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Original Research

CYP3A4 and *CYP3A5* Genes in Cyclophosphamide-treated Chronic Lymphocytic Leukemia Patients: A Pharmacogenetics Study

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Abstract

Background: Approximately 75% of drug metabolism in clinical settings is attributed to cytochrome P450 enzymes. This study aimed to assess the effects of the *CYP3A4*1B* and *CYP3A5*3* genetic variations on the clinical results of individuals with chronic lymphocytic leukemia (CLL) following cyclophosphamide treatment. Furthermore, we aimed to ascertain how well the inflammatory condition affects the therapeutic response. **Methods**: *CYP3A4*1B* and *CYP3A5*3* polymorphisms were examined in 150 Egyptian CLL patients using allele-specific amplification (ASA)-polymerase chain reaction (PCR); serum interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α) levels were also measured to assess the non-genetic inflammatory effect on *CYP3A4* and *CYP3A5* genes. Patients further received chemotherapy and were subsequently followed up. **Results**: The allelic frequencies of the *CYP3A4*1B* gene were (74.3% *A-allele* vs. 25.7% *G-allele*), and for *CYP3A5*3,* these frequencies were (73.4% *A-allele* vs. 26.6% *G-allele*). Patients with the *CYP3A4*1B* and *CYP3A5*3* genes, or both variants, were less likely to respond than the normal patients ($p < 0.001$). Regarding the non-genetic inflammatory effect, patients in the response group who achieved partial remission were characterized by higher IL-6 and TNF-α values than those who achieved complete remission ($p < 0.001$), and patients in the non-response group who had a progressive disease were characterized by higher IL-6 and TNF-α values than those who had a stable disease ($p < 0.001$). **Conclusion**: *CYP3A4*1B* and *CYP3A5*3* variants could be helpful indicators in predicting the response to cyclophosphamide chemotherapy. *CYP3A4* and *CYP3A5* variability should be factored into personalized medicine, which attempts to optimize drug dosing for individual patients by considering genetic and non-genetic factors affecting the response.

Keywords: CLL; SNP; *CYP3A4*; *CYP3A5*; cyclophosphamide; cytokines

1. Introduction

Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disease characterized by a gradual accumulation of nonfunctional monoclonal B-lymphocytes in the peripheral blood, bone marrow, and lymphoid organs [1]. Chemotherapy remains the primary treatment for CLL patients. Most patients respond to initial therapy; nevertheless, the disease has a clinical course characterized by recurrent episodes, resulting in treatment resistance [2].

Cyclophosphamide (CPA) has a wide range of applications that manage CLL. Generally, depending on the age and activity level of the patient, CPA is used in conjunction with other medications in CLL treatment protocols, such as the (fludarabine/cyclophosphamide) regimen. CPA is a classic example of a prodrug, which lies inactive until metabolically activated to provide anti-tumor effects. The plasma pharmacokinetics of CPA varies significantly, with total clearance ranging from 1.0 to 12.6 L/h [3].

The conversion of CPA to its active form, 4-hydroxy cyclophosphamide (4-OH-CPA), is a rate-limiting step in its bioactivation. This step is supposed to occur only in the liver, instead of directly in cancer cells, and depends on hepatic enzymes, mostly cytochrome P450 members CYP3A4 and CYP3A5. Various CYPs have been demonstrated to have the potential to hydroxylate CPA, producing phase I (4-OH-CPA), which is in equilibrium with its tautomer, aldophosphamide. Further, phosphoramide mustard, which is thought to be the primary metabolite responsible for the DNA alkylating activity, is produced when aldophosphamide is absorbed by the cells [4].

Phase I xenobiotic biotransformation is often conducted by the superfamily of heme-containing enzymes known as cytochrome P450 (CYP) [5]. There are 44 subfamilies and 18 families in the 57 CYP enzymes [6]. There is only one subfamily of cytochrome P450 family 3 (CYP3), and it is found on chromosome 7q22.1. Most P450 en-

zymes in the human liver, including CYP3A4, CYP3A5, CYP3A7, and CYP3A43, are found in CYP3A, accounting for 30% of all CYPs. These isozymes metabolize over half of the drugs used in clinical therapy. Furthermore, these P450 enzymes are effective steroid hydroxylases that play a significant role in breaking down several endogenous hormones, such as progesterone, testosterone, and cortisol [7].

The prostate, liver, kidneys, and pancreas generally express CYP3A43 at modest levels; however, it does not deal with medications; instead, it primarily affects the metabolism of endogenous substances. CYP3A4 is not expressed in the fetal liver, but CYP3A7 makes up 30–50% of the total CYP450 enzymes expressed in the liver. Since CYP3A4 increasingly replaces it after birth, CYP3A7 is not often found in the liver of adults at considerable amounts [8]. CYP3A4 is the most expressed CYP enzyme, with a 14.5–37% range in the hepatic P450 pool. In general, CYP3A5 expression is less than that of CYP3A4 [7].

Several genetic, physiological, clinical, and environmental variables affect the expression and activity of CYPs; genetic polymorphisms are one of the leading causes of this variability [9].

The human *CYP3A* polymorphism is dominant and shows substantial changes in protein expression levels between ethnic groups. Several single nucleotide polymorphisms (SNPs) have been identified within the *CYP3A4* gene, including a substitution in the promoter region *(-A392G)* that creates the variant allele *CYP3A4*1B*, which is associated with decreased enzyme expression and activity [10].

The most common polymorphism in the *CYP3A5* gene is a variation in intron 3 *(A6986G)*, which encodes the alternative allele *CYP3A5*3*. This variation generates an abnormal mRNA transcript containing 131 additional nucleotides in intron 3 between exons 3 and 4 and introduces an early termination codon; only a small amount of normal protein is produced by this variation. This lack of active enzymes underlies the significant differences in the inter-individual pharmacokinetics of drugs [11].

Moreover, circulating cytokines function as signaling molecules that cause significant alterations in liver gene expression during infection, inflammation, and cancer. This results in the severe downregulation of numerous drug-metabolizing enzymes. Changes in the activity or expression of drug-metabolizing enzymes may affect hepatic clearance, drug bioavailability, and the biotransformation of various prodrugs processed by CYP enzymes, as the liver is the primary site of drug metabolism [12].

Tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6) are the chief cytokines that exhibit this suppressive impact. The primary mechanism for modifying CYP activity is gene transcription modifications, which are mediated by cytokines [13,14]. Our study aimed to examine the genetic polymorphisms of *CYP3A4* and *CYP3A5* isoforms among Egyptian patients with CLL and their possible impact on the clinical outcome following CPA chemotherapy. In addition, we intended to examine the association between the IL-6 and TNF-α serum levels and the response to CPA as a non-genetic factor affecting *CYP3A4* and *CYP3A5* functions.

2. Subjects and Methods

2.1 Subjects and Samples

This cross-sectional study comprised 150 Egyptian patients referred to the Oncology Center at Mansoura University who were newly diagnosed with CLL according to the National Comprehensive Cancer Network (NCCN) guidelines [15]. The study was approved by the Ethics Committee at the Faculty of Medicine, Mansoura University (R.24.06.2649.R2). Informed consent was obtained from all contributors. The exclusion criteria involved patients with atypical CLL or other lymphoproliferative disorders; typical CLL patients with co-morbidities such as renal impairment, liver disease, and heart diseases; patients with inflammatory diseases and autoimmune disorders; patients with Tp53 mutation/deletion; patients who received chemotherapy other than cyclophosphamide; patients who received any interfering medication (inducers/inhibitors) of *CYP3A4* and *CYP3A5*. The flowchart of the included participants is displayed in Fig. 1.

2.2 Methods

2.2.1 DNA Extraction and Genotyping

DNA was extracted from whole blood samples using a DNA Extraction kit (QIAGEN, Germantown, MD, USA) and collected in EDTA tubes as an anticoagulant. Then, the DNA concentration was detected using a NanoDrop™ 1000 Spectrophotometer (NanoDrop Tech., Inc., Wilmington, NC, USA).

Separate polymerase chain reactions (PCRs) were performed to amplify the normal or variant allele in allele-specific amplifications (ASAs). Two forward primers were also required: one for the variant and one for the normal alleles, alongside a common reverse primer. Genotyping of the *CYP3A4*1B* SNP was conducted via the method reported by Rebbeck *et al.* [16] using a forward wild-type primer (F1), a forward variant primer (F2) and a reverse (R) primer. Primers' sequences are displayed in **Supplementary Table 1**. Each reaction included primers for a gene that could be readily amplified; we used the *β-actin* gene to guarantee no false negative results.

The amplification was conducted using a double PCR reaction mixture with a total volume of 25 µL containing 4 µL extracted DNA, 12.5 µL PCR ready-to-use master mix (ThermoFisher Scientific, Waltham, MA, USA), and 1 µL of each ((F1, R and β-actin F and R) primers in the first reaction, or (F2, R and β-actin F and R)) primer in the second reaction. The overall volume in both reactions was completed using nuclease-free water. The thermal cycler was used according to the cycling program presented in **Supplementary Table 2**.

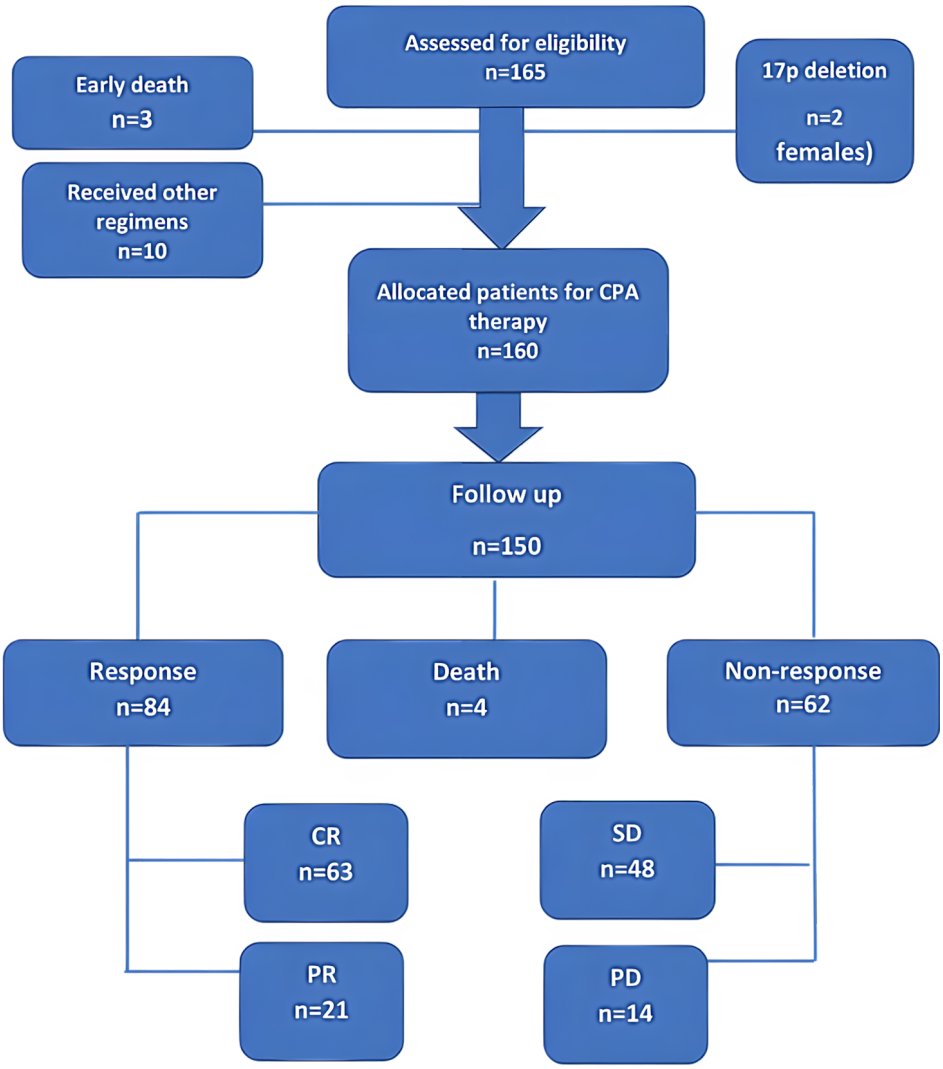

Fig. 1. Flowchart of the studied participants. CR, complete remission; PR, partial remission; PD, progressive disease; SD, stable disease; CPA, cyclophosphamide.

Genotyping of the *CYP3A5*5* SNP was performed according to the method reported by Ashavaid *et al*. [17]. The amplification was conducted in a double PCR reaction mixture with a total volume of 25 µL, containing 4 µL extracted DNA, 12.5 µL PCR master mix, and 1 µL of each (F1 and R) primer in the first reaction, or (F2 and R) primers in the second reaction. The final volume in both reactions was completed using nuclease-free water. The amplification products of both SNPs were electrophoresed at 90 volts for 60 mins, placed onto a 2% agarose gel stained with ethidium bromide using 1X Tris-borate-EDTA (TBE) buffer, and then observed using an ultraviolet transilluminator.

2.2.2 IL-6 and TNF-α Measurements

The levels of TNF-α and IL-6 were determined in the serum of all the participants using Sandwich ELISA kits (Bioassay Technology Laboratory, Shanghai, China) according to the manufacturer's guidelines.

2.2.3 Follow-up

The fludarabine/cyclophosphamide regimen included fludarabine (30 mg/m^2) and cyclophosphamide (250 mg/m^2) administered via i.v. every 28 days on days 1–3 for a maximum of 6 cycles. The IW-CLL defined treatment outcomes depending on the tumor load and bone marrow recovery after chemotherapy as complete remission (CR), partial remission (PR), progressive disease (PD), and stable disease (SD). Patients underwent follow-up, and the response to treatment was assessed following chemotherapy. Patients who exhibited SD or PD after treatment were classified as non-responders, whereas those who attained at least PR were called responders [18].

2.3 Statistical Analysis

Data analysis was performed using the SPSS software version 26 (IBM Corp., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to check data normal-

ity. The parametric data were expressed as the mean ± SD. and processed using Student's and one-way ANOVA tests. In contrast, the non-parametric data were expressed as median (min–max) and processed using the Mann-Whitney and Kruskal-Wallis tests. As appropriate, chi-square and Fisher's exact tests were used to compare qualitative data between groups. The receiver operating characteristic (ROC) curve was performed with an area under the curve (AUC) >0.9, 0.7–0.9, 0.5–0.7, and <0.5, which indicated excellent, good, low, and poor accuracy, respectively. A p-value < 0.05 was reported as statistically significant.

3. Results

3.1 Main Characteristics of the Studied Cases

The enrolled patients included 96 (64%) males and 54 (36%) females, with a median age of 57.7 years, ranging between 36 and 76 years. The results of immunophenotyping following flowcytometry presented that CLL cells in the samples of patients expressed CD5 (98%), CD19 (100%), CD20 (100%), CD23 (96%), CD10 (8%), FMC7 (7%), sIg (7%), CD22 (11%), ƙ-light chain (55%), ƛ-light chain (45%), CD38 (36%), and CD49d (44%).

Upon examination of the patients, 96% of patients possessed splenomegaly, 85.5% had hepatomegaly, 100% had lymphadenopathy, and 44% presented with constitutional B-symptoms (weight loss (13.5%), night sweats (17%), and fever (13.5%)). All patients presented with >30% bone marrow infiltration by mature B-lymphocytes. In addition, 46% of cases were found to have chromosomal abnormalities (26% with 13q14 del., 17% with 11q23 del., 1.5% with combined 13q+11q del., and 1.5% with trisomy 12). The hematological and biochemical test results of CLL patients before starting chemotherapy are shown in **Supplementary Table 3.**

3.2 Analysis of CYP3A4 and CYP3A5 Genes

The genotypes of the *CYP3A4*1B* SNP were classified as wild-type **1A/*1A (AA)*, heterozygous carrier **1A/*1B (AG)*, and homozygous variant **1B/*1B (GG)*. The genotypes of the *CYP3A5*3* SNP were classified as wild-type **1/*1 (AA)*, heterozygous *1/*3 *(AG)*, and homozygous *3/*3 *(GG)*. The PCR results after gel electrophoresis are shown in Figs. 2,3. The genotypic and allelic frequencies of the *CYP3A4*1B* and *CYP3A5*3* variants among CLL patients are shown in Table 1.

3.3 Characteristics of Post-treated CLL Patients

Following receiving cyclophosphamide chemotherapy, 84 (56%) of patients responded, 62 (41.3%) were non-responders, and the remaining 4 (2.7%) died before the end of the treatment. Responder and non-responder patients were divided into four subgroups based on the findings of the tumor load and bone marrow recovery following chemotherapy (Table 2). The responder and non-responder patients were classified as having 63 (42%) CR, 21 (14%) PR, 48 (32%) SD and 14 (9.3%) PD. Patients possessing the *CYP3A4*1B*, *CYP3A5*3*, or both variants were less likely to achieve a response than patients with wild-type alleles (OR = 9.06 for *CYP3A4*1B* and OR = 14.0 for *CYP3A5*3*; $p < 0.001$; Table 3).

3.4 Association of IL-6 and TNF-α Levels With The Clinical Outcome

Fig. 4 displayed the initial levels of IL-6 and TNF-α before treatment. The ROC for IL-6 and TNF-α showed reduced AUC values (0.481 and 0.499, respectively) (Fig. 5), which reflects poor test accuracy in discriminating between the response and non-response groups.

Regarding the different response subgroups (CR, PR, SD, and PD), in the responder patients, there was a significant increase in both IL-6 and TNF-α in the PR group compared with the CR group ($p < 0.001$ for both). In the non-responder patients, there was a significant increase in both concentrations in the PD group compared to the SD group ($p < 0.001$ and $p = 0.006$, respectively). The ROC curves for IL-6 and TNF-α showed high AUC values (0.87 and 0.83, respectively), reflecting the reasonable accuracy of the test to discriminate between the CR and PR groups. Furthermore, in Fig. 5, the ROC curves for IL-6 and TNF-α showed high AUC values (0.89 and 0.82, respectively), reflecting the good accuracy of the test in discriminating between the SD and PD groups.

4. Discussion

CLL is defined by the presence of at least 5×10^9/L monoclonal mature B cells in the blood. CLL exhibits a relapsing and remitting pattern, and many patients can benefit from sequential therapy regimens [19].

Even following the well-established advancements in the use of these regimens, a considerable proportion of patients remain incurable because of drug resistance or unacceptable toxicities that force chemotherapy to end early. Considering the FC regimen, regarded as the chemotherapy backbone of modern CLL, this regimen only produces a 23% to 38% complete remission rate, suggesting that a considerable percentage of patients exhibit some therapy resistance [20,21].

Molecular and cellular markers, such as cytogenetic abnormalities, CD38 and CD49d expressions, *immunoglobulin heavy chain variable* (*IGHV*) mutational status, and β2 microglobulin level, are employed in clinical practice and have substantial prognostic value; however, the predictive value of these markers in the current treatment protocols is still being assessed [22].

Currently, TP53 loss, resulting from *TP53* mutations and/or 17p13 deletion, is the only validated predictive marker that can be used to inform treatment decisions. Defective *TP53* is the most potent adverse prognostic factor and indicates patients who will not respond well to chemotherapy or who will not live to the predicted length.

Fig. 2. Agarose electrophoresis to analyze the *CYP3A4 (-A392G)* SNP. Lane (M): DNA marker 100–1200 bp; 7,8,9,10: (wild-type *AA* genotype at 479 bp); 5,6: (heterozygous carrier *AG* genotype at 479 bp); 1,2,3,4: (homozygous variant *GG* genotype at 479 bp); *β*-actin band was at 321 bp.

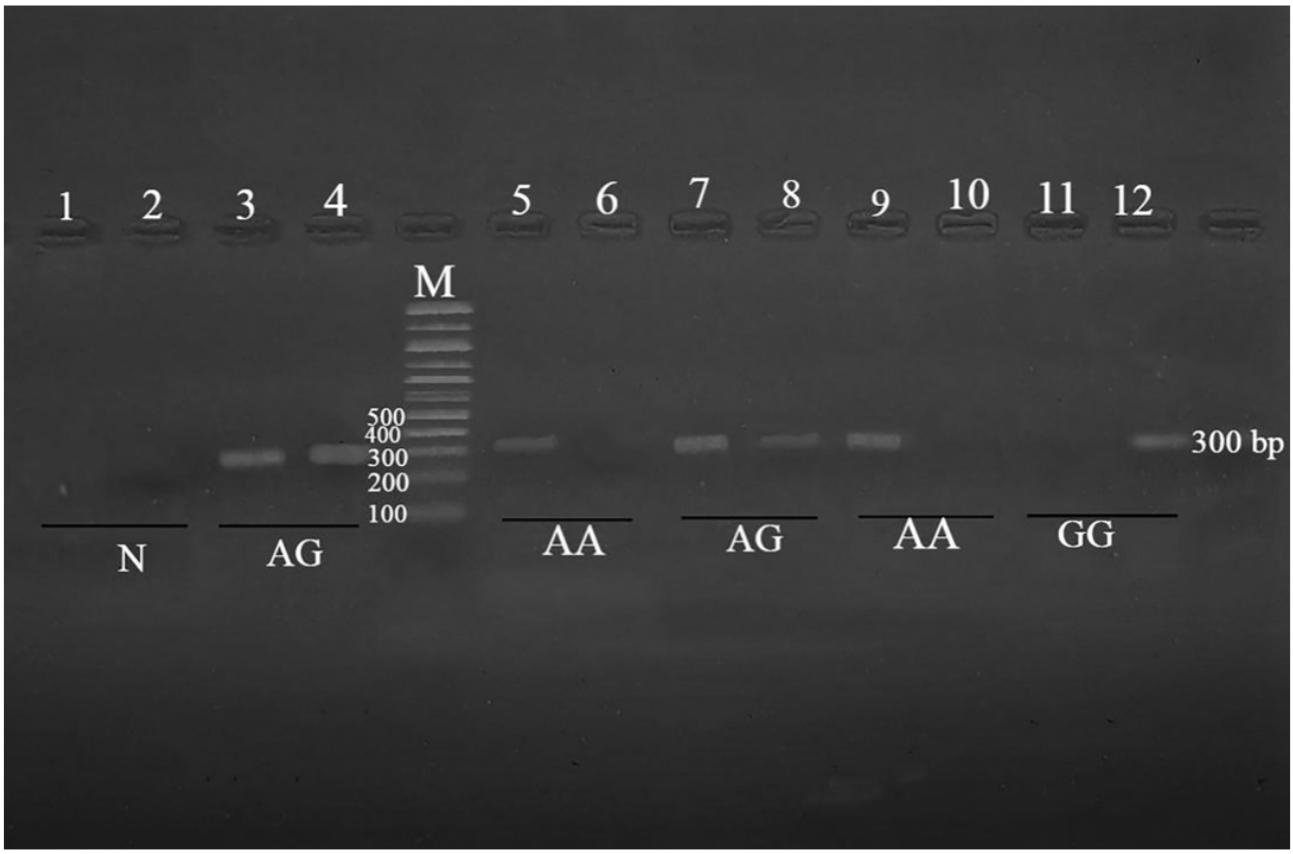

Fig. 3. Agarose electrophoresis to analyze the *CYP3A5 (A6986G)* SNP. Lanes 1,2: the -ve control; 3,4,7,8: (heterozygous carrier *AG* genotype at 300 bp); 5,6,9,10: (wild-type *AA* genotype at 300 bp); 11,12: (homozygous variant *GG* genotype at 300 bp).

Table 1. Genotypic and allele frequencies of the *CYP3A4*1B* and *CYP3A5*3* SNPs.

Genetic polymorphisms		n (%)
*CYP3A4*1B*	Genotypic frequencies, (n=150)	
	AA	87 (58%)
	AG	49 (32.7%)
	GG	14 (9.3%)
	Allelic frequencies, (n=300)	
	A-allele	223 (74.3%)
	G-allele	77 (25.7%)
*CYP3A5*3*	Genotypic frequencies, (n=150)	
	AA	84 (56%)
	AG	52 (34.7%)
	GG	14 (9.3%)
	Allelic frequencies, (n=300)	
	*A-allele (*1)*	220 (73.4%)
	*G-allele (*3)*	80 (26.6%)

Parameters are described as the frequency (percentage). SNPs, single nucleotide polymorphisms.

Table 2. Tumor load and bone marrow recovery among the pretreated, response, and non-response groups.

Parameter		Pretreated	Response	Non-response	p_1	p_2	p_3
		n = 150	n = 84	n = 62			
Tumor load	Lymph nodes♦	0 (0%)	80 (95.2%)	0 (0%)	<0.001*	1.0	<0.001*
	Non-enlarged	150 (100%)	4 (4.8%)	62 (100%)			
	Enlarged						
	Spleen♦	6 (4%)	78 (92.9%)	7 (11.3%)	<0.001*	0.048*	<0.001*
	Non-enlarged	144 (96%)	6 (7.1%)	55 (88.7%)			
	Enlarged						
	Liver	22 (14.7%)	83 (98.8%)	37 (59.7%)	<0.001*	<0.001*	<0.001*
	Non-enlarged	128 (85.3%)	1 (1.2%)	25 (40.3%)			
	Enlarged						
	B-symptoms	84 (56%)	81 (96.4%)	43 (69.3%)	<0.001*	0.01*	0.006*
	Absent	55 (44%)	3 (3.6%)	19 (29.7%)			
	Present						
	Lymphocytes ($\times 10^9$/L)	69.2 (43–177.2)	3.3 (0.39–23)	58.2 (11.6–177)	<0.001*	0.006*	<0.001*
	Marrow infiltration (%)	65 (32–88)	10 (4–50)	66 (18–89)	<0.001*	0.55	<0.001*
Marrow recovery	Hemoglobin (g/dL)	10.97 ± 1.21	11.03 ± 0.99	11.16 ± 0.85	0.69	0.26	0.41
	Neutrophils ($\times 10^9$/L)	3.7 ± 1.41	2.4 ± 1.42	3.4 ± 1.02	<0.001*	0.07	<0.001*
	Platelets ($\times 10^9$/L)	197 (80–269)	130.5 (60–119)	155 (50–221)	<0.001*	0.003*	0.32

*: Significant *p*-value, ♦: (enlarged lymph node and spleen if >1.5 and >13 cm, respectively), p_1: (comparison between pretreated and response), p_2: (comparison between pretreated and non-response), p_3: (comparison between response and non-response).

Table 3. Association between *CYP3A4*1B* and *CYP3A5*3* SNPs and response to cyclophosphamide chemotherapy.

Outcome	Response	Non-response	OR (95% CI)	*p-value*
	n = 84	n = 62		
Normal	63 (75%)	11 (17.7%)	Reference	<0.001*
Single **1B* polymorphism	12 (14.3%)	19 (30.6%)	9.06 (5.15–38.05)	
Single **3* polymorphism	9 (10.7%)	22 (35.6%)	14.0 (5.15–38.05)	
Double **1B*+**3* polymorphisms	0 (0%)	10 (16.1%)	-	

*: Significant *p*-value. OR, Odds ratio; CI, Confidence interval.

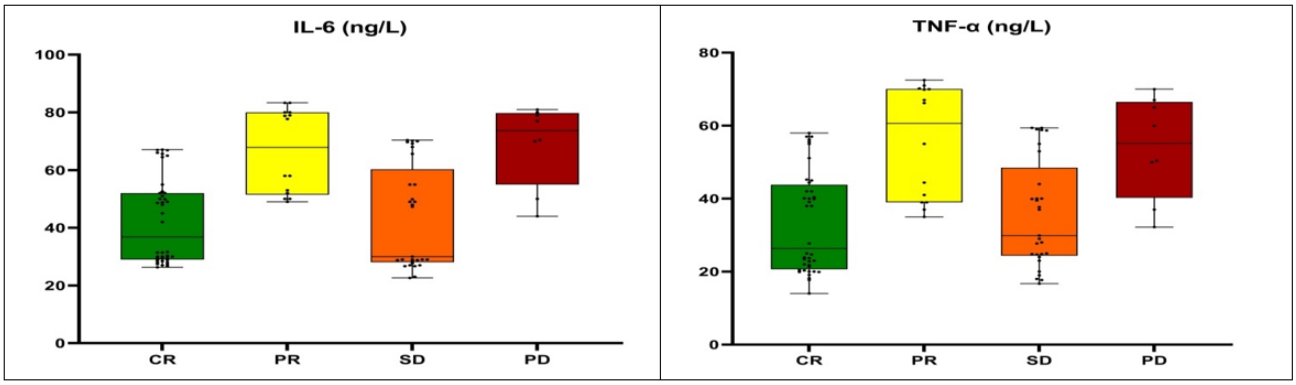

Fig. 4. IL-6 and TNF-α serum levels among different response and non-response groups. IL-6, interleukin 6; TNF-α, tumor necrosis factor-alpha.

Nevertheless, the reason why specific individuals with intact TP53 would not respond well to chemotherapy is not explained by the genetic and molecular profiling of CLL. Consequently, it is vital to consider other factors that may impact the pharmacokinetics or pharmacodynamics of applied medications [22].

Due to changes in the balance between dose and blood levels of the active agent, variations in drug metabolism might result in severe toxicity or therapeutic failure. As a result, this influences the clinical outcome, which can differ significantly throughout individuals and even within the same person. Therefore, we can divide the patients into extensive, intermediate, and poor metabolizers [23]. The genetic variants in drug-metabolizing enzymes, primarily in the cytochrome P450 superfamily, account for a significant portion of this diversity. Pharmacogenetics investigates the relationship between the genotype of an individual and the capacity to metabolize certain substances. CYPs exhibit significant polymorphism, with a few shared SNPs influencing gene expression and enzyme function [24].

The current work studied the association of *CYP3A4*1B* and *CYP3A5*3* polymorphisms with the clinical outcome of Egyptian CLL patients who received cyclophosphamide (CPA). We found that patients with variant alleles **1B*, **3*, or both were less likely to attain remission after chemotherapy than normal ones. The presence of *CYP3A4*1B* and *CYP3A5*3* carried a 9-fold and 14-fold risk of response failure, respectively.

The proportion of patients with variant genotypes in the non-response group was notably higher than that of patients with normal genotypes (82.3% vs. 17.7%; $p < 0.001$). The lower response rates in the individual with variant alleles could be explained by the fact that lowered enzyme activities caused by *CYP3A4*1B* and *CYP3A5*3* polymorphisms had a negative impact on activating the conversion of CPA to its active form, which further affects the rate of formation of the phosphoramide mustard, the primary metabolite responsible for the DNA alkylating activity of cyclophosphamide [4].

CYP3A4 and CYP3A5 are similar in more than 85% of the primary amino acid sequence, which causes related substrate selectivity between them; only limited substrate differences were observed [25]. However, combining variants in both genes is predicted to increase this risk.

The assessment of remission is based on the recovery of hemopoietic function. Consequently, the lower response rate observed in those with the variant genotypes likely represents increased hematologic toxicity rather than worse cytoreduction; an effort was made to ascertain whether this effect was frank [18]. The higher residual tumor burden (lymphocytosis, lymphadenopathy, and hepatosplenomegaly) and the post-treatment values of Hb, neutrophils, and platelets recorded in the SD and PD groups excluded this possibility.

The *CYP3A4*1B* and *CYP3A5*3* variations were significant genetic contributions to the inter-individual variation in CYP3A-dependent drug metabolism in leukemic individuals, consistent with our findings. Individuals with genotypes that indicate poor or intermediate metabolism and lower metabolic activity might be unable to respond to the treatment. Furthermore, the drug will build up in the bodies of these patients and eventually become a carcinogen when used at a regular dosage. Alternatively, a poor prognosis and drug resistance can result from treating these patients with reduced doses of the drug. As a result, care must be taken with these patients [4].

According to the Gor *et al.* [26] study, women with the *CYP3A4 *1A*/*1B* genotype who received adjuvant chemotherapy based on CPA for breast cancer had substantially worse disease-free survival compared to those with the wild-type genotype *CYP3A4 *1A*/*1A* (OR = 2.4). This finding supports our theory that a worse outcome after adjuvant treatment based on CPA is caused by decreased phase I enzymatic activity (via the *CYP3A4*1B* polymorphism), most likely due to delayed CPA activation [26].

Petros *et al.* [27] examined a large panel of phases I and II drug metabolizing enzyme variants in 85 patients with metastatic and inflammatory breast cancer who were

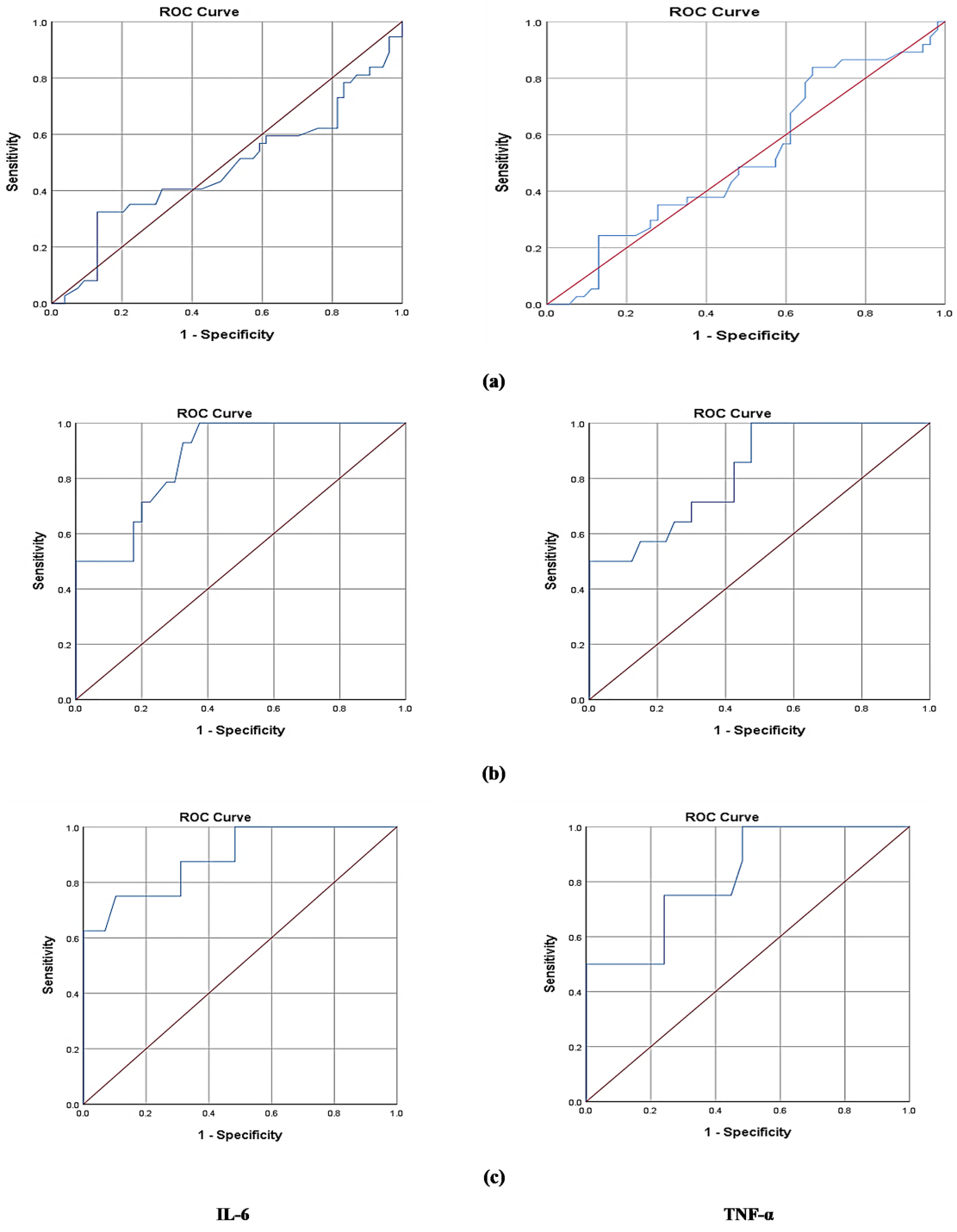

Fig. 5. Receiver operating characteristic (ROC) for IL-6 and TNF-α to discern between (a) response and non-response, (b) CR and PR groups, and (c) SD and PD groups.

receiving high doses of CPA. Petros *et al.* [27] also discovered that patients with *CYP3A4*1B* or *CYP3A5*3* variant alleles had increased levels of parent CPA and inferior overall survival ($p = 0.043$ for both) [27].

After drug delivery, specific metabolic clearance routes are quantified to evaluate the phenotypic activity of CYPs. Phenoconversion is the term for the situation where there is a discrepancy between the observed phenotype and the projected phenotype based on genotyping data. Genetic testing can help predict the activities of CYPs and the phenotypes of patients. Phenoconversion can affect most CYPs for a variety of factors, including age, sex, inflammation, use of specific drugs, and diet [13].

Inflammation is a key non-genetic variable that can significantly affect drug metabolism. This is primarily due to the suppression of CYPs, drug-metabolizing enzymes,

which can cause a temporary phenoconversion. The inflammatory regulation of CYPs can occur via several mechanisms, including cytokine and CYP-specific mechanisms. For this reason, it was also relevant to consider the phenoconversion of these cytokines on the action of CYP enzymes in addition to the genotyping analysis [28].

Several researchers have established that IL-6 effectively inhibits the expression of CYP enzymes in PHHs and HepaRG cell lines. Tanner *et al*. [29] investigated using IL-6 to treat the HepaRG cell line and found that the most significant downregulation of *CYP3A4* was observed in response to IL-6; however, the other CYPs, most notably CYP2D6, appeared to be less affected [29].

IL-6 may use different pathways to affect hepatocytes, as it binds to its receptor and activates three cellular signaling pathways: the Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) pathway, the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway, and the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) pathway. By changing the phosphorylation state of the nuclear receptor proteins, pregnane X receptor (PXR) and constitutive androstane receptor (CAR), the protein kinases implicated in IL-6 signaling, such as protein kinase C and JNK, can suppress the function of the nuclear receptors and subsequently downregulate the expression of CYPs. Furthermore, the CCAAT/enhancer-binding protein beta (C/EBPβ)- liver-enriched transcriptional inhibitory protein (LIP) protein isoform is upregulated by the mitogen-activated protein kinase (MAPK)/ERK1/2 pathway, which subsequently aids in downregulating *CYP3A4* [14].

Additionally, hepatocytes express the tumor necrosis factor receptor 1 (TNFR1), which activates the MAPK/ERK pathway and the NF-κB pathway when bound by TNF-α [30]. Aitken and Morgan [31] discovered that *CYP3A4* mRNA was downregulated when treated with TNF-α, whereas *CYP2C9* and *CYP2C19* mRNA levels were unaffected.

IL-6 and TNF-α concentrations at the diagnosis were compared between response and non-response groups to determine whether their levels would further affect the clinical outcome of CLL patients following CPA treatment. No significant differences were noticed, and the ROC curves plotted to assess the validity of their levels for the discernment between response and non-response groups reflected poor accuracy (AUC values of 0.481 and 0.505, respectively). However, upon comparing the IL-6 and TNF-α values at diagnosis among the different response groups (CR, PR, SD, and PD), we discovered that these cytokines were notably more significant in the PR group than the CR and the PD groups compared to the SD.

The ROC curves for IL-6 and TNF-α reflected the reasonable accuracy of the test to discriminate between the CR and PR groups (AUC values of 0.87 and 0.83, respectively) and between the SD and PD groups (0.89 and 0.82, respectively). These observations lead us to conclude that the IL-6 and TNF-α concentrations were not determinant factors for response, but rather, these concentrations would suppress the activities of the CYP3A4 and CYP3A5 enzymes.

Furthermore, Rubin *et al*. [32] reported that *CYP3A4* mRNA levels and catalytic activity were suppressed when IL-6 was applied to HepaRG cells. Using cultivated human hepatocarcinoma FLC-4 cells, Mimura *et al*. [33] examined the effects of IL-6 and TNF-α on the expression of *CYP3A4* and discovered that these factors drastically lowered the *CYP3A4* mRNA level. Enokiya *et al*. [34] investigated the effect of IL-6 on *CYP3A4* and *CYP3A5* expression *in vitro* using HepG2 and Caco2 cells. IL-6 significantly decreased the expression of *CYP3A4* and *CYP3A5*, with concentration- and time-dependent effects noted in both cell lines [34].

5. Conclusion

*CYP3A4*1B* and *CYP3A5*3* polymorphisms were associated with an unfavorable cyclophosphamide response, indicating these polymorphisms might contribute to poor disease progression and therapeutic outcomes. Additionally, these polymorphisms might be a useful therapeutic marker for predicting how well CLL patients will respond to cyclophosphamide treatment. Individuals with such polymorphisms, specifically those with elevated IL-6 and TNF-α levels, may require aggressive treatment strategies and special consideration for experimental therapy. Neutralizing or blocking some cytokine receptors might decrease the resistance of CLL cells to chemotherapy. Personalized medicine, which aims to improve therapeutic doses for each patient by considering genetic and non-genetic factors that could affect the response, should consider cytochrome P450 variability.

Availability of Data and Materials

The dataset utilized in the preparation of this study will be available from the corresponding author upon reasonable request.

Author Contributions

HME and MAA were responsible for formal analysis, investigation, and statistical analysis. SME, AES and MMY were responsible for to the conception or design. SR was responsible for samples collections and data curation. AEM and ERS were responsible for methodology and investigation. MAA was responsible for writing original draft and visualization. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Informed consent was obtained from all participants in this work. All procedures accomplished in this work,

including those involving human subjects, were in accordance with the ethical guidelines of the institutional research committee at the Faculty of Medicine, Mansoura University (R.24.06.2649.R2) and with the 1964 Helsinki Declaration and its adjustments.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBS36269.

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