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Pharmacogenetic predictors of toxicity to platinum based chemotherapy in non-small cell lung cancer patients

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ABSTRACT

Platinum-based chemotherapy is the standard treatment for NSCLC patients with EGFR wild-type, and as alternative to failure to EGFR inhibitors. However, this treatment is aggressive and most patients experience grade 3–4 toxicities. ERCC1, ERCC2, ERCC5, XRCC1, MDM2, ABCB1, MTHFR, MTR, SLC19A1, IL6 and IL16 gene polymorphisms may contribute to individual variation in toxicity to chemotherapy. The aim of this study was to evaluate the effect of these polymorphisms on platinum-based chemotherapy in NSCLC patients. A prospective cohorts study was conducted, including 141 NSCLC patients. Polymorphisms were analyzed by PCR Real-Time with Taqman® probes and sequencing. Patients with ERCC1 C118T-T allele ($p = 0.00345$; RR = 26.05; $CI_{95\%}$ = 4.33, 515.77) and ERCC2 rs50872-CC genotype ($p = 0.00291$; RR = 4.06; $CI_{95\%}$ = 1.66, 10.65) had higher risk of general toxicity for platinum-based chemotherapy. ERCC2 Asp312Asn G-alelle, ABCB1 C1236T-TT and the IL1B rs12621220-CT/TT genotypes conferred a higher risk to present multiple adverse events. The subtype toxicity analysis also revealed that ERCC2 rs50872-CC genotype ($p = 0.01562$; OR = 3.23; $CI_{95\%}$ = 1.29, 8.82) and IL16 rs7170924-T allele ($p = 0.01007$; OR = 3.19; $CI_{95\%}$ = 1.35, 7.97) were associated with grade 3–4 hematological toxicity. We did not found the influence of ERCC1 C8092A, ERCC2 Lys751Gln, ERCC2 Asp312Asn, ERCC5 Asp1104His, XRCC1 Arg194Trp, MDM2 rs1690924, ABCB1 C3435T, ABCB1 Ala893Ser/Thr, MTHFR A1298C, MTHFR C677T, IL1B rs1143623, IL1B rs16944, and IL1B rs1143627 on platinum-based chemotherapy toxicity. In conclusion, ERCC1 C118T, ERCC2 rs50872, ERCC2 Asp312Asn, ABCB1 C1236T, IL1B rs12621220 and IL16 rs7170924 polymorphisms may substantially act as prognostic factors in NSCLC patients treated with platinum-based chemotherapy.

1. Introduction

Lung cancer is the most common diagnosed type of cancer, being the second tumor in incidence, after prostate and breast, respectively. Estimated cases in 2016 are 117,920 in men and 106,470 in women, with an approximate incidence of 14% [1]. This tumor represents the first cause of cancer death worldwide in both genders [1]. According to the latest cancer statistics, over 224,390 new cases and 158,080 deaths are expected to occur in the United States in 2016.

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The most important types of lung cancer are small cell lung cancer and non-small cell lung cancer (NSCLC). NSCLC accounts for approximately 80–85% of all lung cancer cases and is divided into different subtypes: squamous cell carcinoma, adenocarcinoma and large cell carcinoma. At the time of diagnosis, most patients with NSCLC have advanced stage (IIIB–IV), according to American Joint Committee on Cancer (AJCC) [2–4]. Therefore, five-year survival is low with rates of 5% for IIIB and 1% for IV stages [2–4].

Platinum-based doublet-chemotherapy is the standard treatment for NSCLC for EGFR wild-type patients, and as second line in mutated EGFR patients [5]. It is frequently given together with other agents, such as anti-microtubule agents (taxanes and vinca alkaloids), antifolate agents (pemetrexed), or pyrimidine antagonists (gemcitabine). Platinum-based chemotherapy has showed benefits in terms of survival (10.7 months vs 3.9 months, respectively; $p < 0.001$) and symptom control compared with best supportive care [6,7]. However, it is a very aggressive treatment, which presents high percentages of severe adverse events, such as asthenia (44.0%), gastrointestinal toxicity (33.3%), hematological toxicity (67.1%), neurotoxicity (69.9%) and nephrotoxicity (20–30%) [8–10]. This toxicity profile varies from person to person. Various studies have reported that this inter-individual differences may be due to genetic factors, such as single nucleotide polymorphisms (SNPs), which are involved in platinum pharmacodynamics, metabolism and mechanism of action [11–26].

Cisplatin and carboplatin are the main platinum compounds used on NSCLC therapy. They are heavy metal complexes that interact with DNA, forming platinum-DNA adducts, which result in severe local distortions of the DNA double helix [27,28]. Therefore, this interaction leads to DNA damage, inhibiting DNA replication and transcription and inducing apoptosis. Several pathways are activated in response to this interaction, which include DNA repair and p53 pathways. Deactivation of platinum drugs increase the activity of DNA repair pathways, which involve nucleotide-excision repair (NER), base excision repair (BER), and double-strand break repair (DSB). There are a great variety of proteins involved in detecting and repairing these adducts, such as excision repair cross-complementing group 1 (ERCC1), excision repair cross-complementation group 2 (ERCC2, also known as XPD), excision repair cross-complementation group 5 (ERCC5) and X-ray repair complementing defective repair in Chinese hamster cells 1 (XRCC1) [29,30]. Cell cycle control and apoptosis initiation is mediated by p53 pathway [31]. MDM2 proto-oncogene, E3 ubiquitin protein ligase (MDM2) plays a crucial role in this pathway, because it interacts with p53, leading to its ubiquination and degradation [32]. Genetic alterations, such as SNPs in any of this genes may modulate repair function and apoptosis, promoting individual variation in the toxicity to platinum-based chemotherapy [11–15].

Other mechanisms involved in platinum toxicity are drug transporters, folate metabolism and cytokine signaling [16–24]. Drug transporters are responsible of pumping out the cell platinum compounds [33–35]. The main gene involved in this process is ATP-binding cassette, sub-family B (MDR/TAP), member 1 (ABCB1, also called MDR1). Polymorphisms in this gene may alter its function and expression, leading to an accumulation of platinum drugs outside the cells [36]. Thus, genetic alterations in this gene may affect the inter-individual toxicity profile of platinum-based chemotherapy. Folate metabolism also plays an essential role on platinum cytotoxicity. Polymorphisms in genes involved in this pathway, such as methylenetetrahydrofolate reductase (MTHFR) and methionine synthase (MTR) modify methylation of DNA [37–41]. In fact, genetic alterations in these genes have been associated with lower enzyme activity and its results have been correlated with DNA hypomethylation, which alter sensitivity of tumor cells to platinum compounds [23,25,26,42]. Other gene involved in folate metabolism is the solute carrier family 19 (folate transporter), members 1 (SLC19A1). This protein transports folate drugs into the cell, such as pemetrexed, a drug that is frequently given in combination with platinum compounds [20,43]. Polymorphisms in this gene may alter the cellular entry of this drug and subsequently affects the cytotoxicity of platinum-pemetrexed based chemotherapy [18–22]. Citokine signaling has also showed an association with tumor progression [44,45]. Several studies have reported a connection between chronic inflammation and early stage of neoplastic development [46]. Innate immune cells are activated to battle infection as a physiological process. However, if this damaged becomes chronic, it could lead to a continuous cellular proliferation and subsequently initiates metaplasia and dysplasia [44,45]. A family of cytokines, which are named interleukins (ILs), induce growth, differentiation and activation of immune cells [47,48]. Moreover, they inhibit apoptosis of malignant cells at the site of inflammation [48]. In NSCLC, IL1B, IL6 and IL16 have recently showed a relevant impact on clinical outcomes for patients treated with platinum-based chemotherapy [16,17].

Based on above, we conducted this study to evaluate the effects of ERCC1, ERCC2, ERCC5, XRCC1, MDM2, ABCB1, MTHFR, SLC19A1, IL1B, IL6 and IL16 gene polymorphisms in toxicity to platinum-based chemotherapy in NSCLC patients.

2. Material and methods

A prospective cohorts study was conducted.

2.1. Study population

This study was performed at Complejo Hospitalario Universitario de Granada (CHUG), Granada, Spain. Between December 2012 and January 2016, 141 NSCLC patients $\geq$18 years diagnosed histologically or cytologically as NSCLC (stages I–IV) were enrolled in the study. The eligible patients were those with normal results of hematological function (hemoglobin > 9 g/dl, neutrophil count > 1500/mm^3, and platelet count > 100000/mm^3), liver function (bilirubin <1.5 times the normal upper limit, aspartate aminotransferase and alanine aminotransferase <2.5 times the normal upper limit), renal function (creatine clearance rate >50 ml/s) and measurable disease by chest computed tomography (CT) scan.

All patients were treated intravenously with cisplatin or carboplatin in combination with a third-generation drug (gemcitabine, paclitaxel, pemetrexed and vinorelbine) according to the National Comprehensive Cancer Network (NCCN) guidelines [5]. Hematology and biochemistry analyses were done at the end of each cycle. Based on NCCN version 4. 2016 guideline, patients with lymph node metastasis (N2–N3) and distant metastasis (M1) were not surgical candidates, although after neoadjuvant chemotherapy patients with potentially resectable N2 NSCLC were candidates for surgery [5]. Adjuvant chemotherapy was administered in patients with ECOG 0-1. Patients with unresectable stage IIIA NSCLC were candidates for chemoradiotherapy [5].

EGFR status was measured by cobas® EGFR Mutation Test. This study was approved by the CHUG Ethics and Research Committee and was performed conform the declaration of Helsinki. All patients signed an informed consent form for blood sample collection.

2.2. Sociodemographic and clinical variables

Sociodemographic and clinical data were collected by reviewing clinical records. Clinical and histopathological data collected were: gender, family history of cancer, previous non-lung cancer, previous lung disease, smoking status, age, histology, tumor stage, chemotherapy agents, surgery, concomitant or concurrent radiotherapy, EGFR status and response.

The staging system used to classified tumor was based on the guidelines of the AJCC [49]. Platinum-based chemotherapy response was evaluated based on the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (Version 1.1) [50]. Patients classified as complete response plus partial response were catalogued as responders to treatment and stable disease plus progressive disease as non-responders.

Non-patient identifiers were applied to identify samples and they were preserved confidentially.

2.3. Genetic variables

2.3.1. DNA isolation

DNA was extracted using the QIAamp DNA Mini Kit (QiagenGmBH, Hilden, Germany) according to the manufacturer's instructions for DNA purification from blood and stored at −40 °C.

2.3.2. Detection of gene polymorphisms

ERCC1 C118T (rs11615), ERCC1 C8092A (rs3212986), ERCC2 Lys751Gln (rs13181), ERCC2 Asp312Asn (rs1799793), ERCC2 (rs50872) ERCC2 (rs238416), ERCC5 His46His (rs1047768), ERCC5 Asp1104His (rs17655), XRCC1 Arg194Trp (rs1799782), XRCC1 Gln399Arg (rs25487), MDM2 (rs1470383), MDM2 (rs1690924), ABCB1 C3435T (rs1045642), ABCB1 C1236T (rs1128503), MTHFR A1298C (rs1801131), MTHFR C677T (rs1801133), MTR (rs1805087), SLC19A1 Arg27His (rs1051266), IL1B (rs12621220), IL1B (rs1143623), IL1B (rs16944), IL1B (rs1143627), IL6 (rs1800795), IL16 (rs7170924) gene polymorphisms were analyzed by Real-Time PCR using TaqMan® probes. ABCB1 Ala893Ser/Thr (rs2032582) gene polymorphism was evaluated by sequencing. Genotyping methodology was previously described [51].

2.4. Toxicity variables

Toxicity was evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 [52]. Based on the severity of adverse events, it was classified into grade 0–2 and 3–4. General toxicity was defined as 3–4 when at least one adverse event was present in grade 3–4, and 0–2 otherwise. The occurrence of more than one and more than two adverse events was also analyzed.

The types of adverse events collected were: asthenia, gastrointestinal toxicity, hematological toxicity, infection, neurotoxicity and nephrotoxicity.

2.5. Statistical analysis

Quantitative data were expressed as the mean (±standard deviation) for normally-distributed variables or medians and percentiles (25 and 75) for non-normal distributed variables. The Shapiro-Wilks test was used to assess normality.

The bivariate association between toxicity and polymorphisms was assessed using the Pearson's chi-square or Fisher's exact test, and evaluated by relative risk (RR) and their corresponding 95% confidence intervals (CI).

Logistic regression model (backward stepwise method) was used to determine the influence of polymorphisms on toxicity.

All tests were two-sided with a significant level of $p < 0.05$. Data analysis was performed using R 3.0.1 [53].

Hardy Weinberg equilibrium and pairwise haplotype frequencies were estimated using the free, open-source whole genome association analysis toolset PLINK [54].

3. Results

3.1. Patients characteristics

The study comprised a total of 141 NSCLC Caucasian patients. The baseline characteristics are summarized in Table 1. Mean age was 61 [52,67] years, 104 were male (104/141; 73.76%) and 100 stage IIIB–IV (100/141; 70.92%). All patients received platinum-based chemotherapy in combination with gemcitabine (21/141; 14.89%), paclitaxel (33/141; 23.40%), pemetrexed (37/141; 26.24%) or vinorelbine (50/141; 35.46%). During follow-up, grade 3–4 toxicity profile presented was: 29.79% (42/141) general toxicity, 2.13% (3/141) asthenia, 2.13% (3/141) gastrointestinal toxicity, 24.11% (34/141) hematological toxicity, 3.55% (5/141) infection, 2.13% (3/141) nephrotoxicity and 0.71% (1/141) neurotoxicity.

3.2. Influence of clinico-pathologic characteristics on toxicity

General toxicity was associated with previous non-lung cancer ($p = 0.007653$; RR = 7.99; $CI_{95\%} = 1.73–36.8$; Table S1) and chemotherapy agents ($p = 0.0002537$; Table S1). Hematological toxicity was also associated with previous non-lung cancer ($p = 0.03109$; RR = 6.43; $CI_{95\%} = 1.18-34.91$; Table S2) and chemotherapy agents ($p = 0.002183$; Table S2). Nephrotoxicity was associated with histology ($p = 0.0486$; Table S3). Infection was associated with response to platinum-based chemotherapy ($p = 0.02655$; RR = 9.56; $CI_{95\%} = 1.07–85.21$; Table S4).

Asthenia, gastrointestinal toxicity, neurotoxicity and the occurrence of multiple adverse events were not associated with clinical or demographic characteristics (Tables S5–S9).

3.3. Genotypes distribution

All gene polymorphisms distributions were in agreement with those expected according to the Hardy-Weinberg equilibrium model. Linkage disequilibrium values D' and r^2 are shown in Table S10.

3.4. Influence of gene polymorphisms on toxicity

3.4.1. General toxicity

ERCC1 C118T, ERCC2 rs50872 and ABCB1 C1236T were associated with general toxicity. Patients with CT/TT genotypes for ERCC1 C118T ($p = 0.01864$; RR = 6.78; $CI_{95\%} = 1.38$, 33.4; Table S11), CC for ERCC2 rs50872 ($p = 0.02336$; RR = 2.03; $CI_{95\%} = 1.1$, 3.74; Table S11) or TT for ABCB1 C1236T ($p = 0.01483$; RR = 2.08; $CI_{95\%} = 1.15$, 3.75; Table S11) were in higher risk of grade 3–4 toxicity. Logistic regression analysis adjusted by chemotherapy agents and previous non-lung cancer revealed that ERCC1 C118T-T allele and ERCC2 rs50872-CC genotype were the only genetic independent factors associated with higher general toxicity ($p_{likelihoodratiotest} = 1.898 \cdot 10^{-7}$; Table 2).

ERCC2 Asp312Asn and IL1B polymorphisms were associated with multiple adverse events (>1). In particular, ERCC2 Asp312Asn ($p = 0.03778$; Table S12), IL1B rs12621220-CT/TT ($p = 0.01627$; RR = 1.35; $CI_{95\%} = 1.06$, 1.72; Table S12), IL1B rs1143623-CG/GG ($p = 0.01627$; RR = 1.35; $CI_{95\%} = 1.06$, 1.72; Table S12), IL1B rs16944 ($p = 0.02977$; Table S12) or IL1B rs1143627 ($p = 0.04501$; Table S12) were in risk of experiencing more than one adverse event. A logistic regression analysis revealed that the AG genotype for ERCC2 Asp312Asn and CT/TT for IL1B-rs12621220 were independently associated with the occurrence of more than one adverse event ($p_{likelihoodratiotest} = 0.0009103$; Table 3). Similarly, carriers of ABCB1 C1236T-TT genotype ($p = 0.009664$; RR = 2.32; $CI_{95\%} = 1.23$, 4.39; Table S13), ERCC2 Asp312Asn ($p = 0.04637$; Table S13) or XRCC1 Gln399Arg-AG/GG genotype ($p = 0.04589$; RR = 5.79; $CI_{95\%} = 1.03$,

Table 1
Clinico-pathologic characteristics of the 141 NSCLC patients treated with platinum based chemotherapy.

	n	%
Gender		
Female	37	25.24
Male	104	73.76
Family history of cancer		
YES	85	60.28
NO	56	39.72
Previous non-lung cancer		
YES	23	16.31
NO	118	83.69
Previous lung disease		
YES	34	24.11
NO	107	75.89
Smoking status		
Current-Smokers	72	51.06
Former-smokers	52	36.88
Non-smokers	17	12.06
Age at NSCLC diagnosis	61 [52,67]	
≤60	68	48.23
>60	73	51.77
Histology		
Adenocarcinoma	87	61.70
Squamous cell carcinoma	51	36.17
Unknown	3	2.13
Tumor stage		
I, II or IIIA	41	29.5
Neoadjuvant Chemotherapy	20	48.78
Adjuvant Chemotherapy	16	39.02
Chemoradiotherapy	5	12.20
IIIB or IV	98	70.5
Chemoradiotherapy	37	37.76
Palliative	61	62.24
Chemotherapy agents		
Gemcitabine	21	14.89
Paclitaxel	33	23.40
Pemetrexed	37	26.24
Vinorelbine	50	35.46
Surgery		
YES	33	23.4
NO	108	76.6
Radiotherapy		
YES	45	31.91
NO	96	68.09
EGFR status		
Wild-type	72	51.06
Mutated	16	11.35
Unknown	53	37.59
Response		
CR	38	27.34
PR	60	43.17
SD	8	5.76
PD	33	23.74
Response/No response (divided by stage)		
I, II or IIIA		
Neoadjuvant Chemotherapy	18/2	90.0/10.0
Adjuvant Chemotherapy	15/1	93.8/6.2
Chemoradiotherapy	3/2	60.0/40.0
IIIB or IV		
Chemoradiotherapy	30/7	81.1/18.9
Palliative	31/29	51.7/48.3
Toxicity		
Grade 0–2	99	70.21
Grade 3–4	42	29.79

Qualitative variables: number (percentage).
Quantitative variables.
Normal distribution: mean ± standard deviation.
Non-normal distribution: P_{50} [P_{25}, P_{75}].
CR: Complete Response; PR: Partial Response; SD: Stable Disease; PD: Progressive Disease.

Table 2
Multivariate regression analysis for general toxicity according to clinical characteristics and gene polymorphisms.

	General Toxicity	
	OR ($CI_{95\%}$)	p
Chemotherapy agents		
Gemcitabine (Reference)		
Paclitaxel	0.13 (0.02, 0.52)	0.00587
Pemetrexed	0.10 (0.02, 0.38)	0.00147
Vinorelbine	0.27 (0.07, 0.91)	0.04098
Previous non-lung cancer		
No	11.57 (2.09, 217.62)	0.02245
ERCC1 C118T-CT/TT	26.05 (4.33, 515.77)	0.00345
ERCC2 rs50872-CC	4.06 (1.66, 10.65)	0.00291

32.47; Table S13), showed higher risk of experiencing more than two adverse events. Logistic regression analysis revealed that the G-allele of ERCC2 Asp312Asn and the TT genotype of ABCB1 C1236T were the only independent factors associated with higher risk of more than two adverse events ($p_{likelihoodratiotest} = 0.0007531$; Table 3).

3.4.2. Hematological toxicity

ERCC2 rs50872 and IL16 rs7170924 presented influence on hematological toxicity (Table S14). In fact, patients carrying the CC genotype for ERCC2 rs50872 (p = 0.02228; RR = 2.34; $CI_{95\%}$ = 1.13, 4.85; Table S17) or IL16 rs7170924 T-allele (p = 0.02772; RR = 2.06; $CI_{95\%}$ = 1.08, 3.92; Table S17) presented higher grade of hematological toxicity. A multivariate logistic regression analysis adjusted by chemotherapy agents was used to further investigate the impact of ERCC2 rs50872 and IL16 rs7170924 polymorphisms on hematological toxicity. Both remaining significantly associated to grade 3–4 hematological toxicity ($p_{likelihoodratiotest} = 6.861 \cdot 10^{-5}$; Table 4).

3.4.3. Other toxicity subtypes

The bivariate analysis showed that higher grade of asthenia was associated to IL16 rs7170924 (TT > GT > GG) polymorphism (p = 0.03769; Table S15). No association was found for other genetic or clinical variables. ERCC5 His46His and SLC19A1 Arg27His were associated with gastrointestinal toxicity. Particularly, patients carrying the CC genotype for ERCC5 His46His (p = 0.02699; Table S16) or AA for SLC19A1 Arg27His (p = 0.01431; Table S16) showed greater risk of gastrointestinal toxicity. No association was reached on multivariate logistic regression model. ERCC2 rs238416 was the only polymorphism that showed influence on grade 3–4 infection. Particularly, carriers of AA genotype showed greater risk of grade 3–4 infection to those carrying the G-allele (p = 0.01767; RR = 9.63; $CI_{95\%}$ = 1.56, 59.6; Table S17). No multivariate regression analysis was investigated. The bivariate analysis revealed that IL6 rs1800795-CC, MTR rs1805087-AG/GG and XRCC1 Gln399Arg-GG genotypes were associated with severe nephrotoxicity (p = 0.04286 for IL6 rs1800795, p = 0.0251 for MTR rs1805087 and p = 0.03836 for XRCC1 Gln399Arg) (Table S18). However, multivariate logistic regression analysis did not revealed influence of these polymorphisms on severe nephrotoxicity. The only polymorphism associated with grade 3–4 neurotoxicity was MDM2 rs1470383, particularly the CC genotype (p = 0.04965; Table S19). No multivariate regression analysis was furtherly investigated.

4. Discussion

Platinum-based doublet chemotherapy remains as the standard treatment for NSCLC patients with EGFR wild-type, and as alternative to failure to EGFR inhibitors in mutated EGFR patients [5]. This highly aggressive treatment is generally accompanied by

Table 3
Multivariate regression analysis for multiple adverse events according to gene polymorphisms.

	Number of adverse events	
	OR ($CI_{95\%}$)	p
	>1 adverse events	
ERCC2 Asp312Asn		
AA	0.23(0.07–0.71)	0.01140
(Reference) AG		
GG	0.31(0.13–0.73)	0.00891
IL1B rs12621220-CT/TT	3.37(1.55–7.69)	0.00280
	>2 adverse events	
ERCC2 Asp312Asn-AG/GG	5.92(1.52–40.18)	0.026040
ABCB1 C1236T-TT	4.35(1.67–11.76)	0.002842

Table 4
Multivariate regression analysis for hematological toxicity according to clinical characteristics and gene polymorphisms.

	Hematological Toxicity OR ($CI_{95\%}$)	p
Chemotherapy agents		
Gemcitabine (Reference)		
Paclitaxel	0.15 (0.03, 0.54)	0.00559
Pemetrexed	0.11 (0.02, 0.42)	0.00218
Vinorelbine	0.36 (0.11, 1.10)	0.07525
ERCC2 rs50872-CC	3.23 (1.29, 8.82)	0.01562
IL16 rs7170924- GT/TT	3.19 (1.36, 7.97)	0.01007

severe adverse events (asthenia, gastrointestinal toxicity, hematological toxicity, neurotoxicity and nephrotoxicity) [8–10], a toxicity profile which great inter-individual differences probably due to genetic factors [11–24]. Several SNPS in different genes involved in platinum pharmacodynamics, metabolism and mechanism of action have been proposed as potential causes of this variability. In this study, 141 NSCLC patients from a single institution treated with cisplatin or carboplatin in combination with gemcitabine, paclitaxel, pemetrexed or vinorelbine were recruited to investigate the potential role of ERCC1, ERCC2, ERCC5, XRCC1, MDM2, ABCB1, MTHFR, MTR, SLC19A1, IL1B, IL6 and IL16 gene polymorphisms in chemotherapy toxicity. In these patients, ERCC1 C118T-T allele and ERCC2 rs50872-CC genotype acted as predictors of occurrence of at least one case of grade 3–4 adverse events, along with gemcitabine as combination agent and non-history of previous non-lung cancer (Table 2). Despite the influence of ERCC1 gene polymorphisms on toxicity has been widely investigated in NSCLC, no association has been previously reported [11,24,56–68]. Only a study of 81 Caucasian patients diagnosed with late-stage solid tumors (55.6% lung cancer) has reported a role of ERCC1 gene polymorphisms as predictors of cisplatin-induced nephrotoxicity [69]. In particular, patients carrying the T-allele of ERCC1 rs11615 showed a 12.8% mean decrease in estimated glomerular filtration rates ($p = 0.047$) [69]. The effect of ERCC2 rs50872 polymorphism on platinum-based chemotherapy toxicity had not been previously investigated in NSCLC, although C-allele was associated to higher median overall survival (OS) and progression-free survival (PFS) ($p = 0.009$ and $p = 0.032$, respectively) in 129 unresectable Asian NSCLC patients [70]. In our patients, the occurrence of multiple adverse events was influenced by ERCC2 Asp312Asn G-alelle, ABCB1 C1236T-TT genotype and IL1B rs12621220C-allele (Table 3). Studies with large samples have not reported association between ERCC2 Asp312Asn and toxicity [58–60,64–67,71,72]. However, the G-allele for ERCC2 Asp312Asn was associated with lower frequency of severe hematological toxicity in 55 Polish stage IIIB-IV NSCLC patients (OR = 0.08; $CI_{95\%}$ = 0.01, 0.40; $p = 0.0005$; AG/GG vs AA) [11]. The effect of ABCB1 C1236T polymorphism on toxicity has not previously described, although the CC genotype has been associated with lower OS and PFS in previous studies [73,74]. The influence of IL1B-rs12621220 on platinum-based chemotherapy toxicity has not either been previously investigated, although the heterozygous variant has recently been associated with decreased risk of NSCLC in patients over age 63 from a study including 889 lung cancer and 1005 controls (OR = 0.71; $CI_{95\%}$ = 0.52-0.98; $p = 0.04$) [75].

Since platinum-based chemotherapy present a wide toxicity profile, we analyzed different subtypes of toxicity: asthenia, gastrointestinal toxicity, infection, hematological toxicity, nephrotoxicity and neurotoxicity. According to our results, patients carrying IL16 rs7170924 T-allele were in higher risk of hematological toxicity (OR = 3.19; $CI_{95\%}$ = 1.35, 7.97; Table 4). No studies have evaluated the impact of IL16 rs7170924 polymorphism on this toxicity subtype. However, a protective effect of the IL16 rs7170924 T-allele on PFS has been reported in 651 Caucasian stage I–IV NSCLC patients (HR = 0.65; $CI_{95\%}$ = 0.50, 0.83 for GT/TT vs GG) [17]. In our patients, hematological toxicity adjusted by chemotherapy agents was also associated with ERCC2 rs50872 polymorphism. Particularly, the CC genotype was associated with higher risk of hematological toxicity (OR = 3.23; $CI_{95\%}$ = 1.29, 8.82; Table 4). No studies have been published related to the effect of ERCC2 rs50872 polymorphism on hematological toxicity. In our patients, although some gene polymorphisms were associated to particular subtypes of toxicity in the bivariate analysis (ERCC2 rs238416, ERCC5 His46His, XRCC1 Gln399Arg, MDM2 rs1470383, MTR rs1805087, SLC19A1 Arg27His, IL6 rs1800795), the independency of those associations could not be analyzed, due to the insufficient number of patients who presented severe asthenia (3) gastrointestinal toxicity (3), infection (5), nephrotoxicity (3) and neurotoxicity (1). Other studies have attempted to find a role for MDM2 rs1470383, ERCC5 His46His, SLC19A1 Arg27His and XRCC1 Gln399Arg on toxicity to platinum-based chemotherapy, not always successfully. Whereas MDM2 rs1470383 has been proposed as a risk factor for hematological chemotherapy-related toxicity (OR = 4.10; $CI_{95\%}$ = 1.73, 9.71 for CC vs TT) [15], no significant association was found for ERCC5 His46His in 74 Spanish stage IIIA-IV NSCLC patients [64] or for SLC19A1 Arg27His in 94 Caucasian stage IIIB-IV NSCLC patients [20]. The role of XRCC1 Gln399Arg on toxicity to platinum-based chemotherapy in NSCLC patients has showed conflicting results. Although no effect on nephrotoxicity was found in 55 Caucasian stage IIIB-IV NSCLC patients [11,13,14], the GG genotype has been associated with other subtypes of toxicity, specifically, increased hematological toxicity (OR = 0.323; $CI_{95\%}$ = 0.121, 0.862 for AG/AA vs GG and OR = 0.22; $CI_{95\%}$ = 0.06, 0.82 for AG/AA vs GG) and gastrointestinal toxicity (OR = 0.298; $CI_{95\%}$ = 0.108, 0.825 for AG/AA vs GG) [11,13,14]. On the contrary, other study in 487 Chinese stage IIIA–IV NSCLC patients has shown a protective effect of GG genotype for hematological toxicity (OR = 2.135; $CI_{95\%}$ = 1.207, 3.777 for AG/GG vs GG) [14]. The influence of ERCC2 rs238416, MTR rs1805087 and IL6rs1800795 gene polymorphism on toxicity to chemotherapy in NSCLC patients has not been previously evaluated.

In our study, ERCC2 Lys751Gln polymorphism showed no influence on toxicity, in consonance with other studies developed in larger samples (up to 493 patients) [57–61,64–67,71,72,76,77]. However, small studies developed have reported an association with hematological toxicity and nephrotoxicity [11,24]. The A-allele for ERCC2 Lys751Gln polymorphism was associated with increased grade 2–3 neutropenia ($p = 0.04$) in 62 Spanish stage IIIB-IV NSCLC patients [24] and severe nephrotoxicity in 55 Polish late-stage NSCLC patients (OR = 0.07; $CI_{95\%} = 0.02$, 0.31 for AC/CC vs AA) [11]. ERCC5 Asp1104His was not either associated with toxicity in our patients, despite a previous study with 388 Chinese NSCLC patients had proposed ERCC5 rs17655 as a risk factor for infection ($p = 0.017$) [12]. Similarly, no association was found between ABCB1 C3435T, ABCB1 Ala893Ser/Thr and XRCC1 Arg194Trp in our patients, which is in consonance with previous studies [11,56,64,66,73,78–83]. MDM2 rs1690924-GG genotype has been related to lower gastrointestinal toxicity in 663 Asian stage IIIA-IV NSCLC patients (OR = 2.32; $CI_{95\%} = 1.30$, 4.14 for AG vs AA) [15]. However, MDM2 rs1690924 did not influence toxicity in our study. A similar outcome was observed for polymorphisms in MTHFR, which did not demonstrate an association with toxicity in our patients, despite a previous study in 1004 Chinese stage III–IV NSCLC patients reported that MTHFR C677T-CC and MTHFR A1298C-AA genotype were predictors of higher hematological toxicity (OR = 0.40; $CI_{95\%} = 0.19$, 0.85 for CT vs CC and OR = 0.40; $CI_{95\%} = 0.22$, 2.23 for AC vs AA, respectively) [23]. The effect of IL1B rs1143623, rs16944 and rs1143627 on platinum-based chemotherapy toxicity, which showed no significant association in the present study, has not been previously evaluated.

The patients enrolled for this study were recruited from one hospital, which ensures the homogeneity of the sample, regarding individuals, treatment administered and measure of the toxicity variables. The main limitation of this study is the sample size, which may have been responsible for the lack of association of some SNPs, particularly when investigating those toxicity subtypes which occurred in a very low number of patients. Despite of this, the prognostic value of polymorphisms in ERCC1, ERCC2, ABCB1, IL1B and IL16 genes to predict severe toxicity was evident.

These results suggested that ERCC1 C118T, ERCC2 Asp312Asn, ERCC2 rs50872, ABCB1 C1236T, IL1B rs12621220 and IL16 rs7170924 polymorphisms may substantially act as prognostic factors in NSCLC patients treated with platinum-based chemotherapy. These associations should be validated in a new, independent cohort of patients. Furthermore, the influence of ERCC2 rs238416, ERCC5 His46His, XRCC1 Gln399Arg, MDM2 rs1470383, MTR rs1805087, SLC19A1 Arg27His, IL6 rs1800795 gene polymorphisms on toxicity to chemotherapy in NSCLC patients should be elucidated in further studies with larger samples.

5. Conclusions

Our results suggest that NSCLC patients with ERCC1 C118T-T allele and ERCC2 rs50872-CC genotype have higher risk of general toxicity for platinum-based chemotherapy. The G-alelle of ERCC2 Asp312Asn, ABCB1 C1236T-TT and the IL1B rs12621220-CT/TT genotypes conferred a higher risk to present multiple adverse events. The subtype toxicity analysis also revealed ERCC2 rs50872-CC genotype and IL16 rs7170924-T allele were associated with grade 3–4 hematological toxicity. No influence of ERCC1 C8092A, ERCC2 Lys751Gln, ERCC5 Asp1104His, XRCC1 Arg194Trp, MDM2 rs1690924, ABCB1 C3435T, ABCB1 Ala893Ser/Thr, MTHFR A1298C, MTHFR C677T, IL1B rs1143623, IL1B rs16944, and IL1B rs1143627on platinum-based chemotherapy toxicity was found in our patients.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phrs.2016.08.002.

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