 16 (40%) patients. *Candida* species was the most encountered fungi. It was present in 21(50%) specimens (including 2 specimens were mixed *candida* and mold pathogen) followed by *Aspargillus* in 19 (45.2 %) specimens then *penicillum* in 2 (4.8%) specimens. This result was different from Tang et al.[33] who reported that *Candida spp* still predominated and almost twice as common as *Aspergillus spp*. The reasons for this difference are mostly due to difference in number of patients enrolled, different specimen types and the absence of anti-yeast azole prophylaxis.

The reports in Egypt are very limited; an Egyptian study conducted on high-risk pediatric cancer patients by EL-mahallawy et al.[35] reported that yeast was isolated in (78.6%) of specimen and molds in (21.11%). Among yeasts, *Candida* was the commonest, while the most encountered molds were *Aspergillus spp.* They found that polymicrobial (mixed bacterial and fungal growth) was encountered in 62.5% of specimen, which is in great accordance with our results.

In this study, *non-albicans Candida spp*. (*C. tropicalis and C. parapsilosis*) were common 8/21 (38.1%) as *C. albicans* 13/21 (61.9%). Another study with similar findings postulated that neutropenia is correlated with *non-albican Candida* infections.[36]

Table 3. TLR2 and TLR4 expression level in comparison between patients and control group.

Variable	AML Patients with IFI No (40)	AML patients with no IFI No (20)	Control group No (20)
TLR2%			
Mean ±SE	17.09± 2.31	18.01± 0.91	19.16 ± 0.96
***p*-value**	*P*=0.659	*P*=0.72	
TLR4%			
Mean ±SE	23.18± 2.42	3.71± 0.23	4.86 ±0.37
***p*-value**	*P*=0.001**	*P*=0.69	

Quantitative variables are expressed as Mean Age ± standard error. No: number. T.L.R.: Toll-like receptor. Mann-Whitney Test, **highly significant difference ($p < 0.005$).

Table 4. TLR2 and TLR4 expression level in comparison between AML patients with mixed fungal and bacterial infection and healthy controls.

Variable	AML patients with mixed fungal and bacterial infections No(16)	Healthy controls No(20)
TLR2%		
Mean ±SE	34.47±3.92	19.12±0.9
***p*-value**	*P*=0.002**	
TLR4%		
Mean ±SE	23.57±2.5	4.9±0.4
***p*-value**	*P*=0.001**	

Quantitative variables are expressed as Mean ± standard error. No: number. TLR2: Toll-like receptor 2. TLR4: Toll-like receptor 4. **highly significant difference ($p < 0.005$).

An Egyptian study reported that 75 (44.1%) *Candida spp* (25 (33.3%) *non-albicans Candida spp* and 50 (66.6%) *C. albicans*) were isolated from AML patients on induction chemotherapy.[37] The common site of IFI was the lower respiratory tracts (47.5%, 19/40), and oral mucosa (mucositis grade II or IV) (37.5%, 15/40) followed by mixed infection sites (bloodstream, oral and LRTI) (15%, 6/40). Pagano et al.[38] and Tang et al.[33] reported that lower respiratory tract was the most common site for IFIs (80% and 75.4% respectively); also EL-mahallawy et al.[35] and Kurosawa et al.[30] found an incidence of (35.7% and 55.3% respectively) for IFIs affecting the lung. Few articles evaluated the risk factors of IFIs in AML patients during induction chemotherapy. In this study, we have determined these risk factors as standard induction chemotherapy, febrile neutropenia, elderly and male gender. Tang et al.[35] postulated similar risk factors including standard induction chemotherapy, younger than 40 or older than 60 years, and a poor chemotherapy response for all-category IFIs. Neofytos et al.[39] postulated that mucositis and organ dysfunctions are important risk factors for invasive candidiasis during induction chemotherapy, and male gender is the only risk factor for mold infection. Hammond et al. [29] also reported male gender as risk factors for IFIs. Chen et al.[40] stated that AML patients have multiple risk factors for developing invasive fungal diseases, such a including advanced age, prolonged and profound neutropenia, the presence of indwelling catheters, and individual genetic susceptibilities. Previous results indicate the heterogeneity of the study subjects and treatment protocols.

The exact role of TLRs in the development of invasive fungal infection in AML patients is unknown. Numerous endogenous and exogenous factors affect cell proliferation and play critical roles in cancer development. The expression level of TLRs may depend on the environment, subset, cell type, stimulus and probably age group.[41]

In the current study, no statistically significant differences were found between TLR4 or TLR2 expressions and age, sex, cytogenetic risk and Patient's outcome ($P > 0.05$). Similar results showed by Ramzi et al.[42] postulated that expressions of TLR2 did not show significant differences in cytogenetic abnormalities status ($P = 0.67$). The expression of TLR4 was not different in favorable, intermediate and poor risk groups ($P = 0.97$). Renshaw et al[41] reported that old age could have negative effects on TLR expression and function, and therefore leads to increased susceptibility to infections and poor adaptive immune responses.

The current study included 122 newly diagnosed non-M3 AML patients and reporting no statistically significant differences between TLR4 or TLR2 expressions and type of AML ($P > 0.05$). In the same context, Ramzi et al.[42] observe a higher expression of TLR2 in AML-M3 cases compared to non-M3 AML patients ($P = 0.015$).

Human T cells isolated from peripheral blood reported to express mRNA for most TLRs, with considerable variation in the reported expression levels. Protein expression of TLR2, 3, 4, 5 and 9 has also been detected by flow cytometry.[43] The current study revealed that TLR2 expression in AML patients with IFIs in comparison to healthy controls presented no significant difference ($p = 0.659$), while there was a significant increase in the expression of TLR4 in the same patients group compared to healthy controls ($p = 0.001$). Consistent with these findings is the study of Bellocchio, Montagnoli. They reported that TLR4 but not TLR2 participated in host defense against *A. fumigatus*.[44] In addition, Chai et al.[45] stated that after stimulation with *A. fumigatus* conidia, surface TLR2 expression is markedly reduced compared to TLR4 expression, this

suggests that *A. fumigatus* conidia induced depletion and down—regulation of the TLR2-mediated pathway involved in the receptor internalization together with *Aspergillus* conidia into the phagosome, resulting in decreased TLR2 expression on the cell membrane. Chai et al[45] suggested a possible explanation for these findings as they postulated that the balance between TH1 and TH2 immune system pathways is necessary for the pathogen clearance and limitation of inflammation. TLR4 favors the production of TH1 response with pro-inflammatory cytokine production such as IFN-γ and 1L-12, which induces protective antifungal defense mechanisms. T regulatory cells induced by TH2 response mediated by TLR2 signaling are needed to lower immune response and to avoid collateral damage after antifungal TH1 response mediated by TLR4 signaling.

Our result revealed that TLR2 and TLR4 expression in patients with polymicrobial infection (fungus and bacteria) are significantly increased as compared to healthy controls. This result agreed with the result of Armstrong et al.[46] who reported that expression of TLR2 and TLR4 in septic patients was significantly up-regulated compared with the expression of these receptors in healthy individuals. Tsujimoto et al.[47] stated also that septic patients display significantly up-regulated TLR expression in various organs.

We can conclude that in polymicrobial infection (fungus and bacteria) there is a marked increase of both TLR2 and TLR4 expression and this may be due to the powerful effect of bacterial LPS and other bacterial PAMPs that augment the stimulatory effect of fungal PAMPs.

Susceptibility to infections is determined by the malignant disease and its treatment, environmental factors (e.g. nutritional status and hygiene of the patient), and genetically determined variations of the immune system. Some genetic polymorphisms in the innate immune system, such as profound mannose-binding lectin deficiency and TLR polymorphism associated with an increased risk of infections. Mutations in genes encoding TLRs or downstream signaling proteins increase the risk of infection.[48]

Numerous polymorphisms and mutational inactivation have been described in TLRs and appear to have clinical significance,[48,47] reported that severely septic patients with bad general conditions and the unfavorable clinical outcome did not have increased expression of TLRs,[49] have observed that a decrease in TLR2 expression in patients with invasive candidiasis can lead to severe disseminated infection. On the other side,[50] found that mice with non-functional TLR4 showed increased fungal load in the kidneys and deficiencies in neutrophil upon *C. albicans* challenge when compared to TLR4 responsive mice.

Conclusions. The incidence of IFIs is high in AML patients who received induction chemotherapy in Assiut University Hospitals. TLR2 and TLR4 expressions in AML patients with IFI are related to invasiveness and dissemination of fungal infection. TLRs expressions could be important biological markers for the occurrence of IFI in non-M3 AML patients after intensive induction chemotherapy. Additional larger studies including a larger number of patients and detection of proinflammatory cytokines are necessary to confirm the immunological relation between TLR and fungal infection in AML patients.

Data Availability. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions. M.R.A. and D.S. conceived and designed the research. D.S., M.R.A. and S.M.M. recruited patients, carried out the clinical investigations, collected clinical data. S.G.E. and M.A.E. contributed in the interpretation of data for the work. D.S., M.R.A., S.M.M., S.G.E. and M.A.E. prepared the original draft of the manuscript. All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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