Apatinib combined with temozolomide in the treatment of recurrent glioblastomas: an exploratory study

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Abstract

Background

Glioblastoma (GBM) is the most common primary malignant brain tumor with poor prognosis. At present, there is no standard treatment for GBM who failed first-line treatment. Apatinib is a new type of oral small molecule angiogenesis inhibitor. The goals of this study are to research the curative effect, side effects and changes of immune factors and cytokines of treatment with Apatinib combined with TMZ (NCT:04253873).

Methods

12 patients with recurrent GBM who failed first-line treatment were treated with apatinib combined with TMZ. They received oral apatinib (500 mg/day) in conjunction with TMZ (150 mg/m^2/day). Contrast-enhanced MRI was performed every 4 weeks before and after treatment to measure the tumor volume. The peripheral blood samples were collected and the expressions of soluble PD-1(sPD-1), soluble PD-L1(sPD-L1), IL-6 and IL-10 in peripheral blood were detected by ELISA.

Results

The objective effective rate (ORR) was 41.67% (5/12). The disease control rate (DCR) was 75% (9/12). The median progression-free survival (m PFS) time was 6 months. The 6-month PFS rate was 50%. The most common adverse events were Hypertension and Fatigue. In our study, we did hematological analysis on 5 patients out of 12, we found that the plasma expression of sPD-L1 decreased and the expression of sPD-1 increased in the patients with significant reduced tumor volume, which may be related to longer survival and better prognosis, While the expressions of sPD-L1 increased and sPD-1 decreased in some patients with enlarged tumor volume, which may be related to shorter survival and worse prognosis. The expression levels of IL-6 and IL-10 in blood showed variation in patients with different tumor volume change. And the expression levels of △sPD-1, △sPD-L1, △IL-6 showed significant correlations to the tumor volume changes (△volume) (r=-0.651, p = 0.002; r = 0.898, p < 0.0001; r = 0.463, p = 0.040).The changes in △IL-10 were not correlated with tumor volume changes (△volume) (r = 0.408, p = 0.074).

Conclusion

Apatinib combined with TMZ is safe and effective in the treatment of recurrent GBM. This study showed that immune factors (sPD-1, sPD-L1) may be potential prognostic biomarkers for GBM patients.

Background

Glioma is the most common primary malignant brain tumor with poor prognosis. According to the classification of central nervous system tumors of the World Health Organization (WHO), gliomas are classified as grade Ⅰ-Ⅳ. GBM, the highest-grade glioma, is highly malignant and aggressive. The current standard treatments include surgery, concurrent chemoradiotherapy, and adjuvant chemotherapy [1]. Nevertheless, the recurrence rate is still very high. Recurrent GMB is more aggressive, and there are few treatment options. The study of Nieder C [2]

reported that the survival time of recurrent GBM patients was 6 to 8 months, and the median time to the second progression was 14 weeks. At present, there is no standard treatment for recurrent and refractory GBM [3].

GBM transforms normal blood-brain barrier into blood-tumor barrier through a variety of vascular formation methods, resulting in the formation of microvascular morphology distortion and malformation. This is associated with overexpression of the vascular endothelial growth factor (VEGF-A) [4]. Therefore, anti-angiogenic drugs such as bevacizumab, a recombinant humanized monoclonal antibody to human vascular endothelial growth factor that neutralizes the effect of VEGF-A, have been approved for the treatment of recurrent GBM [5]. At present, there is no standard treatment for GBM who failed first-line treatment.

As a new type of oral small molecule angiogenesis inhibitor, apatinib highly selectively inhibits the activity of vascular endothelial growth factor receptor-2 (VEGFR-2), exerting promising antitumoral effect in tumors. Xue N, et al [6] found that the efficacy of TMZ plus apatinib in the treatment of recurrent or progressive GMB was higher than that of TMZ combined with bevacizumab. Bunevicius A, et al [7] showed that elevated expressions of IL-6 were related to shorter survival in high-grade glioma patients. Albulescu R, et al [8] found that the IL-6 and IL-10 were involved in tumor progression and aggressiveness. Liu S, et al [9] showed that PD-L1 was expressed in a soluble form in the circulation. In gliomas, elevated circulating and CSF sPD-L1 levels were related to aggressive biological activities. sPD-L1 was a promising biomarker for gliomas that can be used in clinical practice.

The goals of study are to observe clinical efficacy, side effects and changes of cytokines and immune factors of treatment with Apatinib combined with TMZ.

Patients And Methods

Study design

This is a single-arm, open, single-center clinical trial (NCT:04253873) to study the clinical efficacy and safety of apatinib combined with GBM in the treatment of recurrent, refractory and unresectable GBM and to observe that the changes of immune factors and cytokines (sPD-1, sPD-L1, IL-6, IL-10) **(Fig. 1)**.

The main research end point is progress-free survival time (PFS), secondary end point is overall survival (OS), objective remission rate (ORR), disease control rate (DCR).

Patients

From July 2018 to December 2019, 12 patients with recurrent GBM were treated in the Department of Radiation Oncology, Affiliated Cancer Hospital of Zhengzhou University. The institutional review boards at Affiliated Cancer Hospital of Zhengzhou University Hospital approved the study before initial patient enrollment. And this study was written informed consents (or assent when appropriate) were obtained from patients or their legal guardians.

Additional eligibility criteria included Age ≥ 18 years old. Patient who was pathologically diagnosed with recurrent GBM had measurable lesions; Karnofsky performance score (KPS) ≥ 70, Eastern Cooperative Oncology Group (ECOG) performance score ≤ 2; The estimated survival time was ≥ 3 months; WBC ≥ 3.0 × 10^9/L, HB ≥ 90 g/L, ANC ≥ 1.5 × 10^9/L, PLT ≥ 80 × 10^9/L; TBIL < 1.5 × ULN, ALT < 2.5 × ULN, AST < 2.5 × ULN, Serum Cr ≤ 1.25 ULN; Postoperative MRI showed total or subtotal resection of the tumor; Written informed consent.

Exclusion criteria included Pregnant or lactating women; Patients with uncontrolled blood pressure (140/90 ≥ mmHg); Patients with bleeding tendency or undergoing thrombolysis or anticoagulation therapy; Patients with chronic diarrhea or intestinal obstruction that significantly affect oral drug absorption; Patients with previous arterial thrombosis, myocardial infarction, cerebrovascular accident and gastrointestinal perforation; Patients with partial resection or biopsy; Pulmonary hemorrhage of ≥ CTCAE grade II and hemorrhage of ≥ CTCAE grade III occurred within 4 weeks before enrollment; Arteriovenous thrombosis events occurred within 6 months before enrollment, such as cerebrovascular accidents, deep venous thrombosis and pulmonary embolism; Patients treated with anticoagulants or vitamin K antagonists such as warfarin, heparin or their analogues; Patients were allowed to use low-dose warfarin (1 mg/day) or low-dose aspirin (between 80 mg/day and 100 mg/day) for preventive purposes on the premise that the internationally standardized ratio of prothrombin time (INR) is less than 1.5; Patients refused to take part in the study.

Experimental reagents and instruments

The soluble human PD-1, PD-L1, IL-6 and IL-10 ELISA kits were purchased from Wuhan Huamei Company, the enzyme labeling instrument (imark) was purchased from Bio-Rad Company of the United States, and the plate washing machine (ELX50) was purchased from BIO-TEK Company of the United States. The Mini centrifuge (mySPIN12) and the high-speed centrifuge were purchased from Thermo Fisher Company. The waterproof constant incubator was purchased from Taiwan Sturst Instruments and equipment.

Clinical specimens

Before taking the medicine, the peripheral blood was collected on an empty stomach at 5 ~ 10 ml in the morning. After taking the medicine, the peripheral blood 5 ~ 10 ml was collected every 4 weeks on an empty stomach. After 30 min-1 h, the supernatant (serum and plasma) was taken after centrifugation of 3000 × g for 10 minutes. Packed in 1.5 ml aseptic centrifuge tube and frozen in 80-degree refrigerator. The expressions of IL-6 and IL-10 in serum, sPD-1, sPD-L1in plasma were measured by ELISA [10].

Treatment method

The patients received apatinib 500 mg/day for 2 weeks, then rested for 1 week, started oral TMZ (150 mg/m^2 /day) for 5 days of each 28-days cycle.

If toxicities were ≤ grade 3 or patients who could benefit from the continued medication were judged by the researchers, this schedule was continued. Initial apatinib dose was 500 mg. The dose was adjusted to 250 mg, as long as grade III or IV toxicity occurred. 12 patients had received prior surgery, concurrent chemoradiotherapy, and adjuvant TMZ treatment.

Curative effect evaluation

MRI scans were performed every 4 weeks (a 28-days cycle) before and after adjuvant treatment. The clinical curative effect was evaluated according to the RANO standard of glioma [11], including complete remission (CR), partial response (PR), disease stable (SD), disease progression (PD).

Adverse events

Evaluation of adverse events according to CTCAE5.0 version standard [12].

Tumor volume measurement

Image acquisition

MRI scans of 12 patients were selected for analysis. The images were affected by many factors, such as the amount of injection contrast agent, the type of NMR (nucler magnetic resonance) machine and scanning technology and soon. The above factors were consistent. MRI sequences contained T1, T1 weighted contrast-enhanced, T2, T2 flair, DWI and other image sets, downloaded in Dicom format.

Volume Segmentation, Measurement and Modeling

Using 3D slicer 4.10.2 is a software package developed by Harvard Medical School for medical image analysis (including registration and interactive segmentation) and visualization (including the 3D rendering), and for image-guided therapy research software platform [13, 14]. Two radiologists with 15 years of experience segmented the image, including the volume of the tumor on T1weighted contrast-enhanced. At the same time, tumor modeling is carried out.

Statistical methods

Statistical analysis was performed using SPSS version 21.0. Kaplan-Meier method was used for survival analysis. Spearman correlation analysis was used to analyze the correlations between the changes of immune factors (△sPD-1, △sPD-L1, △IL-6, △IL-10) and tumor volume (△volume). For data analysis, $P < 0.05$ was considered significant.

Results

Patients

There were 5 males and 7 females, aged 31–70 years. The median age was 49.5 years old ($N = 12$). Patients were pathologically confirmed GBM (WHO grade IV). The heart, lung, liver and kidney functions of the patients were basically normal. The KPS score was ≥ 70, and ECOG score was ≤ 2 (Table 1).

Table 1
Patient characteristics, treatment information and outcome date

Patient no.	Sex	Age(y)	IDH1 Mutation Status	Grade	S + CRT + AC	Apatinib dose(mg)	TMZ dose(mg/m^2)	Efficacy	PFS	OS
1	F	52	-	IV	yes	500	150	SD	6.2	SA
2	M	45	-	IV	yes	500	150	PD	1.5	5.2
3	M	31	-	IV	yes	500	150	SD	4.8	SA
4	F	50	-	IV	yes	500	150	PD	5	5
5	F	34	-	IV	yes	500	150	CR	ST	SA
6	M	35	-	IV	yes	500	150	PR	3.9	SA
7	F	49	-	IV	yes	500	150	SD	ST	SA
8	F	60	-	IV	yes	500	150	PR	ST	SA
9	F	55	-	IV	yes	500	150	PR	6	6
10	M	66	-	IV	yes	500	150	PD	4.6	SA
11	F	70	-	IV	yes	500	150	PR	ST	SA
12	M	33	-	IV	yes	500	150	SD	ST	SA

M: male; F: female; S + CRT + AC: surgery, concurrent chemoradiotherapy, and adjuvant chemotherapy; TMZ: temozolomide; SD: stable disease; PD: progressive disease; PR: partial response; CR: complete response; SA: still alive; ST: still in treatment; PFS: progression-free survival time; OS: overall survival.

Clinical efficacy

Short-term curative effect

The short-term efficacy of 12 patients was CR in 1 case (8.33%), PR in 4 cases (33.33%), SD in 4 cases (33.33%), PD in 3cases (25%); ORR was 41.67% (5/12); DCR was 75% (9/12).

Long-term survival

The median progression-free survival time of apatinib combined with TMZ was 6 months (95% CI: 4.071–7.929), The 6-month PFS rate was 50% (Fig. 2A-B). The correlation analysis between gender, age and survival showed that there was no significant by Kaplan-Meier (Long-rank) method ($P = 0.097$; $P = 0.644$). (Fig. 2C-D).

Immune factors, cytokines and tumor volume

The experimental data measured in the laboratory are shown in Table 2–3 and Fig. 3.

Table 2
Date of immune factors, cytokines and tumor volume with elapsed time (week)

Patient No.	Elapsed time(week)	sPD-1 (pg/ml)	sPD-L1 (pg/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	Volume (cc)
1	0	21.183	30.197	14.901	6.952	12.163
						21.843
						8.529
						7.799
						9.630
	4	19.464	38.718	14.999	10.932	21.843
						21.843
						8.529
						7.799
						9.630
	8	20.272	31.593	14.685	10.230	8.529
	12	21.125	28.653	13.899	9.595	7.799
	16	21.538	28.213	14.405	8.835	7.630
2	0	24.856	16.341	14.059	6.378	1.695
	4	23.228	19.635	14.434	6.454	3.167
	8	22.893	25.216	14.097	6.516	14.380
	12	22.968	25.733	14.146	6.578	14.984
	16	22.669	25.488	14.106	6.765	15.640
3	0	19.786	39.124	14.734	7.132	8.107
	4	19.649	39.765	14.751	7.183	8.319
	8	19.412	39.835	14.633	7.015	8.557
	12	19.362	39.929	14.685	7.077	8.939
	16	19.560	40.314	14.764	7.323	9.009
4	0	23.596	78.679	14.618	8.616	21.561
	4	22.940	100.446	14.761	8.646	34.147
	8	37.992	80.515	14.544	9.658	27.221

0 week: The time of the end of TMZ adjuvant chemotherapy; 4 weeks: Before apatinib combined with TMZ treatment; 8 weeks: The time of 1 cycle after apatinib combined with TMZ treatment; 12 weeks: The time of 2 cycles after apatinib combined with TMZ treatment; 16 weeks: The time of 3 cycles after apatinib combined with TMZ treatment.

Patient No.	Elapsed time(week)	sPD-1 (pg/ml)	sPD-L1 (pg/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	Volume (cc)
	12	21.942	103.464	14.791	8.962	31.763
	16	22.947	105.734	14.758	9.583	38.176
5	0	22.840	33.190	15.343	6.205	0.184
	4	20.383	36.562	15.533	6.329	0.722
	8	19.027	33.714	15.160	5.895	0.645
	12	19.889	33.562	15.058	5.771	0.534
	16	23.534	28.548	14.691	5.786	0.076

0 week: The time of the end of TMZ adjuvant chemotherapy; 4 weeks: Before apatinib combined with TMZ treatment; 8 weeks: The time of 1 cycle after apatinib combined with TMZ treatment; 12 weeks: The time of 2 cycles after apatinib combined with TMZ treatment; 16 weeks: The time of 3 cycles after apatinib combined with TMZ treatment.

Table 3
Changes of immune factors, cytokines and tumor volume with elapsed time (week)

Patient No.	Elapsed time (week)	△sPD-1 (Pg/ml)	△sPD-L1 (Pg/ml)	△IL-6 (Pg/ml)	△IL-10 (Pg/ml)	△volume (cc)
1	4	-1.719	8.521	0.098	3.980	9.671
	8	0.808	-7.125	-0.314	-0.702	-13.305
	12	0.853	-2.940	-0.786	-0.635	-0.730
	16	0.413	-0.440	0.506	-0.760	-0.169
2	4	-1.628	3.294	0.375	0.076	1.472
	8	-0.335	5.581	-0.337	0.062	11.213
	12	0.075	0.517	0.049	0.062	0.604
	16	-0.299	-0.245	-0.040	0.187	0.656
3	4	-0.137	0.641	0.017	0.051	0.212
	8	-0.237	0.070	-0.118	-0.168	0.238
	12	-0.050	0.094	0.052	0.062	0.382
	16	0.198	0.385	0.079	0.246	0.070
4	4	-0.656	21.767	0.143	0.030	12.586
	8	15.052	-19.931	-0.217	1.012	-6.926
	12	-16.050	22.949	0.247	-0.696	4.542
	16	1.005	2.270	-0.033	0.621	6.413
5	4	-2.457	3.372	0.190	0.124	0.538
	8	-1.356	-2.848	-0.373	-0.434	-0.077
	12	0.862	-0.152	-0.102	-0.124	-0.111
	16	3.645	-5.014	-0.367	0.015	-0.458
4 weeks: Before apatinib combined with TMZ treatment; 8 weeks: The time of 1 cycle after apatinib combined with TMZ treatment; 12 weeks: The time of 2 cycles after apatinib combined with TMZ treatment; 16 weeks: The time of 3 cycles after apatinib combined with TMZ treatment.						
Correlation between expression levels of immune factors, cytokines (△sPD-1, △sPD-L1, △IL-6,△IL-10) and tumor volume(△volume): r=-0.651, p = 0.002; r = 0.898, p < 0.0001; r = 0.463, p = 0.040; r = 0.408, p = 0.074.						

The levels of sPD-1and sPD-L1 in blood showed different changes in patients and affected tumor volume change. The expression of sPD-L1 decreased and the expression of sPD-1 increased in the patients with significant reduced tumor volume, while the expression of sPD-L1 increased and the expression of sPD-1 decreased in some patients with enlarged tumor volume, and the expression of sPD-1 and sPD-L1 slightly

changed, the tumor volume did not change significantly. The expression levels of IL-6 and IL-10 in blood showed variation in patients with different tumor volume change.

This study showed that there were significant correlations between the changes of immune factors (△sPD-1, △sPD-L1, △IL-6) and volume changes (△volume) in patients who were treated with apatinib combined with TMZ (r=-0.651, p = 0.002; r = 0.898, p < 0.0001; r = 0.463, p = 0.040).

Toxicities

12 patients developed side effects, including: hypertension in 4 cases (20%), Fatigue in 4 cases (20%), Nausea and vomiting in 3 cases (15%), myelosuppression in 2 cases (10%), gastrointestinal symptom in 2 cases (10%), hand-foot syndrome in 2 cases (10%), Transaminase abnormality in 2 cases (10%), proteinuria in 1 case (5%).

Table 4 Adverse events in the combination therapy of apatinib and TMZ

Table 4
Adverse events in the combination therapy of apatinib and temozolomide

Toxic reactions	Grade 1–2(%)	Grade 3–4(%)	Total (%)
Hypertension	3(15%)	1(5%)	4(20%)
Proteinuria	1(5%)	0	1(5%)
Myelosuppression	1(5%)	1(5%)	2(10%)
Hand and foot syndrome	2(10%)	0	2(10%)
Fatigue	4(20%)	0	4(20%)
Transaminase abnormality	2(10%)	0	2(10%)
Gastrointestinal symptoms	2(10%)	0	2(10%)
Nausea and vomiting	3(15%)	0	3(15%)

Patient 5

A typical case is as follows: A 34-year-old female patient was first diagnosed with high-grade glioma in the right parietal occipital lobe. She performed surgical resection, concurrent chemoradiotherapy (60 Gy/30f; TMZ:75 mg/m^2/day), and adjuvant chemotherapy (TMZ:150 mg/m^2/day). MRI showed the time of the end of TMZ adjuvant chemotherapy on October 19, 2018 (**A**); MRI scans showed recurrence of the disease on November 14, 2018 (**B**); She began to take apatinib combined with TMZ on November 15, 2018. The MRI scans of post-treatment after 1month, tumor volume was 0.645 cc (**C**); The MRI scans of post-treatment after 2 months, tumor volume was 0.534 cc **(D)**; The MRI scans of post-treatment after 3 months, tumor volume was 0.076 cc **(E)**. Up to now, 11 cycles of treatment. the tumor was basically disappeared, and the curative effect was evaluated by complete remission (CR).

Note: Figure A/B/C/D: T1 fse flair contrast-enhanced axial image; T2 fse flair axial image; T1 fse flair contrast-enhanced sagittal image; T1fse flair contrast-enhanced coronal image; Figure E: T1 weighted contrast-enhanced axial image; T2 flair axial image; T1 weighted contrast-enhanced sagittal image; T1 weighted contrast-enhanced coronal image.

Discussion

GBM is highly malignant and invasive. Although the comprehensive treatments of surgery, postoperative radiotherapy and chemotherapy make great progress, GBM still has high recurrence rate. At present, ample studies have shown that relapse or progression of GBM after the standard regimen has a poor response to TMZ monotherapy, so it is necessary to explore new treatments.

GBM enriches vascular endothelial growth factor (VEGF). The growth and recurrence of GBM are closely related to tumor angiogenesis. It has been reported that bevacizumab, as a monoclonal antibody against vascular endothelial growth factor, has been used in the treatment of recurrent GBM, but with serious adverse reactions of hypertension and bleeding [5]. Apatinib is a new type of oral small molecule angiogenesis inhibitor, and highly selectively inhibits the activity of VEGFR-2, thus, it blocks the signal transduction pathway after the binding of vascular VEGF and its receptor, so as to strongly inhibit tumor angiogenesis and exert its anti-vascular effect. Wang L, et al [15] showed that apatinib combined with irinotecan was objectively effective in the treatment of high-grade brain gliomas, and its adverse reactions can be tolerated.

Similar to our study, several studies showed that apatinib was effective in the treatments of advanced gastric cancer and liver cancer, however, with side effects such as hypertension and proteinuria in advanced gastric and liver cancer [16, 17].

In our study, 12 patients with recurrent GBM were observed, and the results showed that the ORR was 41.67% (5/12), the DCR was 75% (9/12), the m PFS was 6 months, the 6-month PFS rate was 50%. The ORR of 43 cases of apatinib combined with TMZ mentioned by Xue N, et al [6] was 53.49% (23/43), m OS was 639 days. The ORR of our study was lower, which may be related to the general health status before treatment in 12 patients [18]. The ORR of 26 patients treated with apatinib combined with TMZ reported by Sun D [19] was 57.6% (15/26), m PFS was 5.9 months. The m PFS was similar to our study. Zhang J, et al [20] reported a clinical phase II study of apatinib plus TMZ in adults with recurrent GBM. The results showed that the ORR was 25.8% (8/31), m PFS was 6.1 months. Compared with the study, m PFS was similar and ORR was higher, which may be related to the small number of patients in our study and the volume of tumor recurrence. Wang Y, et al [21] reported 20 patients of recurrent GBM who were treated with apatinib combined with dose-intensive TMZ. The results showed that m PFS was 6 months, m OS was 9 months, ORR was 45% and DCR was 90%. The results were similar to our study. The main adverse reactions proved by Xue N, were fatigue, hypertension and proteinuria, and mainly concentrated in I-II grade. The main adverse reactions reported by Sun D and Wang Y, were hypertension and hand-foot syndrome. The adverse reactions reported by Zhang J, were myelosuppression and hypertension. The main adverse reactions of our study were basically the same as the results of the above studies, which proved that apatinib combined with TMZ was safe and effective, and the adverse reactions were tolerable and controllable.

The above studies were to observe the clinical efficacy of apatinib and TMZ in the treatment of GMB. Although few cases are included in our study, we are astounded by the results of the combination of apatinib and TMZ in

the treatment of recurrent and progressive GMB. We were interested in evaluating the predictive significance of cytokines and immune factors in GBM patients.

Tumor microenvironment, which is formed by stromal cells, infiltrating immune cells and tumor cells, is a factor promoting carcinogenesis. There are sufficient evidences to support the involvement of immune factors that lead to the occurrence, development, invasion and metastasis of cancer. Landskron G, et al [22] discovered that in the process of chronic inflammation, proinflammatory cytokines such as IL-6 not only potentially leaded to tumor mutation but also stimulated cell proliferation and reduced apoptosis. Moreover, these cytokines were conducive to tumor growth, and played a significant role in angiogenesis and metastasis, while anti-inflammatory cytokines such as IL-10 were involved in tumor immune escape. Some studies demonstrated that the determination of serum cytokine levels, such as IL-6 or IL-10, may be related to tumorigenesis or poor prognosis [23, 24]. Samaras V, et al [25] proved that IL-6 and IL-10 secreted by peripheral blood mononuclear cells of patients with glioma were higher than those of healthy controls. Other studies [26–28] found that IL-6 and IL-10 were involved in the tumor growth and immune response. Shan Y, et al [29] researched that IL-6 in the CSF and serum of glioma may be used to predict the prognosis of these patients. In our study, we observed that the changes in △IL-6 showed a correlation to the tumor volume changes (△volume) ($r = 0.463$, $p = 0.040$). The expression levels of △IL-10 were not correlation to volume changes (△volume) ($r = 0.408$, $p = 0.074$). Previous evidences showed that anti-angiogenic drugs can normalize the abnormal tumor vasculature and potentially reengineer the tumor immune microenvironment towards a more immune-supportive profile [30, 31].There were consistent results with our study of the anti-angiogenic drug apatinib, which inhibited the activity of VEGF-R and thus affected the expression of cytokines. In our study, the expression levels of IL-6 and IL-10 in blood showed variation in patients with different tumor volume change. The overall changes of IL-6 and IL-10 were not significant. This study showed that cytokines (IL-6, IL-10) for predicting prognosis of GBM were not significant.

Previous study showed that the expression level of PD-L1 was associated with poor prognosis in different solid tumors [32]. Cloughesy TF, et al [33] reported that the expression level of PD-L1, in some GBM tumor cells and tumor-infiltrating immune cells, was higher. The higher the grade, the worse the prognosis. In our study, the expressions of sPD-L1 decreased and sPD-1 increased in the patients with significant reduced tumor volume, which may be related to longer survival and better prognosis. While the expressions of sPD-L1 increased and sPD-1 decreased in some patients with enlarged tumor volume, which may be related to shorter survival and worse prognosis. △sPD-1, △sPD-L1 in peripheral blood were significantly correlated with the tumor volume changes (△volume) ($r=-0.065$, $p = 0.002$; $r = 0.087$, $p < 0.0001$). This study showed that immune factors (sPD-1, sPD-L1) may be prognostic biomarkers of GBM.

There are some limitations in the present study. Because of the operational errors in the experiment, including the time of adding samples and whether the washing is sufficient or not, the average value of the complex hole is adopted in the experimental results to reduce the error as much as possible. In the outline of the abnormal volume of the tumor, the manual automatic drawing has a measurement error of 10%. Due to the limitation of small sample size in this prospective study, the correlation between the expressions of sPD-1, sPD-L1, IL-6, IL-10 and patient's ages, gender, tumor location and MGMT methylation status could not be further analyzed. It is necessary to increase the sample size in future trials and design a randomized control group for further study.

Conclusions

Apatinib combined with TMZ was effective in the treatment of recurrent GBM, and the side effects were tolerable. This study showed that immune factors (sPD-1, sPD-L1) may be potential prognostic biomarkers for GBM patients.

Abbreviations

GBM, glioblastoma; temozolomide, TMZ; VEGF, vascular endothelial growth factor; VEGFR-2, vascular endothelial growth factor receptor-2; TMZ, temozolomide; CR, complete response; PR, partial response; SD, disease stable ;PD, disease progression; SA, still alive; ST, still in treatment; PFS, progression-free survival time; OS, overall survival. ORR, objective remission rate; MRI, magnetic resonance imaging; fse, fast spin-echo; flair, fluid attenuated inversion recovery. KPS, Karnofsky Performance Status; ECOG, Eastern Cooperative Oncology Group.

Declarations

Ethics declarations and consent to participate

This study complied with the Declaration of Helsinki. The institutional review boards at Affiliated Cancer Hospital of Zhengzhou University Hospital approved the study before initial patient enrollment (approval number: AHEAD-AHN02). And this study was written informed consents (or assent when appropriate) were obtained from patients or their legal guardians.

Consent for publication

Patients or their legal guardians allow his/her data generated from this study to be published with privacy protection and sign the consent form.

Data availability

All datasets generated for this study are included in the manuscript and/or the supplementary file.

Competing interests

The authors declare that they have no competing interests.

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Contributions

TL, LH and XH performed the experiment. XS, RH, WL and YW contributed to data analysis and discussion.TL, HW and LH designed and wrote the manuscript. All authors listed have made a substantial, direct and intellectual

contribution to the work, and approved it for publication

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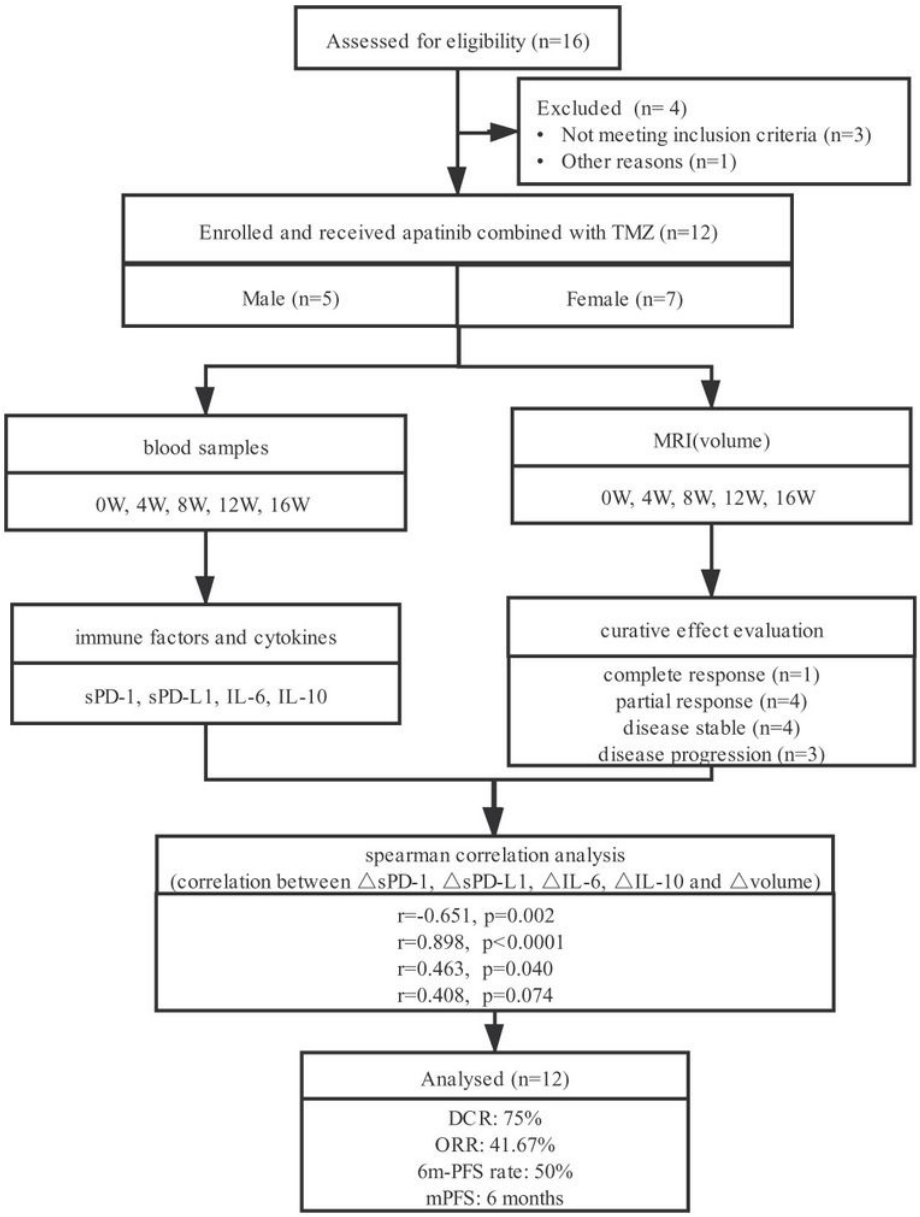
Figures



0 week: The time of TMZ adjuvant chemothearpy; 4 weeks: Before apatinib combined with TMZ treatment; 8 weeks: The time of 1 cycle after apatinib combined with TMZ treatment; 12 weeks: The time of 2 cycles after apatinib combined with TMZ treatment; 16 weeks: The time of 3 cycles after apatinib combined with TMZ treatment.

Fig.1 Patient enrollment and outcomes (flow chart)

Figure 1

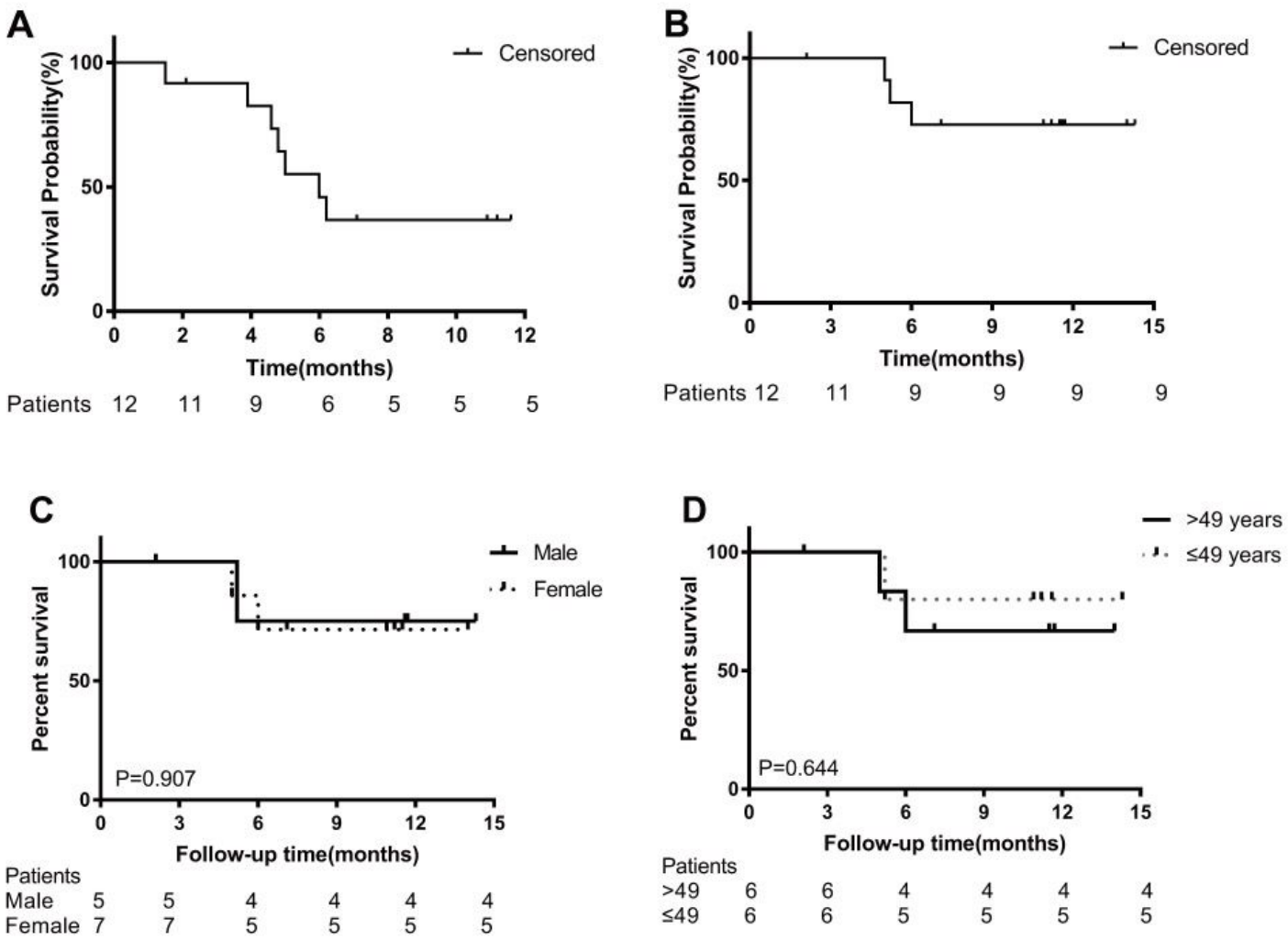


Fig.2 Progression-free survival(A),overall survival(B),genders of patients(C),ages of patients(D).

Figure 2

Progression-free survival(A), Overall survival(B), Genders of patients(C), Ages of patients(D).

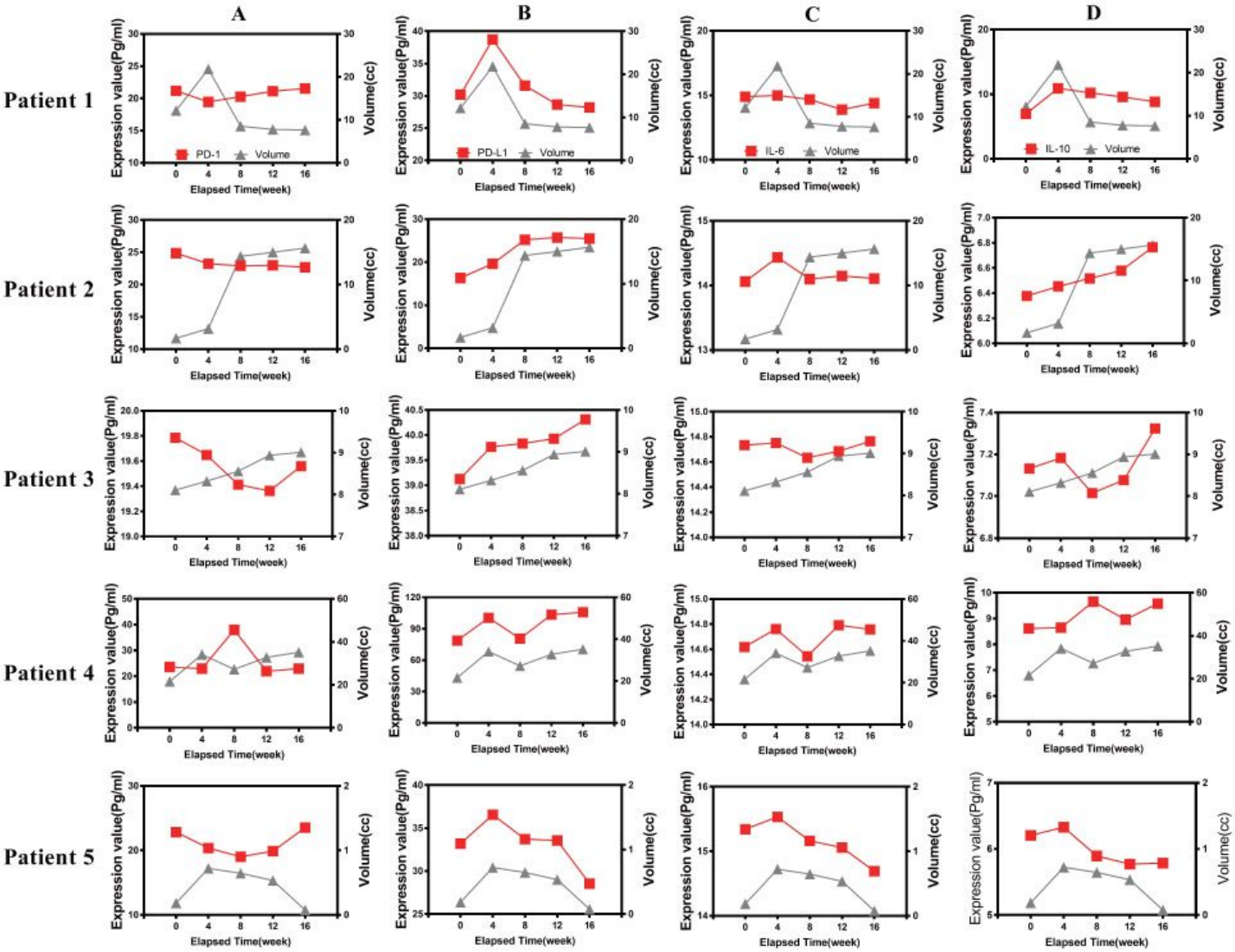


Fig.3 Correlation between immune factors, cytokines and tumor volume with treatment. sPD-1(A),sPD-L1(B), IL-6(C), IL-10(D)

Figure 3

Correlation between immune factors, cytokines and tumor volume with treatment. sPD1 (A), sPD-L1 (B), IL-6 (C), IL-10 (D).

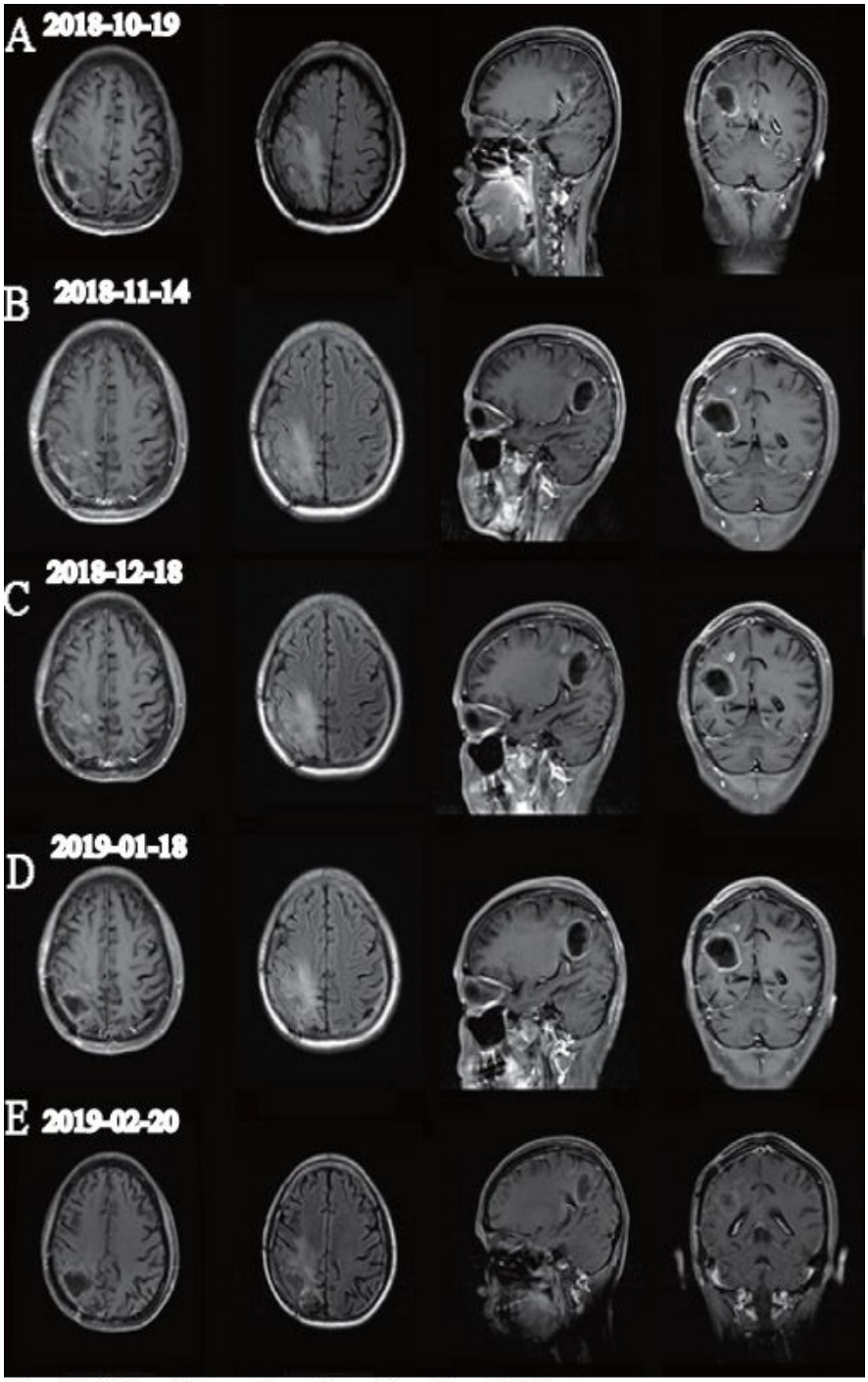


Fig.4 MRI of the patient (no.5) with glioblastoma.

Figure 4

MRI of the patient (no.5) with glioblastoma

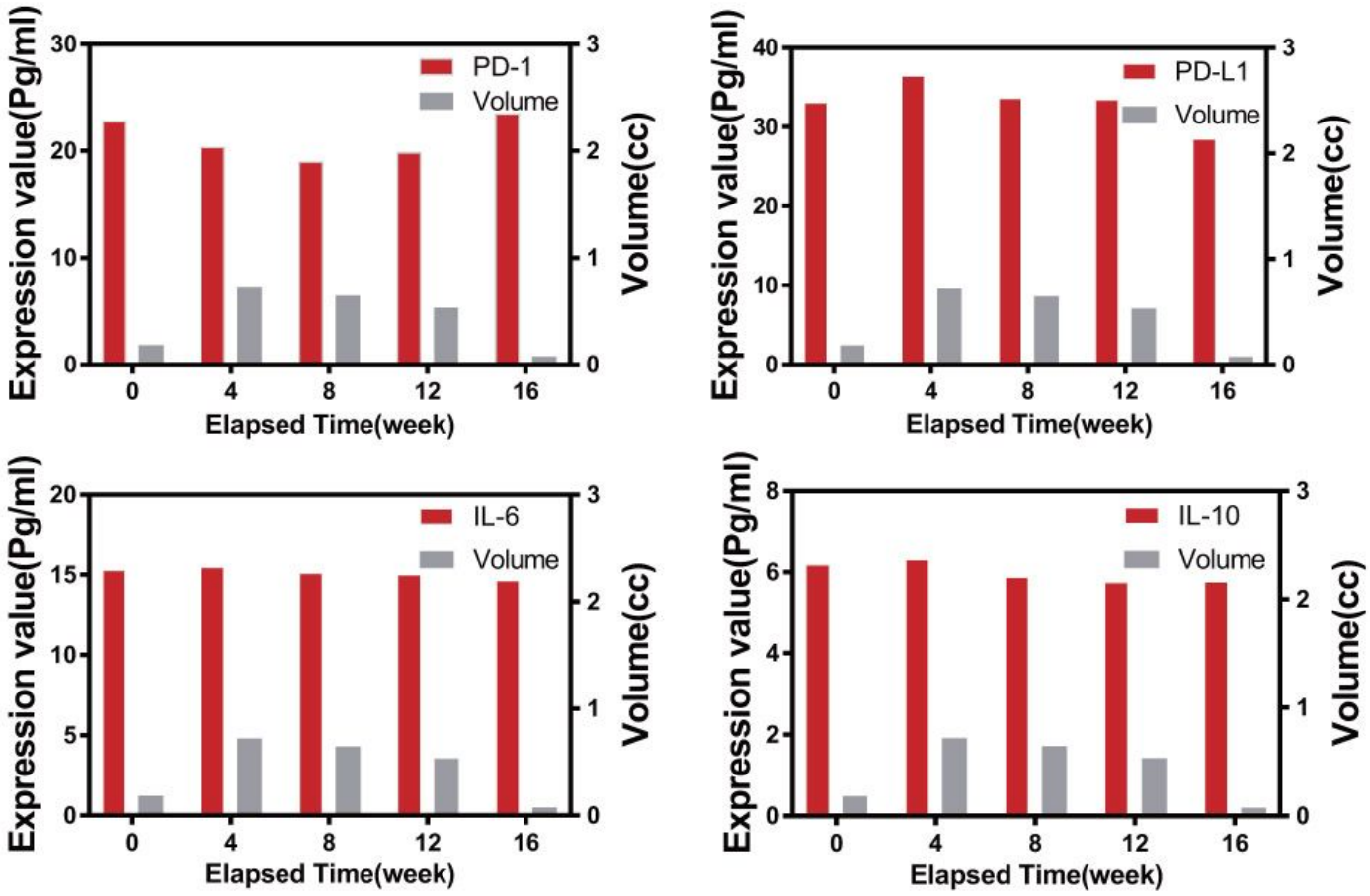


Fig.5 Changes of immune factors, cytokines and tumor volume in a typical patient.

Figure 5

Changes of immune factors, cytokines and tumor volume in a typical patient