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The role of single-nucleotide polymorphism (SNPs) in toxicity of induction chemotherapy based on cisplatin and paclitaxel in patients with advanced head and neck cancer

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ABSTRACT

Background: Induction chemotherapy in locally-advanced head and neck squamous cell carcinoma (LAHNSCC) patients is potentially associated to serious adverse events. Biomarkers associated with toxicity could tailor its indication. This study evaluated the association between single-nucleotide polymorphisms (SNPs) in metabolic genes and toxicity to induction chemotherapy.

Methods: 59 LAHNSCC phase II clinical trial patients (NCT00959387) were assessed regarding 47 metabolic genes (366 SNPs). Toxicities were graded (CTCAE 3.0) and statistical analysis was performed.

Results: The SNPs rs8187710 (*ABCC2*) and rs1801131 (*MTHFR*) were associated to increased risk of gastrointestinal toxicity, whereas the SNPs rs3788007 (*ABCG1*) and rs4148943 (*CHST3*) were associated to decreased risk. Two other SNPs, rs2301159 (*SLC10A2*) and rs2470890 (*CYP1A2*), were associated with increased risk of hematological toxicity. Nevertheless, these SNPs did not remain significant after adjusting for multiple comparisons.

Conclusions: This study could not demonstrate relationship between SNPs and toxicity to induction chemotherapy in LAHNSCC patients. The small number of patients may have affected the results.

Introduction

Head and neck cancer is the fifth most prevalent tumor in the world, being more common in men than women in the ratio of 3:1. The worldwide incidence is about 650,000 new cases per year, which represents almost 5% of the global incidence of cancer. It is responsible for more than 350,000 annual deaths [1]. Squamous cell carcinoma is the predominant histology, corresponding to 90% of the cases, and most of the patients are diagnosed with locally advanced disease - stages III and IVa/b. In this scenario, after concomitant chemoradiotherapy, 40–60% of patients present local recurrence, especially in the first two years, 20–30% develop metastatic disease, and only 30–50% of them are alive in 3 years [2–4].

The role of chemotherapy in the treatment of locoregional disease was evaluated in a meta-analysis with almost 20,000 patients that showed an increased survival when used concomitantly with radiotherapy (HR 0.81; $p < 0.001$), and a reduction in the incidence of distant metastases when used as induction therapy (HR 0.73, $p = 0.001$). However the benefit of induction chemotherapy preceding concomitant chemoradiotherapy still needs to be confirmed, and, mainly due to the high occurrence of serious adverse events with this strategy it is not recommended by guidelines such as National Comprehensive Cancer Network (NCCN) [5–9]. Considering that the toxicity of a given chemotherapy regimen varies widely among patients due to the inherited variability of genes that are involved in drug metabolism [10], the discovery of biomarkers related to toxicity to

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induction chemotherapy could save patients at high risk for its occurrence and avoid harm to concomitant treatment.

Single nucleotide polymorphisms (SNPs) are variations in the DNA sequence that occur in a single nucleotide. They are the most common genetic variations, accounting for 90% of all variations found in the genome, and appear on average every 300 bases, which translates into about 10 million SNPs among the 3 billion bases in the human genome. The vast majority of SNPs have no influence on human health or development. Some of them, however, have already been associated with individual responses to medications, susceptibility to environmental factors and toxins, risk for specific disease development and drug toxicity [11].

Although pharmacogenetic factors have the potential of being used to select patients with lower chance of having toxicity, no validated factors are currently available to improve treatment decision making [12,13]. The main objective of the present study was to identify SNPs in genes associated to drug metabolism or transport that could predict toxicity to induction chemotherapy with cisplatin and paclitaxel in LAHNSCC patients.

Materials and Methods

Patient samples

This study is based on the analysis of patients included in a Institution/Investigator-initiated phase II clinical trial (NCT00959387), designed to evaluated the toxicity and efficacy of a 2-drug regimen for induction chemotherapy followed by chemoradiation in LAHNSCC [14]. Germline DNA was available from whole blood of 59 of the 60 patients enrolled onto phase II clinical trial NCT00959387. Patients received 3 induction chemotherapy cycles of cisplatin 80 mg/m^2 and paclitaxel 175 mg/m^2 followed by concomitant chemoradiotherapy with 3 cycles of cisplatin 100 mg/m^2. The toxicities were classified and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Data on patient characteristics and toxicity were published elsewere [14]. This study was approved by the Institutional Review Board at Barretos Cancer Hospital (project number 436/2011). All patients provided written informed consent.

Selection of genes and SNPs

47 metabolic or transporters genes were selected (*ABCA1, ABCB1, ABCC1, ABCC10, ABCC2, ABCC3, ABCC4, ABCC6, ABCG1, ABCG2, ARNT, BLMH, CHST3, CLPTM1L, COMT, CYP1A1, CYP1A2, CYP1B1, CYP2B6, CYP2C19, CYP2C8, CYP2C9, CYP2D6, CYP3A4, CYP3A5, CYP4B1, DHFR, EPHX1, GALNT14, GSTA1, GSTM3, GSTP1, MTHFR, MTR, NAT2, OPRM1, PPARD, PRDX4, SCL10A2, SLC19A1, SLC22A2, SLC31A1, SLCO1B3, SOD1, SPG7, SULT1C4* and *TPMT*). These genes were chosen based on commercial genetic panels, such as DMET™ (Drug Metabolizing Enzymes and Transporters), Plus Solution (Affymetrix, Santa Clara, CA, USA) and Drug Metabolism PCR Array (SABioscience – Qiagen, Valencia, CA, USA).

Using the research tool of the University of California Santa Cruz (UCSC), based on the human genome version "Feb. 2009 (GRCh37/hg19)", and the SNPs database - dbSNP - from the National Center for Biotechnology Information (NCBI), 366 clinically relevant SNPs (flagged SNPs) on these genes were selected.

Genotyping

Genomic DNA of each patient was extracted from 200 µL of peripheral blood using the QIAsymphony Kit (Qiagen, Valencia, CA, USA) and then quantified using the Qubit™ fluorimeter (Life Technologies, Carlsbad, CA, USA). The enrichment process was performed according to the manufacturer's instructions (HaloPlex Target Enrichment System for Ion Torrent Sequencing, protocol version D.5, 2014, Agilent Technologies, Santa Clara, CA, USA). To validate the enrichment of the fragments generated by Haloplex, the Bioanalyzer High Sensitivity DNA Assay Kit (Agilent Technologies, Santa Clara, CA, USA) was used in the Bioanalyzer 2100 kit (Agilent Technologies, Santa Clara, CA, USA). Five runs of emulsion PCR (ePCR) were performed on the Ion OneTouchTM 2 System (Life Technologies, Carlsbad, CA, USA). The ePCR was performed with the Ion PGM™ Template OT2 200 kit (Life Technologies, Carlsbad, CA, USA).

The sequencing was performed using the Ion Torrent PGM (Life Technology, Carlsbad, CA, USA) and the kit used for sequencing was the Ion PGM™ Sequencing 200 kit v2 (Life Technologies, Carlsbad, CA, USA).

The data were analysed and processed by the software Torrent Suite v5.0.2 and using the Variant Caller tool the SNPs of interest were identified.

Statistical considerations

Patients were categorized as having or not each of the 366 polymorphisms. In regard of toxicity they were classified as present for toxicity ≥ grade 2. The specific toxicities studied were: peripheral neuropathy (motor or sensory), infectious complication, hematologic toxicity (febrile or afebrile neutropenia or anemia or lymphopenia or thrombocytopenia) and gastrointestinal toxicity (nausea or vomiting or diarrhea or constipation).

Statistical analyzes, regarding the association of SNPs with the toxicities to induction therapy, were performed using the statistical software IBM SPSS 19.0 for Windows and R mathematical statistical environment (v3.6). Single marker association analyzes were conducted using frequency comparison by chi-square or Fisher's Exact tests. Multivariate analyzes were performed through multiple logistic regression, including variables that presented $p < 0.2$ in univariate analyzes. Adjustment for multiple comparisons was performed using the Benjamini-Hochberg method for false discovery rate (FDR) [15]. Statistical significance was considered for $p < 0.05$.

Results

Clinical-pathological and SNP characteristics of patients included

Data from 59 patients were used to analyse. The median age was 56 years. There was a clear predominance of males (93.3%) and ECOG-PS 1 (88.1%). The main primary site of disease was oropharynx, corresponding to 52.5%, followed by larynx with 28.8%. The majority of patients presented clinical stage IV, being 66.1% of patients classified as resectable disease. Only 2 patients had no history of smoking (active or past), and among 51 patients tested for p16 (HPV status) only 2 were positive (both with smoking history). Table 1 summarizes the clinical-pathological characteristics of the patients included.

Of the 366 selected SNPs, 108 were found in at least one patient. Sixteen of them were found in a single patient and two of them were found in 55 patients.

SNPs and gastrointestinal toxicity

Gastrointestinal toxicity ≥ grade 2 was observed in 13 patients (22.0%). Univariate analysis showed 4 SNPs associated with gastrointestinal toxicity: rs8187710 (gene *ABCC2*; p = 0.010), rs12613732 (gene *GALNT14;* p = 0.044), rs1801131 (gene *MTHFR*; p = 0.047) and rs4148943 (gene *CHST3*; p = 0.046). None of the clinical variables (diabetes, ECOG-PS, age, dose reduction of cisplatin and dose reduction of paclitaxel) showed a significant association with this toxicity.

In the multivariate logistic regression analysis, the following SNPs showed a statistically significant association with the endpoint gastrointestinal toxicity: rs8187710 (gene *ABCC2*; OR = 33.212; 95%CI = 2.336–470.096; p = 0.010), rs1801131 (gene *MTHFR*;

Table 1
Clinicopathological characteristics of the patients included (59).

	No. of patients	(%)
Gender		
Male	**55**	**(93.3)**
Female	**4**	**(6.7)**
ECOG-PS		
0	**2**	**(3.4)**
1	**52**	**(88.1)**
2	**5**	**(8.5)**
Primary site		
Oral cavity	**3**	**(5.1)**
Oropharynx	**31**	**(52.5)**
Hypopharynx	**8**	**(13.6)**
Larynx	**17**	**(28.8)**
Tracheostomy		
Yes (*baseline*)	**2**	**(3.4)**
Differentiation		
G1	**8**	**(13.6)**
G2	**41**	**(69.5)**
G3	**9**	**(15.2)**
T classification		
T2	**4**	**(6.8)**
T3	**34**	**(57.6)**
T4	**21**	**(35.6)**
N classification		
N0	**13**	**(22.0)**
N1	**8**	**(13.6)**
N2	**29**	**(49.2)**
N3	**9**	**(15.2)**
TNM stage		
III	**18**	**(30.5)**
IV	**41**	**(69.5)**
Resectability		
Resectable	**39**	**(66.1)**
Unresectable	**20**	**(33.9)**
Smoking		
Active	**42**	**(71.2)**
Former	**15**	**(25.4)**
Never	**2**	**(3.4)**
HPV status		
Positive (p16+)	**2**	**(3.5)**
Negative (p16−)	**49**	**(83.0)**

OR = 17.692; 95%CI = 1.733–180.579; p = 0.015), rs3788007 (gene *ABCG1*; OR = 0.045, 95%CI = 0.003–0.599, p = 0.019), and rs4148943 (gene *CHST3*; OR = 0.05, 95%CI = 0.004–0.634, p = 0.021) [Table 2]. Nevertheless, these SNPs did not remain significant after adjusting for multiple comparisons.

SNPs and hematological toxicity

Hematological toxicity ≥ grade 2 was observed in 12 patients (20.3%). Univariate analysis showed that 4 SNPs were significantly associated with hematological toxicity: rs16950650 (gene *ABCC4*; p = 0.039), rs717620 (gene *ABCC2*; p = 0.043), rs2301159 (gene *SLC10A2*; p < 0.010), and rs2470890 (gene *CYP1A2*; p = 0.041). As for gastrointestinal toxicity none of the clinical variables (diabetes, ECOG-PS, age ≥ 60 years, dose reduction of cisplatin and dose reduction of paclitaxel) showed a significant association with hematological toxicity.

In the multivariate logistic regression analysis, the following SNPs showed a statistically significant association with the endpoint hematological toxicity: rs2301159 (gene *SLC10A2*; OR = 25.517; 95%CI = 2.852–228.324; p = 0.004), rs2470890 (gene *CYP1A2*; OR = 10.786; 95%CI = 1.142–101.142; p = 0.038) [Table 3]. However, these SNP were not significant after adjusting for multiple comparisons.

SNPs and infectious complications, nephrotoxicity and neurological toxicity

Infectious complications were observed in two patients (3.4%) and nephrotoxicity was observed in only 1 patient (1.7%). No association between SNPs and such toxicities was found. As no patient presented neurological toxicity, this evaluation could not be done. Similarly, none of the clinical covariates (diabetes, ECOG-PS, age, dose reduction cisplatin and dose reduction paclitaxel) showed a significant association with these toxicities.

Discussion

This is a pharmacogenomic study that evaluated whether SNPs located in drug metabolism genes are associated with toxicity to cisplatin and paclitaxel-based induction chemotherapy in LAHNSCC patients. Our results identified 6 SNPs (in 6 different genes) that were statistically associated to treatment toxicity: rs8187710 (*ABCC2*), rs1801131 (*MTHFR*), rs3788007 (*ABCG1*), rs4148943 (*CHST3*), rs2470890 (*CYP1A2*) and rs2301159 (*SLC10A2*). However, these SNPs were not significant after adjusting for multiple comparisons.

The *ABCC2* gene encodes a protein named MPR2 (Multidrug resistance-associated protein 2) that plays an important role in detoxification and cell chemoprotective, transporting a series of substances out of the cell [16]. The SNP rs8187710 occurs in its coding region. Its presence entails alteration of the amino acid and consequently of the protein [17], and was previously associated with chemotherapy toxicity: in two studies there was an increased risk of cardiac toxicity after exposure to doxorubicin [18,19]. Although previous studies have shown a relationship between rs8187710 SNP and chemotherapy toxicity, we did not replicate these findings in our sample.

The *MTHFR* gene encodes a key protein in the folate metabolizing pathway called 5,10-methylenetetrahydrofolate reductase (FADH2). This enzyme converts the molecule 5,10-methylenotetrahydrofolate into 5-methylenotetrahydrofolate. This reaction is indispensable in the conversion of the amino acid homocysteine to methionine, which in

Table 2
Logistic regression model for gastrointestinal toxicity (n = 59).

Gene	Polymorphism		Odds Ratio	95% CI	p	Adjusted OR*	95% CI	p
ABCC2	rs8187710	GG	ref.		0.001	ref.		0.004
		GA/AA	33.21	2.35–470.10		124.694	4.57–3400.27	
MTHFR	rs1801131	TT	ref.		0.015	ref.		0.014
		TG/GG	17.69	1.73–180.58		23.147	1.90–281.54	
ABCG1	rs3788007	GG	ref.		0.019	ref.		0.034
		GA/AA	0.04	0.003–0.60		0.034	0.002–0.55	
CHST3	rs4148943	CC	ref.		0.021	ref.		0.042
		CT/TT	0.05	0.004–0.63		0.055	0.003–0.90	

* Adjusted for age and performance status.

Table 3
Logistic regression model for hematological toxicity (n = 59).

Gene	Polymorphism		Odds Ratio	95% CI	p	Adjusted OR*	95% CI	p
SLC10A2	rs2301159	GG	ref.		0.004	ref.		
		GA/AA	25.52	2.85–228.32		23.67	2.62–214.01	0.003
CYP1A2	rs2470890	CC	ref.		0.038	ref.		
		CT/TT	10.79	1.14–101.86		14.97	1.32–169.35	0.030

* Adjusted for age and performance status.

turn is important in several processes, including the ability to eliminate toxins [20]. The SNP rs1801131 is located in coding region of the gene, and results in alteration of the amino acid and consequently of the protein. Its relation with toxicity was evaluated in at least 9 studies in patients receiving methotrexate for lymphoma [21], leukemia [22], rheumatoid arthritis [23–27] and psoriatic arthritis [28], and 1 study in colorectal cancer patients receiving 5-FU [29]. In only 1 of them there was an association between the SNP and increased toxicity to chemotherapy [26]. In relation to platinum-containing regimens, a Chinese study evaluated 10 SNPs in the *MTHFR* gene in 1004 patients with non-small cell lung cancer, including the SNP in question [30]. This study demonstrated a reduction of severe gastrointestinal toxicity ($p = 0.004$). However, in the Chinese study, several platinum-based regimens were allowed, and only 31% of the patients received platinum in combination with paclitaxel (31% platinum with vinorelbine, 23.8% platinum with gemcitabine, 8.7% platinum with docetaxel; 4.9% other combinations). Another relevant point of difference between the studies is that, in the palliative context, many Chinese patients probably should have been treated with carboplatin instead of cisplatin, which has a distinct and milder toxicity profile. Thus, it can be assumed that the presence of SNP leads to protein dysfunction, accumulation of chemotherapeutic agents, and consequent increase in toxicity.

The *ABCG1* gene belongs to the same family as the *ABCC2* gene, also related to transporting substances through the cell membrane [31]. The SNP rs3788007 was associated to gastrointestinal toxicity reducing its risk with an odds ratio of 0.045, although, after adjustment, it has lost statistical significance. Considering that the SNP occurs in the intronic region of the gene its presence does not determine amino acid change. However, an intronic variant may interfere with the expression level of the gene, which would justify the different profiles of tolerance to chemotherapy. At least one previous lebanese study specifically evaluated this SNP and chemotherapy toxicity [32]. Thirty-six patients with docetaxel-treated breast cancer were evaluated for the presence of SNP and febrile neutropenia, and a statistically significant relationship was found. Gastrointestinal toxicity was not assessed in this study.

The *CHST3* gene encodes the enzyme carbohydrate sulfotransferase 3 which is involved in the remodeling of the extracellular matrix [33], and may be involved in the process of developing metastases, tumor invasion and endothelial cell adhesion [34]. It is also involved in the metabolism of drugs within the liver [35]. The SNP rs4148943 was associated to a reduction in the risk of developing gastrointestinal toxicity, with an odds ratio of 0.05, although, after adjustment, it has lost statistical significance. Being located in the 3′UTR region of the gene its presence does not determine any alteration in the synthesized protein. Their presence, however, could lead to an increase in protein function with consequent increase in the metabolism of chemotherapeutic agents, which would justify the finding. A previous study in patients with prostate cancer treated with docetaxel and thalidomide also did not find any statistically significant relationship with toxicity, despite having found a relationship of this SNP with treatment response [36].

Two other SNPs were, before adjustment, associated to hematological toxicity, both associated with increased risk (*CYP1A2* - rs2470890 and *SLC10A2* - rs2301159).

The *CYP1A2* gene encodes an enzyme belonging to the cytochrome P450 superfamily and has the function of catalyzing various reactions involving drug metabolism and synthesis of cholesterol, steroids and/or lipid [37]. Reducing the efficacy of its enzyme could lead to the accumulation of the chemotherapy in the intracellular environment, increasing the toxicity to the drug. The SNP rs2470890 was associated to an increase in hematological toxicity in the order of 10.786 times. This SNP occurs in the coding region of the gene, but does not alter the protein. This type of SNP, however, may interfere with the regulation of gene expression. This occurs through the selectivity of microRNAs that bind only to specific alleles - A and not C for example [38]. Study of 2014 demonstrated a relationship between the presence of SNP and increased neurological adverse effects in patients using clozapine [39]. Important to note that after adjustment for multiple comparisons these SNPs were not significant. In the light of our knowledge this is the first study to evaluate the relationship of this SNP with chemotherapy toxicity.

The *SLC10A2* gene encodes a co-transporter protein of sodium and bile, in addition to being critical in cholesterol homeostasis [37]. The SNP rs2301159 is located in the 3′UTR region of the gene, and therefore does not alter the protein synthesis. Its presence was associated to an increase in hematological toxicity of more than 25 times, but after adjustment for multiple comparisons it has lost statistical significance. This is the first study to evaluate its relationship to chemotherapy toxicity.

Regarding infectious complications, in the univariate analyzes two SNPs were statistically associated with infectious complications, but the results did not hold significance in the multivariate analysis suggesting that the reduced number of patients may have interfered negatively with the results.

The study has two main limitations. The first is the small number of patients. However it is important to emphasize that all participants in the clinical trial were included, and all toxicities were collected within the rigor of a clinical trial. The second limitation is the low occurrence of toxicities in the clinical trial. This is because the treatment protocol was specifically designed with the intention of reducing toxicity rates and ensuring that participants, in addition to benefiting from induction chemotherapy, were not impaired in relation to the subsequent phase of concomitant chemoradiotherapy. Also for this reason grade 2 toxicities were included in the analyzes, and not only grade ≥ 3 toxicities. Although of less clinical relevance, there are grade 2 toxicities that lead to delayed doses or possibly suspension of treatment when prolonged, such as haematological toxicities for example.

The discovery of biomarkers is essential to assist the physician towards a tailored and personalized treatment, potentiating benefits and avoiding toxicities. However this study failed to demonstrate a relationship between SNPs and toxicity to induction chemotherapy in LAHNSCC. The small number of patients may have affected the results. Further studies with a larger number of patients are desirable.

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Declaration of Competing Interest

The authors declare no potential conflicts of interest.

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