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The Predictive Value of Pre-therapeutic Serum Gamma-glutamyl transferase in Efficacy and Adverse Reactions to Neoadjuvant Chemotherapy among Breast Cancer Patients

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ABSTRACT

Purpose: Gamma-glutamyl transferase (GGT) has been reported as being involved in tumor progression. Previous studies documented a potential relationship between serum GGT level and survival outcome in several types of human malignancies. However, the association between serum GGT levels and response to neoadjuvant chemotherapy (NAC) has not yet been reported. The present study aimed to evaluate the association between pre-therapeutic serum GGT level and the efficacy, long-term survival, and adverse reactions of NAC and to investigate its role in predicting NAC sensitivity in patients with breast cancer.
Methods: A total of 129 patients were recruited and stratified into 2 groups according to serum GGT level (< 29 U/L and ≥ 29 U/L). The association between pre-therapeutic serum GGT levels and clinicopathological parameters was examined. The correlation between pre-therapeutic serum GGT levels and pathological complete response (pCR) was analyzed using univariate and multivariate logistic regression. Survival analyses of relapse-free survival (RFS) and disease-free survival (DFS) were performed. Pearson's χ^2 test and multivariate logistic regression model were used to analyze the correlation between pre-therapeutic serum GGT levels and adverse reactions.
Results: Pre-therapeutic serum GGT levels were associated with pCR among breast cancer patients treated with NAC. Multivariate analysis showed that low-level GGT significantly increased pCR rate. Patients in the high-level GGT group had poorer survival than those in the low-level GGT group. Subgroup analysis demonstrated that serum GGT level was potentially related to RFS and DFS in the hormone receptor-positive group. Low levels of GGT are significantly associated with a higher incidence of neutropenia.
Conclusion: Pre-therapeutic serum GGT level is an independent and novel biomarker for predicting the efficiency, prognosis, and adverse reactions to NAC in breast cancer patients. Patients with low pre-therapeutic serum GGT levels are more likely to have higher pCR rates, better RFS and DFS, and higher hematologic toxicity.

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Keywords: Breast cancer; Gamma-glutamyltransferase; Neoadjuvant therapy; Chemotherapy; Prognosis

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INTRODUCTION

Neoadjuvant chemotherapy (NAC), an important method for the comprehensive treatment of malignancies, has been widely used in the treatment of locally advanced breast cancer (LABC). Numerous prospective clinical trials have demonstrated that pathological complete response (pCR) after NAC is associated with a long-term survival benefit [1]. However, for those who do not achieve pCR, the survival benefit is still unclear. Thus, it is necessary to identify a biomarker that can effectively predict NAC sensitivity. Peripheral blood molecules are ideal markers as they can be easily detected. In recent years, various peripheral blood markers have been reported in terms of forecasting NAC efficacy [2]. However, a widely recognized peripheral blood molecular marker for this purpose is still lacking.

Gamma-glutamyl transferase (GGT), one of the key enzymes in the metabolism of glutathione (GSH), is considered as an important indicator of oxidative stress [3]. A study suggested that GGT has a crucial role in maintaining intracellular GSH levels, which acts as an antioxidant, neutralizing free radicals and thus protecting cells against oxidative stress during cell metabolism, as well as providing resistance to the toxicity of promoting agents that deplete intracellular GSH and further conferring resistance during pro-oxidant cancer therapy [4]. Strasak et al. [5] investigated the association of GGT with cancer incidence in 92,843 Austrian females, reporting that elevated GGT (> 72.00 U/L) markedly increased the overall cancer risk and several site-specific cancer risks. Moreover, Fentiman and Allen [6] reported a possible association between elevated GGT levels and breast cancer incidence in premenopausal females. Finally, the Swedish AMORIS study, which included 545,460 participants, demonstrated that elevated serum GGT level was an independent risk factor for breast cancer [7].

Several large clinical studies have also documented the potential relationship between serum GGT level and survival outcome in several types of human malignancies, including breast cancer [8], cervical cancer [9], liver cancer [10], renal cell carcinoma [11], and endometrial cancer [12], suggesting that high serum GGT levels are associated with poor prognosis. As Staudigl et al. [8] stated, high pre-therapeutic serum GGT levels (≥ 23.1 U/L) were significantly associated with decreased 5-year overall survival (OS) in patients with primary metastatic breast cancer. Zhu et al. [13] also found that serum GGT level was significantly higher in cervical cancer patients than in healthy people, demonstrating a potential association between GGT and poor disease-free survival (DFS) and OS. Furthermore, Luo et al. [14] indicated that preoperative GGT was an independent prognostic factor of cancer-specific survival and relapse-free survival (RFS) in renal cell carcinoma with venous tumor thrombus and that high GGT (≥ 37.5 U/L) could lead to poor survival. Nevertheless, there have been few reports on the association between pre-therapeutic serum GGT levels and NAC efficacy and prognosis among breast cancer patients.

Based on the findings above, we hypothesized that high serum GGT levels might increase the resistance to NAC, thereby leading to poorer efficacy and prognosis compared to low levels. Thus, we performed a retrospective analysis involving patients undergoing prospective clinical trials on NAC and aimed to evaluate the association between pre-therapeutic serum GGT level and pCR as well as long-term survival after NAC.

METHODS

Patients and clinical management

The study cohort consisted of 129 breast cancer patients participating in the SHPD001 (NCT02199418) and SHPD002 (NCT02221999) clinical trials from January 2013 to January 2017. Patients who presented with pre-existing abnormal liver function and biliary tract diseases were excluded from this study.

The study design and recruitment methods have been previously described in detail [15]. Briefly, all patients were scheduled to receive NAC before surgery. The treatment plan was a combination of weekly paclitaxel (80 mg/m^2 on days 1, 8, 15, 22, out of every 28 days for 4-week cycles) and cisplatin (25 mg/m^2 on days 1, 8, 15, out of every 28 days for 4-week cycles) followed by primary surgery. Human epidermal growth factor receptor-2 (HER-2)-positive patients from SHPD001 and SHPD002 were concomitantly administered with trastuzumab (4 mg/kg for the first dose and 2 mg/kg for subsequent doses) on a weekly basis. For the hormone receptor-positive patients from SHPD002, endocrine therapy (aromatase inhibitor or gonadotropin releasing hormone agonist) was randomized together with chemotherapy according to their menstrual status. All procedures performed in these studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the independent ethics committees of Renji Hospital with the IRB approval number [2017]088. All patients provided written informed consent.

The 3 commonly used definitions of pCR were estimated as NAC efficacy in this study: 1) total pCR (tpCR), i.e., the absence of either invasive cancer or cancer in situ in the breast and axillary lymph nodes (ypT0 ypN0); 2) pCR in the breast (bpCR), i.e., the absence of either invasive cancer or cancer in situ in the breast (ypT0); and 3) near-pCR i.e., the size of the residual cancer being < 0.5 cm, or having only scattered cancer remaining.

Peripheral venous blood samples for evaluation were routinely obtained within one week prior to the first cycle of NAC. All analyses were performed in the clinical laboratory of Renji Hospital. Serum GGT activity was measured using the kinetic method recommended by the International Federation of Clinical Chemistry. At our institution, the normal range of serum GGT levels for female was 7–32 U/L.

Adverse reactions were assessed during each visit and were recorded according to the Common Terminology Criteria for Adverse Events (CTCAE v4.03).

Statistical analyses

Patients were assigned to groups based on final quintile of all patients' baseline serum GGT levels as follows: < 29 U/L is defined as low GGT level, whereas a value ≥ 29 U/L is defined as high level. χ^2 test and Fisher's exact test were used to compare pre-therapeutic serum GGT levels in groups defined by clinicopathological parameters. A comparison of pre-therapeutic serum GGT levels between the pCR and non-pCR groups was assessed using the Mann-Whitney test. The correlation between GGT levels and pCR was assessed using logistic regression. DFS and RFS were assessed as per the following definitions: 1) DFS: the duration from the operation date to the date of first relapse or metastasis (including contralateral breast cancer and other malignant tumors), or death for any reason, or censored with the last follow-

up date if no relapse or metastasis; 2) RFS: the duration from the operation date to the date of first relapse or metastasis (excluding contralateral breast cancer and other malignant tumors), or death for any reason, or censored with the last follow-up date if no relapse or metastasis.

Survival analysis was estimated using the Kaplan-Meier method. Differences between groups were estimated using the log-rank test. Univariate and multivariate survival analyses were performed using Cox's proportional hazards regression model. A 2-sided p-value < 0.050 was considered statistically significant. Statistical software Stata SE 14.1 (STATA Corp., College Station, TX, USA) and Graph Pad Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA) were used for analysis.

RESULTS

Relationship between pre-therapeutic serum GGT level and clinicopathological parameters

A total of 129 patients with LABC were included in this study. The median age of patients at first diagnosis was 52 years (range, 26–70 years). Of these patients, 128 completed all 4 cycles of paclitaxel and cisplatin chemotherapy and underwent subsequent surgery. One patient received only 15 cycles of chemotherapy. No association was found between the pre-therapeutic serum GGT levels and age, menopausal status, body mass index, estrogen receptor (ER), progesterone receptor (PR), HER-2, Ki-67, clinical TNM stage, and tumor molecular classification (**Table 1**).

Relationship between pre-therapeutic serum GGT level and pCR

The tpCR (**Figure 1A**, 37.8% vs. 11.5%, $p = 0.010$) and near pCR (**Figure 1C**, 49.5% vs. 19.2%, $p = 0.007$) rates were both significantly improved among patients in the low GGT group compared to those in the high GGT group. However, the difference in bpCR between these 2 groups was not statistically significant (**Figure 1B**, 39.8% vs. 19.2%, $p = 0.067$).

The inverse association between elevated serum GGT level and pCR rate was confirmed by multivariate logistic regression. Among all breast cancer patients, pre-therapeutic serum GGT level was an independent factor associated with tpCR (**Table 2**; odds ratio [OR], 0.15; $p = 0.026$; 95% CI, 0.03–0.80), bpCR (**Table 2**; OR, 0.23; $p = 0.043$; 95% CI, 0.06–0.96), and near-pCR (**Table 2**; OR, 0.13; $p = 0.005$; 95% CI, 0.03–0.54).

Survival analyses

The median follow-up period was 24 months (range, 6–45 months). Kaplan-Meier survival estimates and log-rank tests for the 2 serum GGT levels among all patients and those in the subgroups showed a significant difference in RFS among HER-2 positive (**Figure 2A**, log-rank $p = 0.001$) and hormone receptor-positive subgroup patients (**Figure 2B**, log-rank $p = 0.011$), suggesting that patients with low serum GGT levels had longer RFS. However, the difference was not statistically significant in the overall group of patients (**Figure 2C**, log-rank $p = 0.157$). Survival analysis for DFS showed similar results (**Figure 3**).

The correlation between elevated pre-therapeutic serum GGT level and poor survival was further verified by multivariate Cox analysis. In all patients, pre-therapeutic serum GGT level was an independent prognostic factor for RFS (**Table 3**; hazard ratio [HR], 4.34; $p = 0.035$; 95% CI, 1.11–16.93) and DFS (**Table 3**; HR, 4.33; $p = 0.035$; 95% CI, 1.11–16.85). In addition,

Table 1. The association between pre-therapeutic serum GGT levels and patients' baseline clinicopathological parameters

| Characteristics	Total No. of patients (n =129)	Low level GGT	High level GGT	χ^2	p-value*
Age at first diagnosis (yr)				1.62	0.203
≤ 50	59 (46.7)	50 (84.7)	9 (15.3)		
> 50	70 (54.3)	53 (75.7)	17 (24.3)		
Menopausal status				2.38	0.123
Premenopausal	57 (44.2)	49 (86.0)	8 (14.0)		
Postmenopausal	72 (55.8)	54 (75.0)	18 (25.0)		
BMI (kg/m²)				4.17	0.108
≤ 23	58 (45.0)	49 (84.5)	9 (15.5)		
> 23	65 (50.4)	51 (78.5)	14 (21.5)		
Unkown	6 (4.6)	3 (50.0)	3 (50.0)		
ER (%)				0.01	0.951
< 10	44 (34.1)	35 (79.6)	9 (20.4)		
≥ 10	85 (65.9)	68 (80.0)	17 (20.0)		
PR (%)				0.42	0.518
< 10	38 (29.5)	29 (76.3)	9 (23.7)		
≥ 10	91 (70.5)	74 (81.3)	17 (18.7)		
Ki-67 (%)				0.63	0.728
< 30	29 (22.5)	22 (75.9)	7 (24.1)		
≥ 30 and < 60	58 (43.0)	48 (82.8)	10 (17.2)		
≥ 60	42 (32.5)	33 (78.6)	9 (21.4)		
HER-2 status				0.05	0.829
Negative	72 (55.8)	57 (79.2)	15 (20.8)		
Positive	57 (44.2)	46 (80.7)	11 (19.3)		
cT				1.04	0.308
cT1–cT2	58 (45.0)	44 (75.9)	14 (24.1)		
cT3–cT4	71 (55.0)	59 (83.1)	12 (16.9)		
cN				2.24	0.134
cN0	22 (17.1)	15 (68.2)	7 (31.8)		
cN1–3	107 (82.9)	88 (82.2)	19 (17.8)		
Molecular classification				5.98	0.112[†]
Luminal A-like	10 (7.8)	7 (70.0)	3 (30.0)		
Luminal B-like	99 (76.7)	82 (82.8)	17 (17.2)		
HER-2 positive (non-luminal)	9 (7.0)	8 (88.9)	1 (11.1)		
Triple negative	11 (8.5)	6 (54.5)	5 (45.5)		

Values are presented as number (%).
The χ^2 test and Fisher's exact test were used to compare pre-therapeutic serum GGT levels in groups defined by clinicopathological parameters.
GGT = gamma-glutamyl transferase; BMI = body mass index; ER = estrogen receptor; PR = progesterone receptor; HER-2 = human epidermal growth factor receptor-2; cT = clinical tumor stage; cN = clinical nodal stage.
*Pearson's χ^2 test; [†]Fisher's exact test.
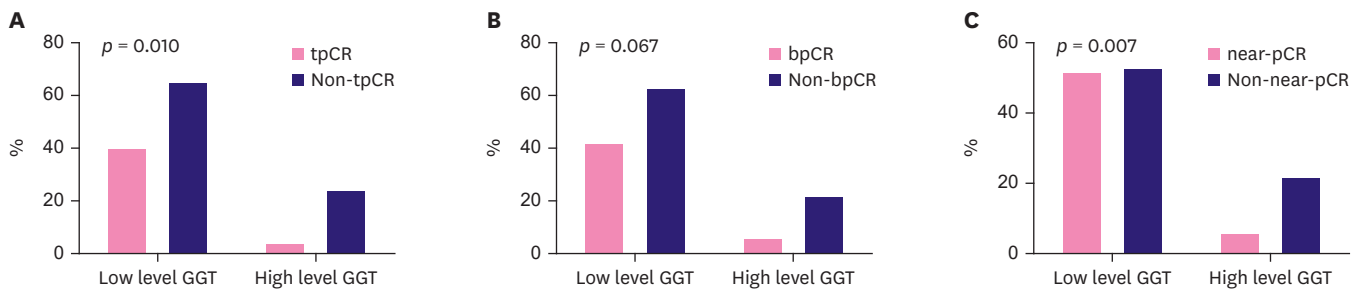
Figure 1. Comparison of pCR rate with pre-therapeutic serum GGT level. The difference of (A) tpCR, (B) bpCR, and (C) near-pCR rate between the low GGT group and the high GGT group were analyzed using the Mann-Whitney test.
GGT, gamma-glutamyl transferase; tpCR, total pathological complete response; bpCR, pathological complete response in breast; pCR, pathological complete response.

elevated Ki-67 and higher clinical tumor stage were associated with poor RFS and DFS based on multivariate analyses.

Table 2. Univariate and multivariate analyses of the predictive markers

Predictive markers	Univariate analysis			Multivariate analysis		
	OR	95% CI	p-value	OR	95% CI	p-value
tpCR						
Age (≤ 50 vs. > 50)	0.78	0.37–1.62	0.500	0.66	0.24–1.82	0.419
BMI (≤ 23 vs. > 23)	0.58	0.27–1.25	0.163	0.48	0.18–1.30	0.151
ER (< 10% vs. ≥ 10%)	0.19	0.09–0.42	< 0.001*	0.25	0.08–0.77	0.016*
PR (< 10% vs. ≥ 10%)	0.55	0.25–1.21	0.137	1.13	0.35–3.65	0.836
Ki-67 expression (< 30% vs. ≥ 30% and < 60% vs. ≥ 60%)	3.54	1.92–6.53	< 0.001*	3.77	1.78–7.98	0.001*
HER-2 status (negative vs. positive)	2.94	1.37–5.26	0.006*	4.04	1.45–11.28	0.008*
GGT level (< 29 vs. ≥ 29)	0.21	0.06–5.26	0.017*	0.15	0.03–0.80	0.026*
cT stage (cT1–2 vs. cT3–4)	0.98	0.47–2.06	0.965	0.74	0.28–1.96	0.546
cN stage (cN0 vs. cN1–3)	1.80	0.61–5.26	0.285	1.04	0.27–4.06	0.956
bpCR						
Age (≤ 50 vs. > 50)	0.88	0.43–1.81	0.723	0.72	0.28–1.88	0.506
BMI (≤ 23 vs. > 23)	0.54	0.26–1.15	0.112	0.45	0.18–1.14	0.091
ER (< 10% vs. ≥ 10%)	0.18	0.08–0.40	< 0.001*	0.31	0.11–0.90	0.031*
PR (< 10% vs. ≥ 10%)	0.42	0.19–0.92	0.030*	0.81	0.27–2.40	0.701
Ki-67 expression (< 30% vs. ≥ 30% and < 60% vs. ≥ 60%)	2.91	1.65–5.14	< 0.001*	2.67	1.35–5.31	0.005*
HER-2 status (negative vs. positive)	2.90	1.38–6.10	0.005*	4.03	1.54–10.54	0.004*
GGT level (< 29 vs. ≥ 29)	0.36	0.13–1.03	0.057	0.23	0.06–0.96	0.043*
cT stage (cT1–2 vs. cT3–4)	0.96	0.46–1.97	0.907	0.80	0.32–2.00	0.630
cN stage (cN0 vs. cN1–3)	2.11	0.72–6.16	0.171	1.43	0.39–5.21	0.590
near pCR						
Age (≤ 50 vs. > 50)	0.65	0.32–1.31	0.228	0.67	0.26–1.74	0.412
BMI (≤ 23 vs. > 23)	0.59	0.28–1.20	0.145	0.55	0.22–1.38	0.200
ER (< 10% vs. ≥ 10%)	0.24	0.11–0.52	< 0.001*	0.27	0.09–0.83	0.023*
PR (< 10% vs. ≥ 10%)	0.59	0.27–1.26	0.174	1.26	0.40–3.94	0.697
Ki-67 expression (< 30% vs. ≥ 30% and < 60% vs. ≥ 60%)	2.65	1.55–4.51	< 0.001*	3.17	1.58–6.33	0.001*
HER-2 status (negative vs. positive)	3.86	1.85–8.07	< 0.001*	4.91	1.89–12.79	0.001*
GGT level (< 29 vs. ≥ 29)	0.24	0.09–0.69	0.008*	0.13	0.03–0.54	0.005*
cT stage (cT1–2 vs. cT3–4)	1.16	0.58–2.34	0.674	0.82	0.32–2.09	0.679
cN stage (cN0 vs. cN1–3)	0.58	0.23–1.47	0.251	0.21	0.06–0.80	0.022*

Univariate and multivariate analyses of the predictive markers of tpCR, bpCR, and near-pCR rate among patients treated with NAC were assessed. Univariate analysis was performed using the χ^2 test and multivariate analysis was performed using logistic regression.
OR = odds ratio; CI = confidence interval; tpCR = total pathological complete response; BMI = body mass index; ER = estrogen receptor; PR = progesterone receptor; HER-2 = human epidermal growth factor receptor-2; GGT = gamma-glutamyl transferase; cT = clinical tumor stage; cN = clinical nodal stage; bpCR = pathological complete response in breast; pCR = pathological complete response; NAC = neoadjuvant chemotherapy.
*$p < 0.050$.

Furthermore, in the subgroup analysis, pre-therapeutic serum GGT level had marginal significance for RFS (**Table 4**; HR, 5.26; $p = 0.050$; 95% CI, 1.00–27.61) and DFS (**Table 4**; HR, 5.19; $p = 0.050$; 95% CI, 1.00–26.96) among hormone receptor (ER and PR)-positive patients but not among HER-2-positive patients.

Relationship between pre-therapeutic serum GGT level and adverse reaction

Based on the adverse reaction data of 114 patients, univariate analysis showed that the evaluated pre-therapeutic serum GGT level was significantly associated with the incidence of increased aspartate transaminase (AST) during NAC ($p = 0.019$). Moreover, serum GGT levels and adverse reactions were found to be correlated based on the multivariate analysis. Patients with low serum GGT levels were associated with a significantly higher risk of grade 3–4 neutropenia (OR, 0.35; $p = 0.047$; 95% CI, 0.12–0.97) but a lower risk of either an increase in alanine aminotransferase (OR, 6.16; $p = 0.009$; 95% CI, 1.57–24.17) or an increase in AST (OR, 4.49; $p = 0.008$; 95% CI, 1.47–13.74) (**Table 5**).
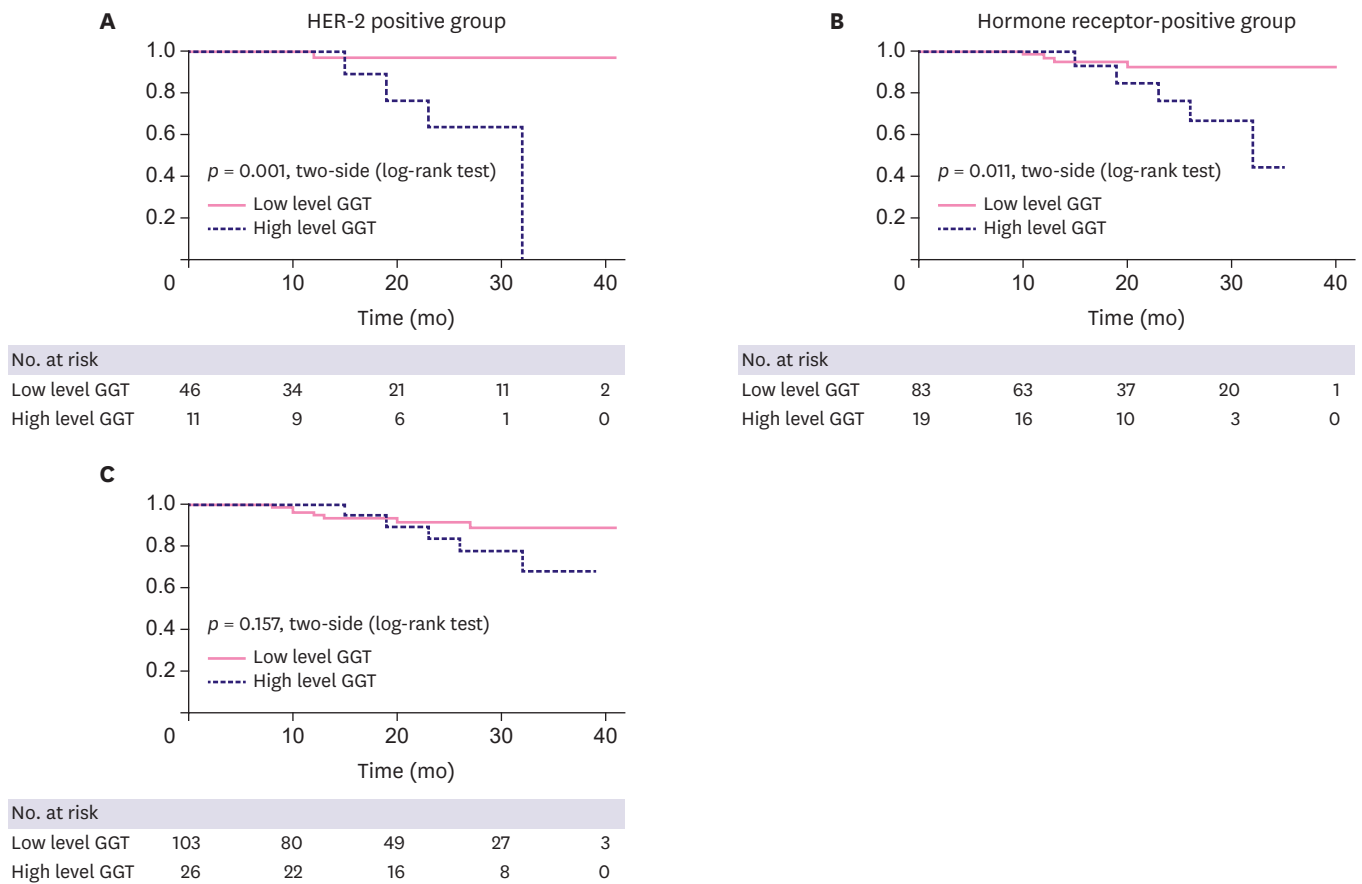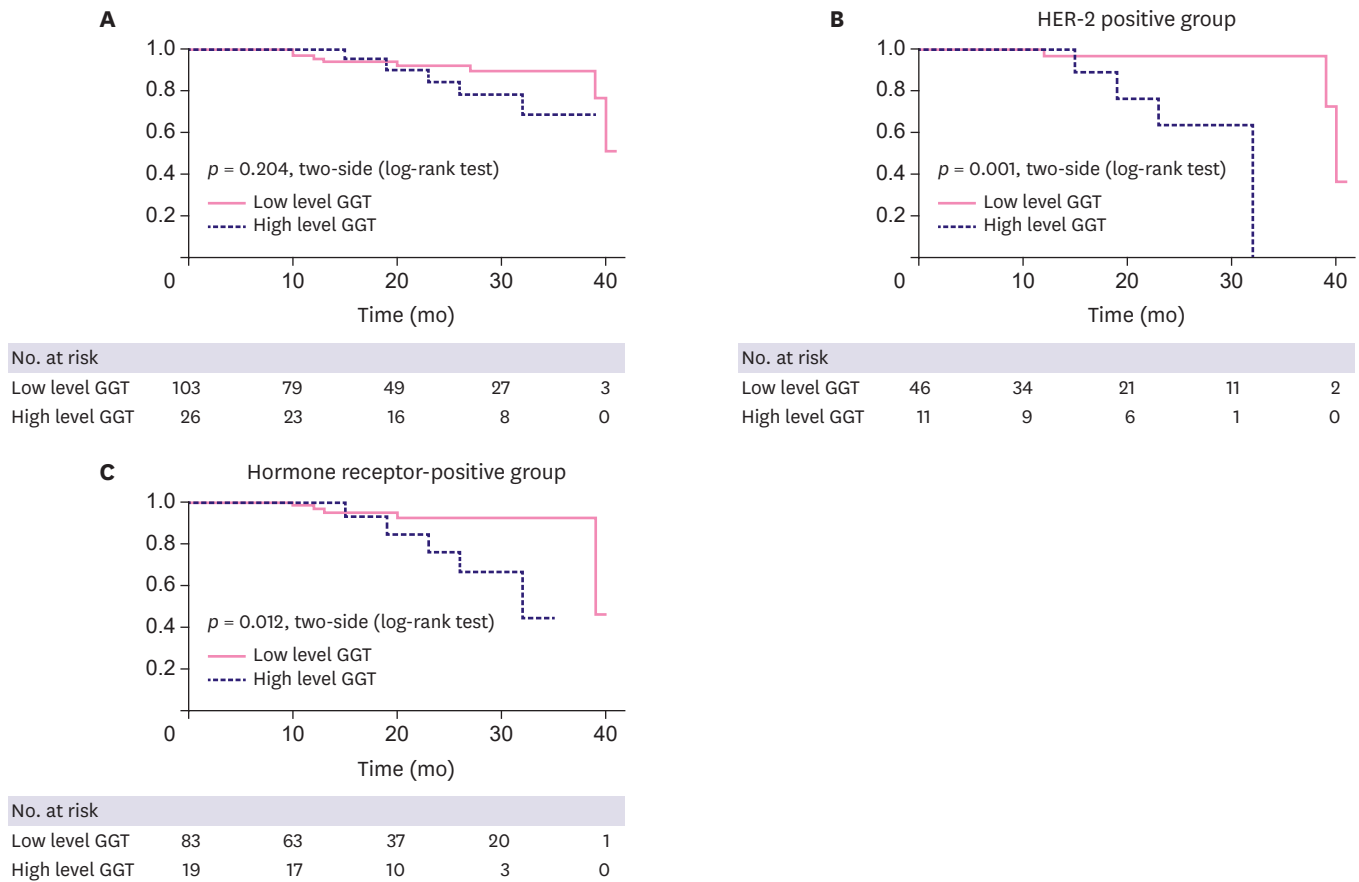
Figure 2. Kaplan-Meier curves for relapse-free survival. Kaplan-Meier survival estimates and log-rank tests were used to analyze the prognostic significance of the pre-therapeutic serum GGT level among (A) HER-2 positive, (B) hormone receptor-positive breast cancer, and (C) all patients treated with neoadjuvant chemotherapy. GGT, gamma-glutamyl transferase; HER-2, human epidermal growth factor receptor-2.

DISCUSSION

To the best of our knowledge, the present study is the first to investigate the predictive value of pre-therapeutic serum GGT level on NAC response in LABC. Based on prospective clinical trials, we found that low serum GGT levels have statistically significant relationships with higher pCR rates, prolonged DFS and RFS, and higher incidence of grade 3–4 neutropenia. Therefore, we speculated that GGT might be a potential predictive biomarker for patients with LABC.

Until now, there has been no consensus on the optimal cutoff value for serum GGT levels. In the current study, we selected the final quintile (29 U/L) of all patients' pre-therapeutic serum GGT levels for stratification, which is also a median value between the average (24 U/L) and the cutoff value of previous GGT cancer risk groups (36 U/L) [8,12,13,16,17]. The final quintile is often used as the stratified value of a risk model in other clinical studies [18,19]; hence, we considered it reasonable to use it in this study. Our findings verified that the baseline serum GGT levels and patients' clinicopathological features, such as age, ER, PR, HER-2, Ki-67 expression, and molecular types, were not correlated. Similarly, it was confirmed in the retrospective study of Staudigl et al., [8] which involved 114 patients with primary metastatic breast cancer, that serum GGT level was not associated with the patient's age, histological type and grade, lymph node involvement, hormone receptor, and HER-2 status [8].

Figure 3. Kaplan-Meier curves for DFS. Kaplan-Meier survival estimates and log-rank tests were used to analyze the prognostic significance of the pre-therapeutic serum GGT level among (A) all, (B) HER-2 positive, and (C) hormone receptor-positive breast cancer patients treated with neoadjuvant chemotherapy. GGT, gamma-glutamyl transferase; HER-2, human epidermal growth factor receptor-2; DFS, disease-free survival.

A number of studies have also indicated that GGT is a prognostic biomarker for several cancers in the adjuvant or rescue settings, such as in cervical cancer [13], endometrial cancer [12], renal cell carcinoma [14], and primary metastatic breast cancer [8]; nevertheless, data for patients receiving NAC are limited. Previous reports have focused on the relationship between serum GGT levels and survival and have not assessed treatment responses. The current study is the first to include the association between serum GGT level and pathological outcome and survival of breast cancer patients who received NAC. We demonstrated that patients with low serum GGT levels (< 29 U/L) are more likely to achieve tpCR, bpCR, and near-pCR compared to those with high serum GGT levels (≥ 29 U/L). Furthermore, we demonstrated that patients with low serum GGT levels have better RFS and DFS.

The results of our clinical study are in accordance with that of other experimental studies. Traditionally, GGT has a vital role in the protection against oxidative stress during cell metabolism, which means that GGT is able to modulate redox-sensitive functions, such as antioxidant defenses and the proliferative/apoptotic balance of cells [4]. Previous research has also demonstrated the increase of serum GGT levels in several malignancies [5]. In this study, the investigators hypothesized that GGT might be associated with tumor development. Wang et al. [20] found that the expression of GGT in tumor cells was increased, which could promote the development and invasion of gastric cancer. Moreover, previous studies have confirmed that GGT can affect the sensitivity of tumor cells to drugs [17,21,22]. Mares et al.

Table 3. Univariate and multivariate RFS and DFS analyses

Variables	Univariate analysis		p-value	Multivariate analysis		p-value
	HR	95% CI		HR	95% CI	
RFS						
ER status	0.82	0.26–2.59	0.739	0.51	0.13–1.98	0.329
PR status	1.08	0.32–3.64	0.892	3.09	0.61–15.66	0.172
Ki-67	2.76	1.10–6.97	0.031*	5.14	1.32–17.39	0.008*
BMI	2.10	0.63–7.00	0.224	1.72	0.41–7.18	0.457
cT stage	3.59	0.96–13.50	0.058	6.64	1.43–30.84	0.016*
cN stage	0.51	0.14–1.90	0.318	0.45	0.09–2.10	0.307
Age	0.74	0.23–2.33	0.604	0.64	0.17–2.44	0.512
GGT level	2.24	0.71–7.09	0.168	4.34	1.11–16.93	0.035*
HER-2	1.01	0.31–3.18	0.988	0.42	0.10–1.67	0.218
DFS						
Age	0.77	0.26–2.24	0.630	0.76	0.20–2.89	0.690
BMI	1.61	0.52–4.95	0.405	1.26	0.34–4.69	0.726
ER status	0.94	0.32–2.81	0.914	0.46	0.12–1.70	0.244
PR status	1.51	0.43–5.27	0.523	3.02	0.63–14.58	0.168
Ki-67 expression	2.68	1.15–6.24	0.023*	5.72	1.69–19.42	0.005*
HER-2 status	1.28	0.44–3.68	0.647	0.58	0.16–2.04	0.393
GGT level	2.04	0.66–6.28	0.214	4.33	1.11–16.85	0.035*
cT stage	3.17	0.98–10.29	0.054	4.98	1.24–20.03	0.024*
cN stage	0.52	0.14–1.91	0.321	0.45	0.09–2.12	0.311

Cox analysis was used to analyze the prognostic significance of pre-therapeutic GGT levels in overall breast cancer patients.
HR, hazard ratio; CI, confidence interval; RFS, relapse-free survival; DFS, disease-free survival; ER, estrogen receptor; PR, progesterone receptor; BMI, body mass index; cT, clinical tumor stage; cN, clinical nodal stage; GGT, gamma-glutamyl transferase; HER-2, human epidermal growth factor receptor-2.
*$p < 0.050$.

Table 4. Univariate and multivariate RFS and DFS analyses

Variables	RFS			DFS		
	HR	95% CI	p-value	HR	95% CI	p-value
HER-2 positive						
Age	0.50	0.02–12.82	0.672	0.43	0.02–11.71	0.620
BMI	1.51	0.04–62.26	0.829	1.19	0.04–35.87	0.920
ER status	0.36	0.01–16.00	0.600	0.38	0.01–17.06	0.620
PR status	11.73	0.18–780.1	0.250	12.76	0.19–866.79	0.237
Ki-67 expression	4.06	0.29–57.40	0.300	5.03	0.46–55.32	0.186
GGT level	11.20	0.54–231.90	0.118	11.83	0.55–254.25	0.114
cT stage	3.77	0.15–94.94	0.420	4.25	0.17–108.93	0.382
cN stage	0.27	0.02–4.42	0.357	0.26	0.02–4.50	0.355
Hormone receptor positive						
Age	1.40	0.29–6.83	0.676	1.42	0.29–6.87	0.662
BMI	1.04	0.20–5.54	0.960	1.04	0.20–5.49	0.968
Ki-67 expression	3.42	1.06–11.04	0.040*	3.40	1.06–10.93	0.040*
HER-2 status	0.78	0.14–4.46	0.781	0.79	0.14–4.47	0.794
GGT level	5.26	1.00–27.61	0.050	5.19	1.00–26.96	0.050
cT stage	4.84	0.92–25.38	0.062	4.91	0.94–25.69	0.060
cN stage	0.60	0.11–3.20	0.551	0.60	0.11–3.18	0.546

Cox analysis was used to analyze the prognostic significance of pre-therapeutic GGT levels in HER-2 and hormone receptor-positive breast cancer patients.
RFS, relapse-free survival; DFS, disease-free survival; HR, hazard ratio; CI, confidence interval; HER-2, human epidermal growth factor receptor-2; BMI, body mass index; ER, estrogen receptor; PR, progesterone receptor; GGT, gamma-glutamyl transferase; cT, clinical tumor stage; cN, clinical nodal stage.
*$p < 0.050$.

[23] found that GGT activity was significantly elevated in cisplatin-resistant glioma cells. On the other hand, Franzini et al. [17] found that the overexpression of GGT reduced the sensitivity of melanoma cells to cisplatin. GGT-overexpressing cells were also shown to be more resistant to other chemotherapy drugs such as doxorubicin and 5-fluorouracil [24]. In our patient cohort, those who had high levels of pre-therapeutic serum GGT showed less sensitivity to NAC than those with low levels, which is consistent with previous findings. Recent studies also demonstrated the potential role of GGT in ferroptosis through the

Table 5. Association of pre-therapeutic serum GGT levels and adverse effects

Toxic reaction	GGT level	Toxic grade No. (%)		χ^2	p-value*	Logit-P†	OR‡	95% CI§
		Grade < 3	Grade ≥ 3					
Neutropenia	Low level	41 (43.2)	54 (56.8)	2.42	0.119	0.047	0.35	0.12–0.97
	High level	13 (61.9)	8 (38.1)					
Leukopenia	Low level	64 (67.4)	31 (32.6)	0.13	0.718	0.486	0.67	0.22–2.02
	High level	15 (71.4)	6 (28.6)					
		Grade < 2	Grade ≥ 2					
Anemia	Low level	64 (67.4)	31 (632.6)	0.62	0.603	0.312	0.55	0.17–1.75
	High level	16 (76.2)	5 (23.8)					
Vomiting	Low level	76 (80.0)	19 (20.0)	1.27	0.357	0.235	0.39	0.08–1.85
	High level	19 (90.5)	12 (9.5)					
Peripheral neuropathy	Low level	61 (64.2)	34 (35.8)	0.39	0.529	0.182	0.46	0.15–1.43
	High level	15 (71.4)	6 (28.6)					
		Grade < 1	Grade ≥ 1					
Hand-foot syndrome	Low level	65 (68.4)	30 (31.6)	0.49	0.604	0.233	0.49	0.15–1.57
	High level	16 (76.2)	5 (23.8)					
ALT increased	Low level	79 (83.2)	16 (16.8)	2.94	0.083	0.009	6.16	1.57–24.17
	High level	14 (66.7)	7 (33.3)					
AST increased	Low level	54 (90.0)	6 (10.0)	5.50	0.019	0.008	4.49	1.47–13.74
	High level	41 (73.2)	15 (26.8)					

GGT, gamma-glutamyl transferase; OR, odds ratio; CI, confidence interval; ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor-2; cT, clinical tumor stage; cN, clinical nodal stage.
*Pearson χ^2 test; †p-values were analyzed with adjustment for age, BMI, ER, PR, HER-2, Ki-67, cT, and cN; ‡OR was analyzed using multivariate logistic regression; §95% CI was analyzed using multivariate logistic regression.

mediation of GSH metabolism and the production of iron-dependent reactive oxygen species [25]. These studies have explored the possible effects of GGT on tumor cell biology. Based on these findings, we believe that GGT can enhance tumor cell resistance to chemotherapy, indicating that serum GGT level might be a potential predictor of pCR. Nevertheless, the role of serum GGT in different breast cancer molecular classifications remains to be elucidated.

In addition, previous studies have suggested that cisplatin-induced kidney damage is associated with oxidative stress [26]. It was found in vitro that knockout or inhibition of GGT resulted in increased susceptibility to cisplatin and kidney injury [27]. However, more recent studies propose a different view. Fliedl et al. [28] found that after inhibiting GGT expression through a specific inhibitor, the renal toxicity of cisplatin decreased. These findings are indicative of a relationship between cisplatin nephrotoxicity and GGT-mediated drug metabolism. In order to explore the relationship between serum GGT levels and adverse effects, we analyzed the incidence of adverse reactions during NAC. Multivariate analysis showed that patients with high serum GGT levels had a significantly lower incidence of grade 3–4 neutropenia. This fact implied that patients with high serum GGT levels have a better tolerance to chemotherapeutic drugs compared to those with low serum GGT levels. However, the correlation between serum GGT levels and renal damage remained unclear in our study. This may due to the fact that only one patient had a grade 3–4 creatinine increase, which meant that the incidence of severe kidney damage was pretty low. Therefore, the relationship between serum GGT level and cisplatin nephrotoxicity needs further research. In summary, we hold the view that serum GGT levels have a predictive value in the incidence of adverse reactions among breast cancer patients during NAC.

A limitation of our investigation was the sample size, which might undermine the critical value of GGT. However, since the majority of the participants in this study were patients undergoing prospective clinical studies who have complete and highly reliable clinical and pathological information, the limitation above most likely had little effect on our results. Moreover, although

our study only included patients receiving weekly paclitaxel and cisplatin regimens, other researchers have already depicted the association between GGT and chemotherapy response in liver metastatic colorectal cancer patients treated with FOLFOX4 with or without bevacizumab [29], supporting the hypothesis that GGT could be used as a predictive factor for successful patient response in other malignancies and chemotherapy regimens. Serum GGT level serves as an indicator of oxidative stress and is clinically used to assess liver disease and excessive alcohol consumption. Its serum concentration is affected by an array of factors, including hepatobiliary diseases and anti-tumor therapy. In this study, we collected baseline patient information, excluding patients with abnormal hepatobiliary function, and thus eliminating the influence of liver disease and anti-cancer therapy. Despite these potential limitations, our results are clinically valuable and may serve as a basis for future studies. Nevertheless, it is indispensable to further increase the sample size and add other chemotherapy regimens to better verify our findings.

REFERENCES

1. Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet 2014;384:164-72.
   PUBMED | CROSSREF

2. Chen Y, Chen K, Xiao X, Nie Y, Qu S, Gong C, et al. Pretreatment neutrophil-to-lymphocyte ratio is correlated with response to neoadjuvant chemotherapy as an independent prognostic indicator in breast cancer patients: a retrospective study. BMC Cancer 2016;16:320.
   PUBMED | CROSSREF

3. Lim JS, Yang JH, Chun BY, Kam S, Jacobs DR Jr, Lee DH. Is serum gamma-glutamyltransferase inversely associated with serum antioxidants as a marker of oxidative stress? Free Radic Biol Med 2004;37:1018-23.
   PUBMED | CROSSREF

4. Hanigan MH. Gamma-glutamyl transpeptidase: redox regulation and drug resistance. Adv Cancer Res 2014;122:103-41.
   PUBMED | CROSSREF

5. Strasak AM, Pfeiffer RM, Klenk J, Hilbe W, Oberaigner W, Gregory M, et al. Prospective study of the association of gamma-glutamyltransferase with cancer incidence in women. Int J Cancer 2008;123:1902-6.
   PUBMED | CROSSREF

6. Fentiman IS, Allen DS. Gamma-glutamyl transferase and breast cancer risk. Br J Cancer 2010;103:90-3.
   PUBMED | CROSSREF

7. Van Hemelrijck M, Jassem W, Walldius G, Fentiman IS, Hammar N, Lambe M, et al. Gamma-glutamyltransferase and risk of cancer in a cohort of 545,460 persons - the Swedish AMORIS study. Eur J Cancer 2011;47:2033-41.
   PUBMED | CROSSREF

8. Staudigl C, Concin N, Grimm C, Pfeiler G, Nehoda R, Singer CF, et al. Prognostic relevance of pretherapeutic gamma-glutamyltransferase in patients with primary metastatic breast cancer. PLoS One 2015;10:e0125317.
   PUBMED | CROSSREF

9. Polterauer S, Hofstetter G, Grimm C, Rahhal J, Mailath-Pokorny M, Kohl M, et al. Relevance of gamma-glutamyltransferase--a marker for apoptotic balance--in predicting tumor stage and prognosis in cervical cancer. Gynecol Oncol 2011;122:590-4.
   PUBMED | CROSSREF

10. Hu G, Tuomilehto J, Pukkala E, Hakulinen T, Antikainen R, Vartiainen E, et al. Joint effects of coffee consumption and serum gamma-glutamyltransferase on the risk of liver cancer. Hepatology 2008;48:129-36.
    PUBMED | CROSSREF

11. Hofbauer SL, Stangl KI, de Martino M, Lucca I, Haitel A, Shariat SF, et al. Pretherapeutic gamma-glutamyltransferase is an independent prognostic factor for patients with renal cell carcinoma. Br J Cancer 2014;111:1526-31.
    PUBMED | CROSSREF

12. Seebacher V, Polterauer S, Grimm C, Rahhal J, Hofstetter G, Bauer EM, et al. Prognostic significance of gamma-glutamyltransferase in patients with endometrial cancer: a multi-centre trial. Br J Cancer 2012;106:1551-5.
    PUBMED | CROSSREF

13. Zhu Y, Zhang AJ, Wu DB, Shen Z, Chen G, Shi YY, et al. Prognostic significance of the pretreatment serum gamma-glutamyltransferase levels in Chinese patients with non-metastatic cervical cancer. Oncotarget 2017;8:115701-8.
    PUBMED | CROSSREF

14. Luo C, Xu B, Fan Y, Yu W, Zhang Q, Jin J. Preoperative gamma-glutamyltransferase is associated with cancer-specific survival and recurrence-free survival of nonmetastatic renal cell carcinoma with venous tumor thrombus. BioMed Res Int 2017;2017:3142926.
    PUBMED | CROSSREF

15. Zhou L, Xu S, Yin W, Lin Y, Du Y, Jiang Y, et al. Weekly paclitaxel and cisplatin as neoadjuvant chemotherapy with locally advanced breast cancer: a prospective, single arm, phase II study. Oncotarget 2017;8:79305-14.
    PUBMED | CROSSREF

16. Grimm C, Hofstetter G, Aust S, Mutz-Dehbalaie I, Bruch M, Heinze G, et al. Association of gamma-glutamyltransferase with severity of disease at diagnosis and prognosis of ovarian cancer. Br J Cancer 2013;109:610-4.
    PUBMED | CROSSREF

17. Franzini M, Corti A, Lorenzini E, Paolicchi A, Pompella A, De Cesare M, et al. Modulation of cell growth and cisplatin sensitivity by membrane gamma-glutamyltransferase in melanoma cells. Eur J Cancer 2006;42:2623-30.
    PUBMED | CROSSREF

18. Gasparini M, Klersy C, Leclercq C, Lunati M, Landolina M, Auricchio A, et al. Validation of a simple risk stratification tool for patients implanted with cardiac resynchronization therapy: the VALID-CRT risk score. Eur J Heart Fail 2015;17:717-24.
    PUBMED | CROSSREF

19. Padwal R, Leslie WD, Lix LM, Majumdar SR. Relationship among body fat percentage, body mass index, and all-cause mortality: a cohort study. Ann Intern Med 2016;164:532-41.
    PUBMED | CROSSREF

20. Wang Q, Shu X, Dong Y, Zhou J, Teng R, Shen J, et al. Tumor and serum gamma-glutamyl transpeptidase, new prognostic and molecular interpretation of an old biomarker in gastric cancer. Oncotarget 2017;8:36171-84.
    PUBMED | CROSSREF

21. Pompella A, De Tata V, Paolicchi A, Zunino F. Expression of gamma-glutamyltransferase in cancer cells and its significance in drug resistance. Biochem Pharmacol 2006;71:231-8.
    PUBMED | CROSSREF

22. Daubeuf S, Leroy P, Paolicchi A, Pompella A, Wellman M, Galteau MM, et al. Enhanced resistance of HeLa cells to cisplatin by overexpression of gamma-glutamyltransferase. Biochem Pharmacol 2002;64:207-16.
    PUBMED | CROSSREF

23. Mares V, Lisá V, Malík R, Kozáková H, Sedo A. Cisplatin induced gamma-glutamyltransferase up-regulation, hypertrophy and differentiation in astrocytic glioma cells in culture. Histol Histopathol 2003;18:687-93.
    PUBMED

24. Hochwald SN, Rose DM, Brennan MF, Burt ME. Elevation of glutathione and related enzyme activities in high-grade and metastatic extremity soft tissue sarcoma. Ann Surg Oncol 1997;4:303-9.
    PUBMED | CROSSREF

25. Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, et al. Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. Cell 2017;171:273-85.
    PUBMED | CROSSREF

26. Hanigan MH, Devarajan P. Cisplatin nephrotoxicity: molecular mechanisms. Cancer Ther 2003;1:47-61.
    PUBMED

27. Paolicchi A, Sotiropuolou M, Perego P, Daubeuf S, Visvikis A, Lorenzini E, et al. Gamma-glutamyl transpeptidase catalyses the extracellular detoxification of cisplatin in a human cell line derived from the proximal convoluted tubule of the kidney. Eur J Cancer 2003;39:996-1003.
    PUBMED | CROSSREF

28. Fliedl L, Wieser M, Manhart G, Gerstl MP, Khan A, Grillari J, et al. Controversial role of gamma-glutamyl transferase activity in cisplatin nephrotoxicity. ALTEX 2014;31:269-78.
    PUBMED | CROSSREF

29. Denić K, Tarabar D, Obradovic S, Ristić M, Spasić J, Radosavljević D, et al. Biochemical liver function test parameter levels in relation to treatment response in liver metastatic colorectal patients treated with FOLFOX4 with or without bevacizumab. Srp Arh Celok Lek 2016;144:615-20.
    PUBMED | CROSSREF