

RESEARCH ARTICLE

Clinical Observations on Associations Between the UGT1A1 Genotype and Severe Toxicity of Irinotecan

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Abstract

Background: Severe toxicity is commonly observed in cancer patients receiving irinotecan (CPT-11). UDPglucuronosyltransferase1A1 (UGT1A1) catalyzes the glucuronidation of the active metabolite SN-38 but the relationship between UGT1A1 and severe toxicity remains unclear. Our study aimed to assess this point to guide clinical use of CPT-11. **Materials and Methods:** 89 cancer patients with advanced disease received CPT-11-based chemotherapy for at least two cycles. Toxicity, including GI and hematologic toxicity was recorded in detail and UGT1A1 variants were genotyped. Regression analysis was used to analyse relationships between these variables and tumor response. **Results:** The prevalence of grade III-IV diarrhea was 10.1%, this being more common in patients with the TA 6/7 genotype (5 of 22 patients, 22.7%) ($p<0.05$). The prevalence of grade III-IV neutropenia was 13.4% and also highest in patients with the TA 6/7 genotype (4 of 22 patients; 18.2%) but without significance ($p>0.05$). The retreatment total bilirubin levels were significantly higher in TA6/7 patients (mean, 12.75 μ mol/L) with compared to TA6/6 (mean, 9.92 μ mol/L) with $p<0.05$. **Conclusions:** Our study support the conclusion that patients with a UGT1A1*28 allele (s) will suffer an increased risk of severe irinotecan-induced diarrhea, whether with mid-or low-dosage. However, the UGT1A1*28 allele (s) did not increase severe neutropenia. Higher serum total bilirubin is an indication that patients' UGT1A1 genotype is not wild-type, with significance for clinic usage of CPT-11.

Keywords: CPT-11 - UGT1A1 genotype - toxicity

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Introduction

Irinotecan (CPT-11) is an S-phase-specific, semisynthetic derivative of camptothecin that interferes with DNA replication and cell division by inhibiting topoisomerase I (Fukuoka et al., 1992) and is now used widely, especially for colorectal and lung cancers (Negoro et al., 1991; Cunningham, et al., 1998; Kudoh et al., 1998; Rougier et al., 1998; Masuda et al., 1999; Uygun et al., 2013; Wei et al., 2013; Wu et al., 2013; Bozkurt et al., 2014; Zhang et al., 2014). Although it prolongs survival, patients who treated with chemotherapy include irinotecan is often accompanied by unpredictable and severe side effects. It causes severe diarrhea and neutropenia in 20% to 35% of patients treated (Negoro et al., 1991; Rothenberg et al., 1993; Rougier et al., 1998; Saltz et al., 2000; Othenberg et al., 2001; Vanhoefer et al., 2001; Fuchs et al., 2003). Fatal events (up to 5.3% prevalence) during single-agent irinotecan treatment have been reported (Vanhoefer et al., 2001). An innovative way of predicting the toxicity is strongly required.

CPT-11 is metabolized to the active form, SN-38 which exerts its antitumor effect by carboxylesterase and is then

converted to the inactive form is by UGT (EC2.4.1.17) to yield its β -glucuronide (Kawato et al., 1991). The glucuronide is excreted in the small intestine via bile, where bacterial glucuronidase resolves the glucuronide into the former SN-38 and glucuronic acid (Takasuna et al., 1996). Interindividual differences in pharmacokinetics of SN-38 are suggested to cause the variation in drug effect (Gupta et al., 1994; Kudoh et al., 1995). Pharmacokinetic studies of irinotecan have reported large variations among individuals, as assessed by area under the concentration-time curves of the active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38) and the inactive glucuronide metabolite (SN-38G), which is conjugated to UDP-glucuronosyltransferase (Gupta et al., 1994). UGT1A1 genotype affect the UGT enzyme activities and is believed to be the isoform primarily responsible for SN-38G formation, and interindividual variability in SN-38G formation is due to various UGT1A1 genotypes (Iyer et al., 1998). The human UGT1A locus is defined by 13 first exons, which are alternatively spliced to four common exons, leading to mRNA isoforms, nine of which conduct to functionally active enzymes. In Asian, dinucleotide repeats in the atypical TATA-box region of the UGT1A1

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promoter (UGT1A1*28 allele) have two sequences: (TA)₆, (TA)₇, compose 3 genotypes: TA₆/TA₆ Wild-type UGT1A1*1/*1, TA₆/TA₇ Heterozygous (UGT1A1*1/*28) and TA₇/TA₇ Homozygous (UGT1A1*28/*28). An increased number of dinucleotide TATA-box region leads to a decreased rate of transcription initiation/expression of UGT1A1 (Beutler, 1998). Several studies suggest that patients homozygous for UGT1A1*28 are more likely to develop dose-dependent severe neutropenia and diarrhea compared with individuals with the no clinical characteristics factors contributing to increased toxicity risk using multivariable analyses reference genotype (*1/*1) (Beutler, 1998; Iyer, 2002; Innocenti et al., 2004; Marcuello et al., 2004; Carlini et al., 2005; Soepenberg et al., 2005; Toffoli et al., 2006; Ichikawa et al., 2008).

Here we evaluated the association between UGT1A1 genetic variation and prevalence of severe toxicity with advanced lung and gastrointestinal tumors patients to find the relationship between UGT1A1 genetic variants and severe toxicity of CPT-11. In addition, this study also aimed to identify baseline clinical characteristics contributing to increased toxicity risk.

Materials and Methods

Patients

Patients were required to be pathologically/cytologically diagnosed with advanced lung and gastrointestinal tumors in Jiangsu Cancer Hospital and Research Institute from June 2006 to February 2014; to sign an informed consent before treatment; to have a score of Karnofsky performance status ≥ 60 ; to be 18 to 80 years of age. Other eligibility criteria included: adequate hematological (white blood cell count $> 3.0 \times 10^9$ and platelet count $> 150 \times 10^9$), liver (bilirubin and transaminases < 1.5 times the upper normal limit) and renal function (creatinine level < 1.5 times the upper normal limit); patients were excluded from this study if they failed to complete two cycles of chemotherapy, or with any serious medical or psychiatric condition, or other malignancies. Pregnant or lactating women are also excluded from this study. We collect the patients' baseline clinical characteristics include sex, age, pathological reports, KPS, sites of metastasis, history of chemotherapy, serum total bilirubin levels before therapy and their UGT1A1 genotype. These factors were analyzed for importance for the side effects.

CPT-11-based chemotherapy treatment

Patients were treated with CPT-11-based chemotherapy. The dosage and schedule of irinotecan administration that patients received were determined by their basic clinic characters and their UGT1A1 genotypes after they had the pharmacogenetic testing. The dosage adopted in our study is 100 mg² to 175 mg². We divide them into high-dose group (≥ 125 mg²) and low-dose group (< 125 mg²). For lung cancer patients, CPT-11 was used with one Platinum include Cisplatin, Nedaplatin, Carboplatin or Lobaplatin. For Gastrointestinal cancer patients, modified FOLFIRI regimen was used or CPT-11 was with Platinum, Fluorouracil, Pemetrexed or Raltitrexed. Modified FOLFIRI regimen was used every 14 days, the others were

Table 1. National Cancer Institute Common Terminology Criteria

Toxicity	I	II	III	IV
Leucopenia ($\times 10^9/L$)	3.0-4.0	2.0-3.0	1.0-2.0	$< 1.0^*$
Neutropenia ($\times 10^9/L$)	1.5-2.0	1.0-1.5	0.5-1.0	$< 0.5^*$
Anemia (g/L)	100-110	85-100	65-85	< 65
Thrombocytopenia ($\times 10^9/L$)	75-100	50-75	25-50	< 25
Diarrhea (times/d)	< 4	4-6	> 6	life-threatening
Vomit (times/d)	< 3	3-5	≥ 6	life-threatening

every 21 days. Because toxicity of irinotecan results in leukopenia and diarrhea is dose-limiting (Negoro et al., 1991), once patients occurred grade IV toxicity, the dosage would be adjusted next chemotherapy. No restriction was put on whatever the previous chemotherapy regimens were. No standard was set to reduce the dose of first-course CPT-11 in patients according to their basic clinic characters and their UGT1A1 genotypes. No standard was set to reduce the dose of second-course CPT-11 in patients who suffered severe diarrhea and neutropenia in the first course. The next course therapy was started after their neutrophil levels recovered to the normal levels.

Toxicity assessment

Toxicity was evaluated according to National Cancer Institute Common Terminology Criteria or Adverse Events version 3.0 criteria (Table 1). The toxicity endpoints consisted of both GI and hematologic toxicities, and were analyzed separately. For GI toxicities, all patients were recorded the incidence and severity of vomiting and diarrhea every day after irinotecan infusion. For hematologic toxicities, laboratory parameters were collected before, during, and after each cycle of chemotherapy. The most severe toxicities of every patient detected were used for data analysis. And we defined "severe toxicity" in this research as grade III-IV of toxicity. Once the toxicities occurred, the corresponding therapy would be implemented. For vomiting, antiemetics include 5-HT₃ receptor antagonists and seroids were used. For diarrhea, Imodium was given: 4mg po once it happened, then 2mg po every 2 hours until the 12 hours after the last diarrhea. If time last over 48h, octreotide was given. For hematologic toxicities, G-CSF was for leucopenia or neutropenia, IL-11 for thrombocytopenia and ferrous succinate or EPO for anemia.

UGT1A1*28 genotype analysis

Genomic DNA was extracted with DNA extraction kit (Qiagen Inc, Valencia, CA, USA) for PCR amplification. Prime sequences were designed as follows: upstream: 5'-TCCCTGCTACCTTTGTGGAC-3', downstream: 5'-AGCAGGCCCGAGGACaagt-3'. The PCR mix was 25ul: 2ul of 10xPCR buffer with 15mM MgCl₂, 2ul of dNTP (2.5 mM), 1ul of primer (10 um), 1ul of DNA Taq polymerase (5 U/ul), and 18.8ul of ddH₂O. Amplification procedure was as follow: a initial denaturation step at 94°C for 5 min; followed by 40 cycles of denaturation (94°C for 15s), annealing (55°C for 25s) and extension (72°C for 50s), and finally, extension at 72°C for 7min. If the PCR product hand is clear by electrophoresis, take 5ul of eligible specimen, mixed by adding 2ul SAP, kept at 37°C for 60min and 80°C for 15min, and then

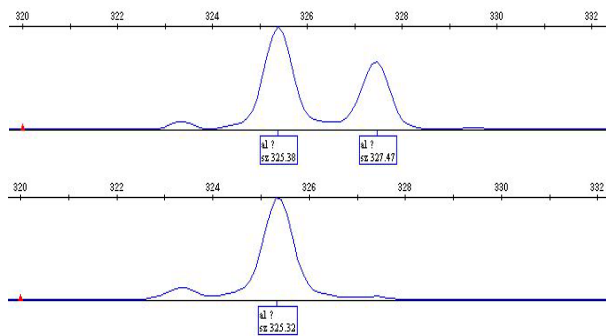


Figure 1. Sequencing Results for (TA)6/(TA)6 and (TA)6/(TA)7 Genotypes with GeneMapper Software. A) (TA)6/(TA)6 genotype, B) (TA)6/(TA)7 genotype

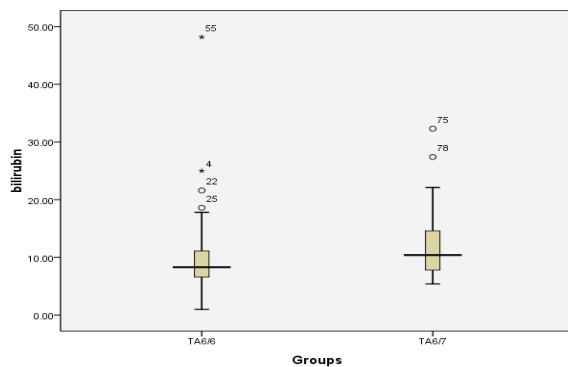


Figure 2. Serum Total Bilirubin Distribution of Different Genotypes

saved at 4°C. Took 3 ul positive PCR hydrolysate, 1ul sequencing reagent (bigdye), and 2ul sequencing primer for PCR amplification: initial denaturation at 96°C for 1min, followed by 25 cycles of denaturation (96°C for 10s), annealing (50°C for 5s) and extension (60°C for 4min), and then stored at 4°C. The sequencing product was sequenced in DNA sequencing instrument (ABI-373, PE corp., USA) after being purified. Sequencing results were displayed and analyzed with GeneMapper software.

Statistical methods

We investigated the associations between side effects of CPT-11 and patients' UGT1A1 genotype characteristics included sex, age, pathological reports, KPS, sites of metastasis, history of chemotherapy and dosage used univariate analysis and multivariable logistic regression model. The relationship between serum total bilirubin levels and UGT1A1 genotype using rank test. And we also conduct the relationship between CPT-11-induced diarrhea and Leukopenia using correlation Analysis. SPSS version 16.0 software (SPSS Inc., USA) was used for all statistical analyses.

Results

89 patients were involved in this study with age between 18 and 80; male 68 and female 21; Lung cancer 24, Esophageal cancer 6, Gastric Cancer 9 and Colorectal Cancer 50. All patients finished two cycle chemotherapy at least. The 6/6 variant of UGT1A1 was detected in 67 (75.3%) participants, the 6/7 variant was 22 (24.7%), while no 7/7 variant was detected. 9 (10.1%) patients

Table 2. Toxicities of Patients

Toxicity	Grade				
	0	I	II	III	IV
Diarrhea	62 (69.7%)	8 (9.0%)	10 (11.2%)	8 (9.0%)	1 (1.1%)
Vomit	62 (69.7%)	17 (19.1%)	9 (10.1%)	1 (1.1%)	0 (0.0%)
Leucopenia	32 (36.0%)	23 (25.8%)	25 (28.1%)	9 (10.1%)	0 (0.0%)
Neutropenia	45 (50.6%)	20 (22.5%)	12 (13.5%)	9 (10.1%)	3 (3.4%)
Anemia	34 (38.2%)	20 (22.5%)	26 (29.2%)	8 (9.0%)	1 (1.1%)
Thrombocytopenia	52 (58.4%)	16 (18.0%)	14 (15.7%)	5 (5.6%)	2 (2.2%)

Table 3. The Relationship between Baseline Clinic Characters and UGT1A1 Genotype

Clinic character	UGT1A1 genotype		p	OR
	TA6/TA6	TA6/TA7		
Age	≥60	27	0.426	0.675
	<60	40		
Sex	Male	48	0.095	0.267
	Female	19		
KPS	90-100	28	0.408	1.538
	70-80	39		
Chemotherapy history	First line	21	0.909	1.065
	No first line	46		
Number of metastasis	0-1	57	0.882	0.900
	≥2	10		

Table 4. Serum Total Bilirubin of Different Genotypes

Genotype	Bilirubin before Treatment	p
TA6/TA6	9.92 μmol/L	0.036
TA6/TA7	12.75 μmol/L	

occurred severe toxicity of CPT-11-induced diarrhea, while 9 (10.1%) occurred severe toxicity of leukopenia (Table 2). The relationships between the side effects and their UGT1A1 genotypes or their baseline clinic characters were as followed. We also analyzed the factors of UGT1A1 genotype include serum total bilirubin and their baseline clinic characters.

The relationship between UGT1A1 genotype and serum total bilirubin and their baseline clinic characters

The different UGT1A1 variants are showed in Figure 1. The univariate regression analysis of genotype of UGT1A1 and baseline characters include age, gender, KPS score, history chemotherapy and metastasis number shows no statistical differences with $p > 0.05$ (Table 3). It turns out that baseline characters don't influence the genotype of UGT1A1. While the serum total bilirubins distribution of different UGT1A1 variants are showed in Figure 2. We can find that the serum total bilirubin of TA6/7 is higher than TA6/6 with their mean 12.75 μmol/L and 9.92 μmol/L. The TA6/7 has a higher serum total bilirubin level than TA6/6 ($p < 0.036$, Mann-Whitney U test; Table 4).

The relationship between side effects and CPT-11 dosage

The CPT-11-induced diarrhea and leukopenia were dose-limited, we adopted different dosage intensity of CPT-11 according to patients' baseline clinic conditions and genotype, then divide them into two groups (≥ 125 mg/m² and < 125 mg/m²). Among the 89 patients, 56 are ≥ 125 mg/m², 10.7% (6) of them appeared grade III-IV diarrhea, 10.7% (6) appeared grade III-IV leukopenia

Table 5. The Relationship between Severe Toxicity and Dosage

Severe toxicity	Dosage		p	OR
	≥125 mg/m ²	<125 mg/m ²		
Diarrhea	6(10.7%)	3(9.1%)	0.806	1.2
Vomit	0(0%)	1(3.0%)	0.997	0
Leucopenia	6(10.7%)	3(9.1%)	0.806	1.2
Neutropenia	6(10.7%)	6(18.2%)	0.23	0.474
Anemia	7(12.5%)	2(6.1%)	0.341	2.214
Thrombocytopenia	5(8.9%)	2(6.1%)	0.629	1.52

Table 6. The Relationship between Severe Toxicity and UGT1A1 Genotype

Severe toxicity	Genotype		p	OR
	TA6/TA6	TA6/TA7		
Diarrhea	4(6.0%)	5(22.7%)	0.034	0.216
Vomit	0(0%)	1(4.5%)	0.977	0
Leucopenia	5(7.5%)	4(18.2%)	0.161	0.363
Neutropenia	8(11.9%)	4(18.2%)	0.46	0.61
Anemia	7(10.4%)	2(9.1%)	0.885	1.167
Thrombocytopenia	5(7.5%)	2(9.1%)	0.806	0.806

or neutropenia. while the other 33 are (125 mg/m², 9.1% (3) appeared grade III-IV diarrhea, 9.1% (3) appeared grade III-IV leucopenia and 18.2% (6) appeared grade III-IV neutropenia. Although the overall severe toxicity occurrence of ≥125 mg/m² turns out higher than <125 mg/m², there is no statistical differences between the two groups. So are the severe toxicity of vomit, anemia and thrombocytopenia by univariate analysis (Table 5). It turns out that the dosage around the 125 mg/m² doesn't influence the severe toxicity

The relationship between side effects and UGT1A1 genotype

Of the 67 TA6/6 patients, 4 (6.0%) cases appeared grade III-IV diarrhea, 5 (7.5%) cases appeared grade III-IV leucopenia, 8 (11.9%) cases appeared grade III-IV neutropenia; while of the 22 TA6/7 patients, 5 (22.7%) cases appeared grade III-IV diarrhea, 4 (18.2%) cases appeared grade III-IV leucopenia or neutropenia. By the univariate analysis, we find that UGT1A1 genotype affects the incidence of diarrhea with statistical significance ($p < 0.05$). While, although the incidence of leucopenia or neutropenia is higher in TA6/7 than TA6/6, UGT1A1 genotype shows no statistical difference by the univariate analysis with $p > 0.05$. So are vomit, anemia and thrombocytopenia (Table 6). UGT1A1 genotype is a significant factor with statistical difference for the CPT-11-induced diarrhea.

Multivariate analysis

Because CPT-11 causes severe diarrhea and neutropenia in 20% to 35% of patients treated (Negoro et al., 1991; Rothenberg, et al., 1993; Rougier et al., 1998; Saltz et al., 2000; Rothenberg et al., 2001; Vanhoefer et al., 2001; Fuchs et al., 2003), and fatal events (up to 5.3% prevalence) during single-agent irinotecan treatment have been reported (Vanhoefer et al., 2001). We associated these two kind side effects with UGT1A1 genotypes,

Table 7. Multivariate Logistic Analysis of Severe Diarrhea

Factor	RR	p
Age	1.027	0.535
Sex	1.537	0.688
KPS	0.487	0.436
Number of metastasis	0	0.998
Chemotherapy history	0.421	0.425
Genotype	6.108	0.048
Dosage	1.742	0.542
Leucopenia	2.336	0.302
Neutropenia	0.779	0.656
Primary cancer	1.032	0.965
Pathological type	0.707	0.648

Table 8. Multivariate Logistic Analysis of Severe Neutropenia

Factors	OR	p
Age	1.025	0.434
Sex	2.784	0.193
KPS	0.892	0.875
Number of metastasis	1.786	0.529
Chemotherapy history	1.253	0.791
Genotype	1.224	0.797
Dosage	0.426	0.251
Primary cancer	0.824	0.728
Pathological type	1.002	0.998
Diarrhea	1.626	0.078

clinic characters and each other to detect the relationship between them by multivariate logistic analysis

Multivariate logistic analysis of Diarrhea

We took the sex, age, KPS, number of metastasis, history of chemotherapy, UGT1A1 genotype, dosage, leucopenia, neutropenia, primary cancer and pathological type into risk factors. By Multivariate logistic analysis, we can see that UGT1A1 genotype is the only factor with statistical difference, its OR is 6.108. It turns out that the risk rate to occur diarrhea of TA6/7 is 6.108 times of TA6/6, While sex, age, KPS, number of metastasis, history of chemotherapy, leucopenia, neutropenia, primary cancer and pathological type don't show statistical significance with $p > 0.05$ (Table 7).

Multivariate logistic analysis of neutropenia

For the neutropenia, we took the sex, age, KPS, number of metastasis, history of chemotherapy, UGT1A1 genotype, dosage, diarrhea, primary cancer and pathological type into risk factors. By multivariate logistic analysis, we don't find a factor that can effect the risk of neutropenia with $p > 0.05$ (Table 8). It worth attention that the diarrhea may have some kind relationship with neutropenia ($p = 0.078$). It means that the more severe the diarrhea occurs, the more severe neutropenia will be.

Discussion

CPT-11 is a broad-spectrum anticancer drugs with determinate effect that can prolong patients' survival.

But its usage always be limited by its severe side effects especially diarrhea and myelosuppression which are dose-limiting toxicities (Negoro et al., 1991; Rothenberg et al., 1993; Rougier et al., 1998; Saltz et al., 2000; Rothenberg et al., 2001; Vanhoefer et al., 2001; Fuchs et al., 2003). Clinical administration dosage is generally calculated according to the body surface area or weight presently which is the group average dose (Reilly and Workman, 1993). but only a part of drugs calculated as above may get satisfactory effects and tolerable toxicity. The small changes in plasma concentration that affected by drug absorption, distribution, metabolism, and excretion may cause efficacy differences and lead to serious adverse effects. Irinotecan therapy is also complicated by the significant inter-subject variations in such toxicity and pharmacokinetic parameter estimates (Gupta et al., 1994; 1997) So it is very necessary that makes anti-tumor therapy entering the era of individualized treatment through the use of biological markers, drug metabolism genes, or pharmacokinetics detection, etc. UGT1A1 encodes the UGT which is essential for the inactivated of SN-38 that exert the antitumor effect of CPT-11. The UGT is also important for bilirubin metabolism. The abnormality in the promoter region of the gene has increased bilirubin and appears to be necessary for Gilbert's syndrome (Beutler, 1998). Our study shows that the serum total bilirubin of TA6/7 is higher than TA6/6. It is consistent with Bosma PJ' study. It makes sense that the higher serum total bilirubin excluded the definite diseases have more possibility of UGT1A1*28. Also the distribution of UGT1A1*28 has a significant difference between human races. The frequency of UGT1A1*28 homozygote is not so high as compared to the Caucasian population (Araki et al., 2006). For Chinese, the TA6/6 of UGT1A1 is 79.6%, T6/7 is 18.2% , and TA7/7 is only 2.2% (Ma Dong, et al., 2011). In our study, the distribution is consistent with the above.

UGT1A1 is a important allele for avoiding the sever adversr events such as neutropenia and/or diarrhea caused by SN-38, the active form of CPT-11 (Ando et al., 2000). Pharmacogenetic testing of this allele is recommended prior to treat cancer patients with CPT-11 in US (Evaluation of Genomic Applications in Praticce and Prevention (EGAPP) Working Group. 2009). Our study shows that the usage of dosages adjusted by UGT1A1 genotype and patients' clinic charatcters caused the lower incidence of severe toxicity compared with 20%-30% showed in some studies (Negoro et al., 1991; Rothenberg et al., 1993; Rougier et al., 1998; Saltz et al., 2000; Rothenberg et al., 2001; Vanhoefer et al., 2001; Fuchs et al., 2003). It illustrates that pharmacogenetic testing of UGT1A1 is meaningful in clinic. In a Hoskins' meta-analysis of 821 cases in 9 clinic trials, severe toxicity was not increased in pediatric patients with the 7/7 genotype when treated with a low-dose (<150mg/m²) protracted schedule of irinotecan, while mid-dosage in 150 ~ 250mg/m², the risk is fixed (Hoskins et al., 2007). In our study, the dosage is around 100-175 mg/m² and it belong to the low-mid dosage in Hoskins' study, but UGT1A1 still make a statistical difference here. Our conclusion may make a Bias due to the reasons below: 1, The distribution of UGT1A1 is different between races, our study don't

include 7/7 genotype. 2, Chemotherapy drugs Combined weren't included in hazard evaluation. 3, The treatment of toxicities is different now. It need more studies whether the UGT1A1 can influence the severe toxicities incidence or not.

On the other hand, in our study, UGT1A1 is a independent risk factor for the increased CPT-11-induced diarrhea by univariate and Multivariate logistic analysis. There are many researches detecting the relationship between them. Some suggest that specific SNPs in UGT1A, other than UGT1A1*28, may influence irinotecan toxicity and increased the risk of diarrhea (Innocenti et al., 2004; Raynal et al., 2010; Ma Dong et al., 2011). While some other suggest that UGT1A1*28 polymorphism is of some relevance to toxicity, however, it is less important than discussed in some trials (Rouits et al., 2004; Toffoli et al., 2006). But these studies all give a common conclusion: TA7/7 homozygous of UGT1A1 have a increased risk of diarrhea. There is still no unified conclusion about TA6/6 and TA6/7 variations. Whatever in our study the risk rate of TA6/7 is 6.108 times of TA6/6 and should be detected to help guide the clinic use of CPT-11.

While for neutropenia caused by CPT-11, we don't come out that UGT1A1*28 can increase the risk of it. Many studies abroad suggest that UGT1A1 genotypes are strongly associated with severe neutropenia, and could be used to identify cancer patients predisposed to the severe toxicity of irinotecan (Innocenti et al., 2004; Rouits et al., 2008; EGAPP Working Group, 2009). Some indicate that the risk of leucopenia or neutropenia in CPT-11-based chemotherapy is higher as the increase on dosage, especially in those who are administrated with medium or high irinotecan doses (>125mg/m²) (Hu et al., 2010). While some others show that there is no relation ship between polymorphism of UGT1A1 (Marcuello et al., 2004). In our study, we didn't find the difference between TA6/6 and TA6/7 variations in severe neutropenia. Chemotherapy drugs combined and chemotherapy cycles are main factors interfering our result. And more controlled studies with large patients should be carried out to confirm this question.

Moreover, we observed that patients who occurred CPT-11-induced diarrhea always experience neutropenia few days after it happened. Although there is no definite mechanism to explain the association between them. But it worth a clinic note that when patients with CPT-11 occur diarrhea, we should test neutrophils more often to prevent the sever neutropenia happened and the more sever diarrhea is, the more sever neutropenia will be.

On Conclusion, Our study supports the result that UGT1A1*28 allele (s) will suffer an increased risk of sever irinotecan-induced diarrhea whether in mid-dosage or in low-dosage. While the UGT1A1*28 allele (s) doesn't increase the severe neutropenia. Higher serum total bilirubin is an indication that patients' t UGT1A1 genotype is not wide-type. And UGT1A1 genotype is significant for clinic usage of CPT-11.

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