Research Article

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Serum Homocysteine and Vitamin B12 as Biomarkers for Haematological Toxicity in Lung Adenocarcinoma Treated With Pemetrexed

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Abstract: Background Serum homocysteine (Hcy) and vitamin B12 (VitB12) were investigated as serological markers for the prediction of pemetrexed induced haematological toxicity in patients with adenocarcinoma of the lung.

Material and Methods A total of 35 lung adenocarcinoma patients who received pemetrexed chemotherapy as first-line treatment were included in the present study. The patients received pemetrexed 500 mg/m² once every three weeks until disease progression. Serum Hcy and VitB12 levels were analysed prior to chemotherapy. Haematological toxicities (leucopenia, neutropenia and thrombocytopenia) were graded for each cycle of chemotherapy. Serum Hcy and VitB12 concentrations were compared between grades 0-1 and 2-4 haematological toxicity groups.

Results A total of 151 chemotherapy cycles were administered to 35 lung adenocarcinoma patients. However, the serum Hcy and VitB12 concentration were only examined and recorded in 61 out of the 151 chemotherapy cycles. For the 61 cycles, grade 2-4 leucopenia, neutropenia and thrombocytopenia were observed in 21, 20 and 10 cases, respectively. Serum Hcy levels were 14.91±4.67 µg/ml, 15.50±4.35 µg/ml and 16.04±4.90 µg/ml for grade 2-4 leucopenia, neutropenia and thrombocytopenia, respectively, which were significantly higher than those of grade 0-1 groups ($p < 0.05$). However, serum VitB12 were not statistically different between

grade 0-1 and 2-4 haematological toxicity groups ($p > 0.05$). The area under the ROC curve (AUC) were 0.73 (0.58-0.88), 0.80 (0.66-0.94), 0.75 (0.57-0.93) for serum Hcy and 0.65 (0.50-0.79), 0.64 (0.49-0.78), 0.68 (0.49-0.87) for serum VitB12 as predictive biomarkers of grade 2-4 leucopenia, neutropenia and thrombocytopenia, respectively.

Conclusion Pre-chemotherapy serum Hcy appeared to correlate with haematological toxicity and may be a useful biomarker for predicting severity of pemetrexed induced haematological toxicity.

Keywords: Adenocarcinoma of the lung; homocysteine; VitB12; pemetrexed; haematological toxicity.

Introduction

Epidemiology studies indicated that lung cancer was the most diagnosed malignant carcinoma world wide and the leading cause of cancer-related deaths for males and second for females [1]. The majority of diagnosed lung cancer cases were of an advantage stage and often unresectable [2]. Chemotherapy was the most common treatment for these advanced lung cancer cases; however, only ~30% of patients typically benefit from first-line platinum-based chemotherapy [3]. Recently, several prospective randomized clinical trials demonstrated that overall survival (OS) and disease-free survival (DFS) could be improved for lung adenocarcinoma patients who received pemetrexed chemotherapy regimen as first-line treatment [4-7]. However, haematological toxicities such as leucopenia, neutropenia and thrombocytopenia were common and therefore limited its clinical application. Consequently, the ability to predict pemetrexed induced haematological toxicity and thus reduce these instances was of vital importance. Previous studies have demonstrated that serum Hcy levels were correlated with the severity of pemetrexed induced haematological toxicity [8]. The higher the pre-chemotherapy serum Hcy level, the higher the risk of developing pemetrexed

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induced haematological toxicity. In this study, we included 35 lung adenocarcinoma patients and evaluated the efficacy of serum Hcy and VitB12 concentrations as serological markers of haematological toxicity when treated with pemetrexed.

Material and Methods

Patients

This work was approved by the Medical Ethics Committee of Tianjin Union Medical Center (Tianjin People's Hospital), Tianjin, P.R. China. The research related to human use had complied with all the relevant national regulations, institutional policies and in accordance with the Helsinki Declaration, and had been approved by the Tianjin Union Medicine's institutional review board or equivalent committee. A total of 151 chemotherapy cycles were administered to the 35 lung adenocarcinoma patients. Serum Hcy and VitB12 concentrations were determined in 61 of the 151 chemotherapy cycles. The pathology type was adenocarcinoma of the lung. The Eastern Cooperative Oncology Group (ECOG) performance score was 0, 1 or 2. The expected survival time was more than three months. Routine blood test, liver and kidney function test and myocardial enzyme test were all in accordance with the indications of chemotherapy. Informed consent was given by all included patients. Patients with hyperhomocysteinemia were excluded as well as those with previous long-term use of either VitB12, VitB6 or folic acid.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the Medical Ethics Committee of Tianjin Union Medical Center (Tianjin People's Hospital), Tianjin, P.R. China.

Informed consent: Informed consent has been obtained from all individuals included in this study.

Chemotherapy regimen

The patients received a 500 mg/m² dose of pemetrexed (Eli Lilly and Company) intravenous drip once every three weeks (single cycle) until disease progression. One-week prior to pemetrexed administration, 40 µg folic acid (Tianjin Lisheng Pharmaceutical Co., Ltd) was administered orally.

Haematological toxicity evaluation

The lowest concentrations of leukocytes, neutrophils and platelets after chemotherapy were used as the criteria for evaluating the severity of haematological toxicity. The chemotherapy-related haematological toxicity severity was described using the Common Terminology Criteria for Adverse Events (CTCAE) v3.0 [9].

Serum Hcy and VitB12 measurement

Five mL of peripheral venous blood was collected one-day prior to chemotherapy and injected into the biochemical test tube. The blood was centrifugated at 3000 r/min for five minutes to obtain serum. Serum Hcy and VitB12 levels were examined by Roche CO-BAS E601 automatic immune analyzer via an electrochemiluminescence method. All operations were carried out in strict accordance with the manufacturer's instructions.

Statistical analysis

SPSS 18.0 statistical software package (SPSS, Inc., Chicago, IL, USA) was used for data analysis. Measurement data were expressed as mean ± standard deviation and analyzed by student t-test. The enumeration data were expressed with a relative number, and the comparison between groups was made by chi-square test. Serum Hcy and VitB12 in the prediction of the severity of chemotherapy-related haematological toxicity was analyzed by receiver operating characteristic (ROC) curves and area under ROC curve (AUC). Two-tailed $p < 0.05$ was considered statistically significant.

Result

General characteristics of the included lung adenocarcinoma patients

The 35 lung adenocarcinoma patients had a mean age of 63.4 ± 11.2 years. Sixteen patients received first-line pemetrexed chemotherapy, and the other 19 cases received second-line pemetrexed treatment. The general characteristics of the included 35 cases were shown in **Table 1**.

Table 1: General characteristics of the included 35 lung adenocarcinoma patients.

Characteristic	N=35
Gender	
Male	11
Female	24
Pemetrexed treatment	
First line	16
Second line	19
Smoking	
Positive	14
Negative	21
ECOG performance score	
0/1	26
2	9
Differentiation	
Well/moderate	10
Poor	25
Stage	
	16
	19

ECOG: Eastern Cooperative Oncology Group

Table 2: The number of pemetrexed related toxicities per chemotherapy cycle.

Toxicity	Total	Cycle 1	Cycle 2	Cycle 3	Cycle 4
Leucopenia					
0-1	40	15	10	9	6
2-4	21	7	5	5	4
Neutropenia					
0-1	41	15	10	11	5
2-4	20	7	5	3	5
Thrombocytopenia					
0-1	51	20	13	11	7
2-4	10	2	2	3	3

Table 3: Serum Hcy and VitB12 concentrations for each haematological toxicity grade/group.

Haematological toxicity	Hcy($\mu\text{g/ml}$)	VitB12(pg/ml)
Leucopenia		
0-1	11.25 $\pm$ 3.04	984.30 $\pm$ 331.00
2-4	14.91 $\pm$ 4.67	915.90 $\pm$ 300.00
t	3.69	1.95
p	0.001	0.06
Neutropenia		
0-1	11.06 $\pm$ 2.97	976.50 $\pm$ 339.80
2-4	15.50 $\pm$ 4.35	8263.60 $\pm$ 283.90
t	4.69	1.76
p	<0.001	0.09
Thrombocytopenia		
0-1	11.82 $\pm$ 3.51	960.90 $\pm$ 323.60
2-4	16.04 $\pm$ 4.90	750.30 $\pm$ 308.70
t	3.25	1.90
p	0.002	0.06

Haematological toxicity

A total of 151 chemotherapy cycles were given to the 35 lung adenocarcinoma patients. However, the serum Hcy and VitB12 concentration were only analysed from 61 of the 151 chemotherapy cycles. Of the 61 cycles, grade 2-4 leucopenia, neutropenia and thrombocytopenia were observed in 21, 20 and 10 cases, respectively. Grade 2-4 haematological toxicities of each chemotherapy cycle were shown in **Table 2**. Only one-cycle of chemotherapy resulted in grade 4 neutropenia and thrombocytopenia.

Correlation between serum Hcy and VitB12 concentrations and haematological toxicity

The serum Hcy concentrations were 14.91 $\pm$ 4.67 $\mu\text{g/ml}$, 15.50 $\pm$ 4.35 $\mu\text{g/ml}$ and 16.04 $\pm$ 4.90 $\mu\text{g/ml}$ for grade 2-4 leucopenia, neutropenia and thrombocytopenia respectively, which were significantly higher than those of grade 0-1 groups ($p < 0.05$), **Table 3**. However, the serum VitB12 concentrations were not statistically different between grade 0-1 and 2-4 haematological toxicity groups ($p > 0.05$), **Figure 1**.

Figure 1: Serum Hcy and VitB12 distribution for each haematological toxicity group. Serum Hcy distribution in grade 0-1 and 2-4 leucopenia(a), neutropenia(c) and thrombocytopenia(e). Serum VitB12 distribution in grade 0-1 and 2-4 leucopenia(b), neutropenia(d) and thrombocytopenia(f).

Table 4: Severity of haematological toxicity prediction by serum Hcy and VitB12 (95%CI).

Marker	Hcy			VitB12		
	Leucopenia	Neutropenia	Thrombocytopenia	Leucopenia	Neutropenia	Thrombocytopenia
Sensitivity	71.43 (47.82-88.72)	70.00 (45.72-88.11)	70.00 (34.75-93.33)	66.67 (43.03-85.41)	60.00 (36.05-80.88)	70.00 (34.75-93.33)
Specificity	72.50 (56.11-85.40)	90.24 (76.87-97.28)	76.47 (62.51-87.21)	62.50 (45.80-77.27)	63.41 (46.94-77.88)	56.86 (42.25-70.65)
AUC	0.73(0.58-0.88)	0.80(0.66-0.94)	0.75(0.57-0.93)	0.65(0.50-0.79)	0.64(0.49-0.78)	0.68(0.49-0.87)
Cut off	12.97	14.43	14.23	898.10	888.30	898.10

AUC: area under the roc curve

Severity of haematological toxicity predicted by serum Hcy and VitB12 concentrations

The AUCs were 0.73 (0.58-0.88), 0.80 (0.66-0.94), 0.75 (0.57-0.93) for serum Hcy and 0.65 (0.50-0.79), 0.64 (0.49-0.78), 0.68 (0.49-0.87) for serum VitB12 as biomarkers in prediction of grade 2-4 leucopenia, neutropenia and thrombocytopenia, respectively, **Table 4** and **Figure 2**.

Discussion

Pemetrexed known as folate antimetabolites is similar to folic acid. The anti-cancer activity of pemetrexed inhibited thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT) [10] in purine and pyrimidine synthesis. Pemetrexed can inhibit the formation of precursor purine and pyrimidine nucleotides, preventing the formation of DNA and RNA, which are required for the growth and survival of both normal and cancerous cells [11, 12].

Clinical trials have shown that compared to other platinum-based chemotherapy regimens, pemetrexed alone or in combination with platinum can improve the prognosis of patients with non-squamous cell lung carcinoma [7, 13]. Kreuter and his colleges [14] performed a randomized phase 2 clinical trial and evaluated the clinical efficacy of adjuvant chemotherapy with cisplatin and pemetrexed versus cisplatin and vinorelbine for early-stage lung adenocarcinoma. They found that adjuvant chemotherapy with cisplatin and pemetrexed was safe, less toxic with superior dose delivery when compared with cisplatin and vinorelbine. However, grade 3/4 pemetrexed induced haematological toxicity was a major obstacle for high-dose clinical application. Several studies have

shown haematological toxicities during pemetrexed chemotherapy regimens for lung carcinoma were common [15-17]. Studies have also shown that haematological toxicity was not only related to pemetrexed dosage but also with the serum level of Hcy, VitB12 and folate [18, 19]. Folic acid administration prior to chemotherapy reduced pemetrexed related haematological toxicity [20]. In addition, serum Hcy concentration also correlated with haematological toxicities in patients receiving pemetrexed chemotherapy [21]. However, the serological markers for haematological toxicity in lung adenocarcinoma treated with pemetrexed were seldom reported. Cao et al. [22] evaluated the correlation of peripheral blood Hcy, VitB12 and folic acid levels with pemetrexed induced haematological toxicity. The results indicated that severe neutropenia correlated with serum Hcy concentrations and patients with elevated serum Hcy had an increased risk of developing severe neutropenia. However, this work did not discuss the predictive performance of serum Hcy, VitB12 and folate as a serological marker for the prediction of haematological toxicity. In our study, we found that the serum Hcy levels were correlated with pemetrexed haematological toxicity, including leucopenia, neutropenia and thrombocytopenia, in accordance with the conclusion of Cao et al. [22]. In addition, we also evaluated the predictive value of serum Hcy and VitB12 for pemetrexed induced haematological toxicity and found that pre-chemotherapy serum Hcy levels can predict the risk of grade 2-4 haematological toxicity.

Conclusion

Pre-chemotherapy (pemetrexed) serum Hcy appeared to correlate with haematological toxicity and may be used as a serological marker to predict its severity.

Figure 2: ROC curve of serum Hcy as a biomarker for prediction of grade 2-4 leucopenia(a), neutropenia(c) and thrombocytopenia(e). Serum VitB12 as a biomarker for prediction of grade 2-4 leucopenia(b), neutropenia(d) and thrombocytopenia(f).

However, due to the small sample size and the fact that patients were recruited from a single medical center, limited statistical power and patient selection bias were ineluctable. Another limitation was that some data relating to chemotherapy related toxicities were used more than once, and therefore, it is important to note that the statistical power may be exaggerated. Consequently, we suggest that a well-designed, prospective randomized clinical trial in lung adenocarcinoma patients is required to further investigate serum Hcy and VitB12 concentrations as biomarkers of pemetrexed induced haematological toxicity.

Conflict of interest: Authors state no conflict of interest

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