RESEARCH ARTICLE

Editorial Process: Submission:06/30/2021 Acceptance:03/14/2022

Clinical Implication of Toll-Like Receptors (TLR2 and TLR4) Polymorphisms in Adult Patients with Acute B-cell Lymphoblastic Leukemia

Salah Aref[1]*, Alshaimaa Abdelmaksoud[1], Sherin Abdellaziz[1], Ahmed Al Tantawy[2], Mohamed Mabed[3], Doaa Atia[1]

Abstract

Backgrounds: Neutropenia after intensive chemotherapy of acute lymphoblastic leukemia (ALL) could lead to infectious complications that affect outcome of acute leukemia patients. Many single-nucleotide polymorphisms (SNPs) of Toll-like receptors (TLRs) can affect the genetic susceptibility to infections. We investigated the impact of different SNPs on the incidence of developing sepsis and pneumonia in patients with newly diagnosed B-ALL following induction chemotherapy. **Subjects and methods:** We analyzed three SNPs in the TLR2 (Arg753Gln) and TLR4 (Asp299Gly& Thr399Ile) genes using polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) in a case control study of 40 precursor B-ALL patients and 50 control subjects. The risk of developing sepsis and pneumonia were assessed by multiple logistic regression analyses. **Results:** The presence of the TLR-2 AG polymorphism was significantly associated with pneumonia in B-ALL patients. Furthermore, TLR4 Thr399Ile AG was a risk factor for sepsis in B-ALL patients. Moreover; Significant association between TLR-2 AA, TLR-4 CC and TL-4 AA genotypes and longer OS were detected in studied B-ALL patients. **Conclusion:** We concluded that TLR-4 (AG and CT) genotypes are associated with high susceptibility to sepsis and pneumonia respectively; while, TLR-2, TLR-4 AA and TLR-4 CC genotypes could predict good B-ALL patients outcome.

Keywords: TLR- SNP- ALL

Asian Pac J Cancer Prev, **23 (3)**, 823-828

Introduction

Leukemia is a type of blood cancer that results from an abnormal proliferation of white or red blood cells. It may occur in acute or chronic form. Acute leukemia are divided into 2 categories, leukemia evolving from the myeloid/granulocyte cell line is called acute myelogenous leukemia (AML) and that from lymphocytic precursors gives rise to acute lymphocytic leukemia (ALL). Also chronic leukemia is divided into 2 categories, chronic myeloid leukemia (CML), and chronic lymphocytic leukemia (CLL) (Yamaguti et al., 2010).

B- ALL is most common in childhood, with a peak incidence at 2–5 years of age and another peak in old age. AML is common in adults, with a median age of onset of 50 years (Seiter et al., 2014). The diagnosis of acute leukemia is established by the presence of 20% or more blasts in the bone marrow or peripheral blood (Döhner et al., 2010). Diagnosis of B-ALL is established by morphology, flow cytometry, Immunophenotyping and cytogenetic testing. Lumbar puncture with CSF analysis is essential at the time of diagnosis to evaluate CNS involvement. If the CNS is involved, brain MRI should be performed. Other investigations include complete blood count with differential and smear, coagulation profiles and serum chemistries. Baseline uric acid, calcium, phosphate and lactate dehydrogenase should be recorded to monitor for tumor lysis syndrome. Lymphoblasts tend to be small to medium size with low degree of cell to cell variability. It has very high nucleocytoplasmic ratio with small rim of cytoplasm which is moderately basophilic, not vacuolated and a granular. In contrast to AML, the chromatin show more condensation with faint nucleoli. L3 differs slightly from L1 and L2 and is characterized by medium to large sized cells with intense basophilic, moderately abundant cytoplasm and prominent cytoplasmic vacuolation (Terwilliger and Abdul-Hay, 2017).

Induction chemotherapy of acute leukemia results in a long period of neutropenia that is associated with a high risk of infectious complications (Syrjala et al., 2010). Infectious complications continue to be one of the major causes of morbidity and mortality in patients with acute

*[1]Department of Hematology Unit Clinical Pathology, Mansoura University, Egypt. [2]Oncology Unit, Department of Internal Medicine Oncology Center, Mansoura University, Egypt. [3]Hematology Unit, Department of Internal Medicine Oncology Center Mansoura University, Egypt. *For Correspondence: salaharef @ yahoo.com*

leukemia (Schnetzke et al., 2015). Sepsis and pneumonia represent the most important infectious complications and are associated with an increased mortality after induction chemotherapy (Mukherjee et al., 2019; Aref et al., 2020). The occurrence of infectious complications represents a crucial factor affecting the prognosis of acute leukemia patients, so prophylaxis and treatment of infections have an important role in the clinical management of these patients (Gupta et al., 2010). The innate immune system is important for both the direct defense of microorganisms by induction of cellular signaling pathways to induce immune response genes, including inflammatory cytokines and the activation of the adaptive immune system (Grigoryeva and Cianciotto, 2021).

TLRs are key players in maintaining defense against any invading pathogen. These molecules are microbial sensing proteins which detect pathogen-associated molecular patterns and induce the body's innate immune system to elicit a response against invading pathogens. In addition to their immune function, these proteins play important role in cancer biology. Accumulating a diverse thread of cancer-associated TLR modulation and infection susceptibility has several caveats. Some cancer-associated TLR modulation increases susceptibility to particular infections, while increased expression of certain TLR was found to help in the carcinogenic process through inducing inflammation (Zhu et al., 2013; Khan et al 2016; Browne, 2020; Li et al 2021).

TLR-2 and TLR-4 are two of the most extensively studied TLRs to have an important role in the recognition of both bacterial and fungal pathogens (El-Zayat et al., 2019). Several studies have investigated the functional consequences of TLR-4 single-nucleotide polymorphisms (SNPs) (Ferwerda et al., 2008). Structural analysis of TLR-4 has revealed evidence of an impaired binding to its ligands for the most frequent polymorphisms of TLR-4: Asp299Gly (rs4986790) and TLR-4 Thr399Ile (rs4986791) (Ohto et al., 2012). The TLR-2 polymorphism Arg753Gln (rs5743708) leads to a functional deficiency concerning hetero-dimerization with TLR-6 resulting in a diminished activation of intracellular signaling pathways (Xiong et al., 2012). Only a few retrospective analyses investigate whether polymorphisms of innate immunity influence the risk of severe infections in acute leukemia patients. Importantly, these studies do not demonstrate any correlation between different SNPs and important clinical events such as fungal infections or sepsis (Klostergaard et al., 2010). Several studies have been assessed the impact of TLR-2 and TLR-4 SNPs on the susceptibility of occurrence of sepsis and pneumonia in AML patients (Aref et al., 2020), however in ALL patients is not previously addressed.

In this study, we hypothesize that TLR-2 and TLR-4 SNPs could affect susceptibility of patients with B-ALL towards developing sepsis and pneumonia during severe neutropenia following intensive chemotherapy.

Materials and Methods

Subjects and Methods

This case control study includes 40 B-ALL patients (28 male, 12 female; median age 37 years, range 18–75 years) versus 50 healthy control subjects (24 male, 26 female; median age 47 years, range 26–62 years) from Egypt. B-ALL diagnosis was established by morphology, immunophenotyping and cytogenetic analysis. All patients enrolled in this study underwent curative-intent induction chemotherapy at Oncology Center Mansoura University, Egypt, between 2016 and 2017. The study was approved by Faculty of Medicine; Mansoura University Institutional Revision Board (IRB) and was done according to declarations of Helsinki.

Inclusion Criteria

Newly diagnosed precursor B-ALL patients before start of therapy with age more than 18 years were included in the current study.

Exclusion criteria

B-ALL patients under treatment with age less than 18 years were not included in our study.

Analysis of TLR polymorphisms

Genotyping of TLR2 Arg753Gln (R753Q, rs5743708), TLR4 Asp299Gly (D299G, rs4986790) and TLR4 Thr399Ile (T399I, rs4986791) was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Briefly, genomic DNA was isolated from peripheral blood or bone marrow samples using Gene JETTM Whole Blood Genomic DNA Purification Mini Kits from Thermo Scientific (lot no k0781, Lithuania, EU). PCR was done by amplification of extracted genomic DNA via thermal cycler (ARKTIK Thermal Cycler, Thermo Scientific Co.). The primers used in amplification of studied genes (TLR2, TLR4) are shown in Table 1.

Reactions were performed in a 25 µl volume containing 12.5 µL of red master mix, 0.1µL of both forward and reverse primer, 1 µL of DNA, 11.3 µL of distilled water (DW).

One hundred nano-grams of genomic DNA was amplified using the following cycling conditions for PCR

Table 1. Primers used for Amplification of TLR-4 and TLR-2

TLR4 Asp299Gly	forward primer, 5′- AGCATACTTAGACTACTACCTCCATG-3′
	reverse primer: 5′- GAGAGATTTGAGTTTCAATGTGGG-3′
TLR4 Thr399Ile	forward primer, 5′-GGTTGCTGTTCTCAAAGTGATTTTGGGAGAA-3′
	reverse primer: 5′-GGAAATCCAGATGTTCTAGTTGTTCTAAGCC-3′
TLR2 Arg753Gln	forward primer, 5′-TTGACTCCATTGAAAAGAGC-3′
	reverse primer, 5′-TAAATATGGGAACCTAGGAC-3 ′.

of TLR4 Asp299Gly: 95°C for 15 min, 35 cycles of 95°C for 30 s, 55°C for 60 s and 72°C for 30 s followed by a final extension of 72°C for 10 min. The PCR protocol for TLR-4 Thr399Ile was the same as for TLR-4 Asp299Gly except that annealing temperature was 53°C. The PCR protocol for TLR-2 Arg753Gln was the same as for TLR-4 Asp299Gly except that annealing temperature was 54°C for 30 s and the final extension of 72°C for 5 min.

The PCR products were then digested at 37°C with HinfI for Thr399Ile polymorphism for 5-15 min (New England Bio-Labs Inc, lot no 0411604), NcoI for Asp299Gly polymorphism for 25 min (New England Bio-Labs Inc, lot no 0061605), PstI for Arg753Gln polymorphism for 5-15 min (New England BioLabs Inc, lot no 0021504). The digests were run on a 2% agarose gel and visualized under UV light using ethidium bromide.

Digestion of PCR product for TLR-4 Asp299Gly using NcoI enzyme produced a fragment of 190 bp for homozygous wild type, produced two fragments of 168 and 20 bp for homozygous mutant type, and produced three fragments of 190, 168 and 20 bp for heterozygous mutant type. Digestion of PCR product for TLR-4 Thr399Ile using HinfI enzyme produced a fragment of 124 bp for homozygous wild type, produced two fragments of 98 and 26 bp for homozygous mutant type, and produced three fragments of 124, 98 and 26 bp for heterozygous mutant type. Digestion of PCR product for TLR-2 Arg753Gln using PstI enzyme produced a fragment of 300 bp for homozygous wild type, produced two fragments of 190 and 110 bp for homozygous mutant type, and produced three fragments of 300, 190 and 110 bp for heterozygous mutant type.

Statistical analysis

The statistical analysis of the data was performed by using excel (Microsoft office 2013) program and SPSS (Statistical Package for Social Science) program (SPSS, Inc, Chicago, IL) version 20. Chi square test was used to compare groups. Quantitative data were presented as median and range, mean and standard deviatio. For comparison between two groups Mann-whitney test (for non-parametric data) was used. For comparison of more than two groups; Kruskal-Wallis (for non-parametric data) was used. Univariate analysis used to evaluate association of polymorphism with occurrence of sepsis, pneumonia and fever. For survival analysis, Kaplan-Meier curves were used and compared by log-rank test.

Table 2. The Clinical Characteristics of the Investigated Patients

Patients characteristics		
Median age (years)		42 (18 -77)
Gender	Male	n=28 (70%)
	Female	n=12 (30%)
WBCs at diagnosis, median (range)		31.85 (1.60 -377.0)
Hemoglobin at diagnosis, median (range)		8.40 (3.60 -12.90)
Peripheral blood blast (%), median (range)		24.50 (0 - 90)
Bone marrow blast (%), median (range)		80.0 (23-95)
Patients with sepsis		16 (40%)
Patients with pneumonia		8 (20%)
Patients Outcome	Lost	n=2 (5)
	Alive	n=18 (45%)
	Dead	n=20 (50%)
Induction remission response	Remission	n=34 (85%)
	Incomplete remission	n=2 (5%)
	Resistant	n=2 (5%)
	Died	n=2 (5%)

Table 3. Association of TLR Polymorphisms with Pneumonia in B-ALL Patients after Induction Chemotherapy

		Absence of Pneumonia (N=32)	Presence of Pneumonia (N=8)	OR	95% CI	P
TLR2 AA	Count	24	0	1	-	R
	%	75	0			
TLR-2AG	Count	4	8	45	1.79-128.4	0.02
	%	12.5	100			
TLR-2GG	Count	4	0	5	0.078-316.7	0.447
	%	12.5	0			
TLR4 Asp299Gly AA	Count	24	4	1	-	R
	%	75	50			
TLR-4 Asp299Gly AG	Count	6	4	4	0.388-41.22	0.244
	%	18.8	50			
TLR-4 Asp299Gly GG	Count	2	0	1.66	0.051-53.92	0.773
	%	6.2	0			
	Count	26	2	1	-	R
TLR 4 Thr399Ile CC	%	81.2	25			
	Count	6	6	13	0.977-172.9	0.052
TLR-4 Thr399Ile CT	%	18.8	75			
	Count	0	0	9	0.126-642.1	0.312
TLR-4 Thr399Ile TT	%	0	0			

Table 4. Effect of TLR Polymorphisms on the Occurrence of Sepsis in B-ALL

		Absence of sepsis (N=24)	Presence of sepsis (N=16)	OR	95% CI		P
TLR-2 TLR2 (Arg753Gln) AA	Count	14	10	1	-	-	R
	%	58.3	62.5				
TLR-2 TLR2 (Arg753Gln) AG	Count	8	4	0.7	0.09	5.43	0.733
	%	33.3	25				
TLR-2 TLR2 (Arg753Gln) GG	Count	2	2	1.4	0.069	28.12	0.826
	%	8.4	12.5				
TLR-4 Asp299Gly AA	Count	22	6	1	-	-	R
	%	91.7	37.5				
TLR-4 Asp299Gly AG	Count	2	8	14.66	1.161	185.2	0.037
	%	8.3	50				
TLR-4 Asp299Gly GG	Count	0	2	9.85	0.323	300.4	0.189
	%	0	12.5				
TLR-4 Thr399Ile CC	Count	20	8	1	-	-	R
	%	83.3	50				
TLR-4 Thr399Ile CT	Count	4	8	5	0.64	39.06	0.124
	%	16.7	50				
TLR-4 Thr399Ile TT	Count	0	0	2.33	0.039	136.9	0.683
	%	0	0				

Results

Patients' characteristics are shown in Table 2. The study included 24 males (60%) and 16 females (40%), 31 patients (77.5%) achieved complete induction of remission (CR) and 2 patients died (5%); resistant 2 (5%). Patients acquired pneumonia were 8 (20%), while those acquired sepsis were 16 (40%). The median haemoglobin levels was 8.4 g/dl (range:3.60 -12.90); the median total white cells count was 31,850/cmm (range: 1,600-377,000). Moreover; the median blast cells count was 80.0% (range: 23-95).

Association of TLR polymorphisms with pneumonia in B-ALL patients after induction chemotherapy

We evaluated the association between TLR genotypes and the prescience of pneumonia which was documented by new radiographic infiltrates and clinical criteria. The statistical analysis revealed that in ALL patients with TLR-4 CT genotypes is the only one that has a significant higher risk of pneumonia as compared to other genotypes. On the other hand, TLR-2 and TLR4 Thr399Ile had lowest impact for the susceptibility of Pneumonia (Table 3).

Effect of TLR polymorphisms on the occurrence of sepsis in B-ALL

Analysis the association between the studied phenotypes and susceptibility of B-ALL patients to harboring sepsis reveled that there is significant association between TLR4 Asp299Gly and high susceptibility to sepsis with OR 14.66(CI: 1.161-185.2). The other genotypes had insignificant impact on susceptibility of sepsis in B-ALL patients (Table 4).

Impact of TLR-2 Arg753Gln, TLR-4 Thr399Ile and TLR-4 Asp299Gly genotype variants on OS of patients with B-ALL

Evaluation the impact of studied TLR genotypes on patients OS and DFS revealed that the significant association between TLR-2 genotypes and mean OS

Table 5. Impact of TLR-2 Arg753Gln, TLR-4 Thr399Ile and TLR-4 Asp299Gly Genotype Variants on OS of Patients with B-ALL

Cases		Survival	Cumulative survival (%)	Mean (months)	CI 95%	P value
TLR-2 TLR2 (Arg753Gln)	AA	OS	49.6	8.54	7.232-9.85	
	AG	OS	11.8	5.2	3.68-6.72	0.001
	GG	OS	20	1.46	0.625-2.29	
TLR-4 Thr399Ile	CC	OS	49.4	7.74	6.29-9.20	
	CT	OS	11.1	5.93	4.34-7.52	0.062
	TT	OS	50	5.25	1.82-8.68	
TLR-4 Asp299Gly	AA	OS	44.9	7.63	6.29-8.97	
	AG	OS	7.3	5.76	3.95-7.58	0.104
	GG	OS	33.3	5.33	0.012-10.84	

Table 6. Impact of TLR-2 Arg753Gln, TLR-4 Thr399Ile and TLR-4 Asp299Gly Genotype Variants on DSF of Patients with B-ALL

		Survival	Cumulative survival (%)	Mean (months)	CI 95%	P value
Cases		DFS	55.2	7.9	6.62-9.19	-
TLR-2 TLR2 (Arg753Gln)	AA	DFS	63.2	8.69	7.19-10.19	
	AG	DFS	41.2	7.41	4.92-9.89	0.002
	GG	DFS	20.0	1.26	0.452-2.06	
TLR-4 Thr399Ile	CC	DFS	71.2	9.03	7.54-10.53	
	CT	DFS	22.6	5.53	3.67-7.39	0.015
	TT	DFS	0.0	2.0	2.00-2.00	
TLR-4 Asp299Gly	AA	DFS	57.3	8.22	6.73-9.72	
	AG	DFS	53.3	7.565	4.85-10.27	0.482
	GG	DFS	33.3	4.83	0.021-10.61	

survival times recorded by months were as follow AA (7.74); AG (5.2); GG(1.26) (P=0.001); for TLR-4 Thr399Ile genotypes were CC (7.47); CT(5.93); TT (5.25) (P=0.06); and for TLR-4 Asp299Gly genotypes were AA (7.63); AG (5.76); GG (5.33) (P=1.104) (Table 5)

Impact of TLR-2 Arg753Gln, TLR-4 Thr399Ile and TLR-4 Asp299Gly genotype variants on DFS of patients with B-ALL

The mean DFS times/months for TLR-2 Arg753Gln genotypes were as follow AA (8.09); AG (7.41); GG (1.26) (P=0.002); for TLR-4 Thr399Ile genotypes CC (9.03); CT(5.53); TT(2.0) (P=0.015); and for TLR4 TLR-4 Asp299Gly genotypes were AA (8.22); AG(7.56); GG (4.83); (P= 0.482) (Table 6).

Discussion

Toll-like receptors (TLRs) are major elements of the innate immune system which recognize pathogen-associated molecular patterns. TLRs are expressed on a wide variety of effector immune and stromal cell types, as well as hematopoietic stem and progenitor cells (HSPCs), and signaling through these receptors is essential to mounting an effective immune response to an acute infection or injury (Monlish et al., 2016).

This study was done to investigate the impact of (TLR-2 and TLR-4) polymorphism on the susceptibility of infection in patients with B-ALL who received intensive induction chemotherapy as first-line treatment.

We found a significant correlation between having TLR2 (Arg753Gln) SNP and TLR-4 (Thr399Ile) SNP and the development of sepsis and pneumonia in B-ALL patients who underwent induction chemotherapy. Long-lasting neutropenia following intensive chemotherapy can be considered as the main risk factor for bacterial and fungal infections in acute leukemia patients. Wahlund et al., (2020) showed that both the TLR-4 rs10759931 and rs11536889 polymorphisms increase the risk of viral infections during febrile neutropenia, but not to the number of episodes of febrile neutropenia in general. Furthermore, earlier studies (Pehlivan et al., 2014, Schnetzke et al., 2014) mainly have associated TLR4 sequence variants to the increased risk of Gram-negative bacterial infections, these findings were rather surprising .

In the current study, there is significant association between TLR4 Asp299Gly and high susceptibility to sepsis with OR 14.66 (CI: 1.161-185.2) in the studied cohort of patients. Many studies have looked for associations of polymorphisms in TLR4 with infection. Infections and sepsis with Gram-negative bacteria, Respiratory Syncytial Virus bronchiolitis, and disseminated candidiasis have all been linked to TLR4 polymorphisms. TLR4 was suggested to have role of recognizing Gram-negative lipopolysaccharide as well as other bacterial, fungal, and viral antigens (Maglione et al., 2015).

The statistical analysis revealed that in ALL patients with TLR-4 CT genotypes is the only one among studied genotypes that has a significant higher risk of pneumonia as compared to other genotypes. On the other hand TLR-2 and TLR4 Thr399Ile had low impact for the susceptibility of pneumonia. Similar findings were reported by Schnetzke et al., (2015) who found that TLR4 polymorphisms Asp299Gly and Thr399Ile were independent risk factors for the development of both sepsis and pneumonia (OR: 3.55; 95% CI: 1.21-10.4, P=0.021 and OR: 3.57, 95% CI: 1.3-9.86, P=0.014, respectively).

TLR- 2 AA , TLR-4 CC and TLR-4 AA genotypes had the longest OS and DFS. This could be attributed to altered ability to recognize pathogens with increased recognition ability of pathogen's ligands and persistent stimulation of inflammatory response against invading pathogens (Khan et al., 2016). Similar findings was reported by Rybka et al 2021 who concluded that SNPs in the genes coding for TLR3, TLR4 and TLR9 may be implicated in clinical outcome of AML and is related with increased risk of infectious complications.

In conclusion, we concluded that TLR-4 and TLR-2 (AG) genotypes are associated with high susceptibility to sepsis and pneumonia in adult ALL patients. While TLR-2 AA, TLR-4 AA, and TLR-4- CC genotypes were associated with good B-ALL patients outcome.

Recommendations

Extension of the study on large number of patients should be considered to validate our results. Further studies are needed to elucidate such molecular markers as SNPs of innate immunity to refine strategies of

antimicrobial prophylaxis or to adjust pharmacological treatment of infections in immune-compromised patients.

Author Contribution Statement

Salah Aref; Conception and supervision. Sherin Abdellaziz, Interpretation or analysis of data. Alshaimaa Abdelmaksoud Interpretation or analysis of data; Laboratory Work; Ahmed Al Tantawy; Clinical Data, Mohamed Mabed, Clinical Follow up of the patients, Doaa Atia, Laboratory work, All authors, Preparation of the manuscript; Interpretation or analysis of data, Revision for important intellectual.

Acknowledgements

The authors of the current manuscript acknowledge the patients who participate in this study as well as the technicians in the clinical pathology department laboratories; Mansoura University.

Ethical issue

The study was approved by Mansoura Faculty of Medicine IRB

Availability of data

The data was available upon request to the corresponding authors.

Funding statement

The study is a part of Master degree thesis of Alshaimaa Abdelmaksoud and was funded by authors of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Aref S, Abd Elmaksoud S, Abd Elaziz S, et al (2020). Clinical implication of toll-like receptors (TLR2 and TLR4) in acute myeloid leukemia patients. *Asian Pac J Cancer Prev*, **21**, 253-9.

Döhner H, Estey EH, Amadori S, et al (2010). Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European Leukemia Net. *Blood*, **115**, 453-74.

Browne E (2020). The Role of Toll-Like Receptors in Retroviral Infection. *Microorganisms*, **8**, 1787.

El-Zayat S, Sibaii H, Mannaa F (2019). Toll-like receptors activation, signaling, and targeting: an overview. *Genet Med*, **43**,187.

Ferwerda B, McCall MB, Verheijen K, et al (2008). Functional consequences of toll-like receptor 4 polymorphisms. *Mol Med*, **14**, 346–52.

Grigoryeva L, Cianciotto N (2021). Human macrophages utilize a wide range of pathogen recognition receptors to recognize Legionella pneumophila, including Toll-Like Receptor 4 engaging Legionella lipopolysaccharide and the Toll-like Receptor 3 nucleic-acid sensor. *PLOS Pathogens*, https://doi.org/10.1371/journal.ppat.1009781.

Gupta A, Singh M, Singh H, et al (2010). Infections in acute myeloid leukemia: an analysis of 382 febrile episodes. *Med Oncol*, **27**, 1037–45.

Khan A, Khan Z, Warnakulasuriya S (2016). Cancer-associated toll-like receptor modulation and insinuation in infection susceptibility: association or coincidence?. *Ann Oncol*, **27**, 984–97.

Klostergaard A, Steffensen R, Moller JK, et al (2010). Sepsis in acute myeloid leukaemia patients receiving high-dose chemotherapy: no impact of chitotriosidase and mannose-binding lectin polymorphisms. *Eur J Haematol*, **85**, 58–64.

Li H, Liu S, Han J, et al (2021). Role of Toll-Like Receptors in Neuroimmune Diseases: Therapeutic Targets and Problems. *Front Immunol*, https://doi.org/10.3389/fimmu.2021.777606.

Maglione P, Simchoni N , and Cunningham-Rundles C (2015) Toll-like receptor signaling in primary immune deficiencies. *Ann N Y Acad Sci U S A*, **1356**, 1–21.

Monlish A, Bhatt I, Laura G, Schuettpelz L (2016). The role of toll-Like Receptors in Hematopoietic Malignancies. *Front Immunol*, https://doi.org/10.3389/fimmu.2016.00390.

Mukherjee S, Huda S, Sinha Babu SP (2019). Toll-like receptor polymorphism in host immune response to infectious diseases: A Review. *Scand J Immunol*, **90**, e12771.

Ohto U, Yamakawa N, Akashi-Takamura S, et al (2012). Structural analyses of human Toll-like receptor 4 polymorphisms D299G and T399I. *J Biol Chem*, **287**, 40611–17.

Pehlivan M, Sahin H, Ozdilli K, et al (2014). Gene polymorphisms and febrile neutropenia in acute leukemia—No Association with IL-4, CCR-5, IL-1RA, but the MBL-2, ACE, and TLR-4 Are Associated with the Disease in Turkish Patients: A Preliminary Study. *Genet Test Mol Biomarkers*, **18**, 474–81.

Rybka J, Bogunia-Kubik K, Kuszczak B, et al (2021). Analysis of polymorphism in the genes TLR3, TLR4 and TLR9 in patients with acute myeloid leukemia. *Blood*, **138**, 4470.

Seiter K, Sarkodee-Adoo C, Talavera F, Sacher RA, Besa EC (2014). Acute Lymphoblastic Leukemia. Medscape Reference. WebMD.

Schnetzke U, Spies-Weisshart B, Yomade O, et al (2014) Polymorphisms of Toll-like receptors (TLR2 and TLR4) are associated with the risk of infectious complications in acute myeloid leukemia. *Genes Immun*, **16**, 83–8.

Terwilliger T, Abdul-Hay M (2017). Acute lymphoblastic leukemia: a comprehensive review and 2017 update. *Blood Cancer J*, **7**, e577.

Xiong Y, Song C, Snyder GA, et al (2012). R753Q polymorphism inhibits Toll-like receptor (TLR) 2 tyrosine phosphorylation, dimerization with TLR6, and recruitment of myeloid differentiation primary response protein 88. *J Biol Chem*, **287**, 38327–37.

Wahlund M, Appell M, Myrberg I, et al (2020). Genetic sequence variants in TLR4, MBL or IL-1 receptor antagonist is not associated to increased risk for febrile neutropenia in children with ALL. *Children*, **7**, 296-7.

Yamaguti GG, Moody CJ, Pagnamenta A, et al (2010). Increased risk for acute lymphoblastic leukemia in children with cytochrome P450A1 (CYP1A1) and NAD (P) H: quinone oxidoreductase 1 (NQO1) inherited gene variants. *Acta Haematol*, **124**, 182–4.

Zhu L, Yuan H, Jiang T, et al (2013). Association of TLR2 and TLR4 polymorphisms with risk of cancer: A Meta-Analysis. *PLoS One*, **8**, e82858.