Complete response to immunotherapy combined with an antiangiogenic agent in multiple hepatic metastases after radical surgery for advanced gallbladder cancer: a case report

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Abstract: Most advanced gallbladder cancers (GBCa) are unresectable or metastatic once diagnosed, and even patients who undergo surgery have a high risk of recurrence and metastasis. Immunotherapy, especially immune checkpoint inhibitors (ICIs), combined with an antiangiogenic agent, is an emerging prospective treatment for GBCa. However, the efficacy and safety of this combination therapy have not yet been investigated. We report the case of a 70-year-old female patient with recurrent metastatic GBCa (stage IVB) after radical surgery. Immunohistochemical examination revealed that 10% of the tumor cells expressed programmed cell death protein-1 (PD-1) and programmed cell death receptor ligand 1 (PD-L1). Whole-exome sequencing showed cancer tissues with a low tumor mutational burden (TMB) and microsatellite stability (MSS). The patient received Camrelizumab (200 mg, every three weeks) and Apatinib (40 mg/d). The clinical and immunological responses were observed, and the patient achieved a complete response after five cycles. This is the first case describing the efficacy and safety of Camrelizumab plus Apatinib in a GBCa patient with weak PD-1 and PD-L1 expression, and low TMB and MSS. The treatment had a tolerable safety profile and a complete response in the patient. Also, we found that the cluster of differentiation (CD)16+CD56+natural killer (NK) cell ratio in peripheral blood was increased after the combined treatment. Immunotherapy with antiangiogenic drugs may be a potential treatment option for patients with recurrent GBC or GBCa.

Keywords: Immune checkpoint inhibitor (ICIs); antiangiogenics; advanced gallbladder cancer; $CD16^{+}CD56^{+}$NK cells; case report

Submitted Jun 01, 2020. Accepted for publication Oct 18, 2020.
doi: 10.21037/atm-20-4420
View this article at: http://dx.doi.org/10.21037/atm-20-4420

Introduction

Gallbladder cancer (GBC) is among the most frequently fatal manifestations of biliary tract cancer (BTC). It is associated with a poor prognosis and has a median survival time of less than 1 year, partly because most patients are diagnosed at advanced stages (1). According to the National Comprehensive Cancer Network (NCCN) guidelines, curative surgery is the recommended front-line therapy; however only 10% of GBC patients can undergo surgery. However, even patients who undergo surgery have a high risk of recurrence and metastasis due to the ability of the GBC to infiltrate adjacent organs (2). Extensive clinical analysis has revealed that the efficiency of traditional therapeutic options for advanced GBC (GBCa), such as surgery, radiation, and chemotherapy, is limited (3). Thus,

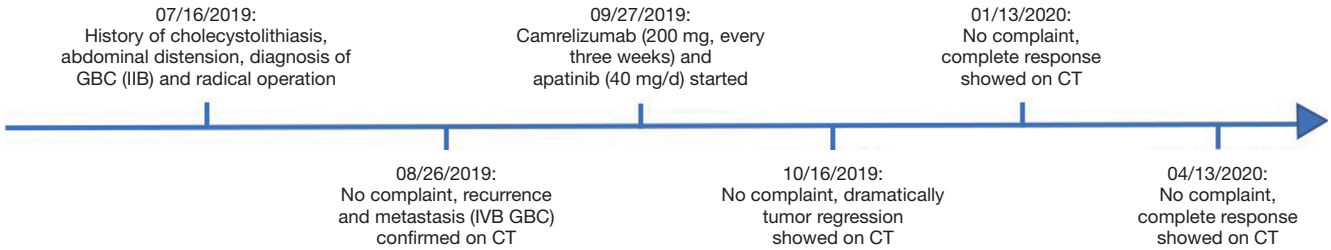

Figure 1 Timeline of the clinical course.

there is an urgent need for the development of more effective treatments for GBCa.

Patients with GBCa usually have a history of chronic gallbladder disease, including calculus and cholecystitis. Considering that chronic inflammatory conditions result in an immunosuppressive microenvironment within the gallbladder, immune checkpoint inhibitors (ICIs) have become a promising therapeutic option for GBCa. The interaction between programmed cell death protein-1 (PD-1) receptor and its ligand, programmed cell death receptor ligand 1 (PD-L1), causes immune exhaustion, which is similar to the immune escape mechanism of cancer cells; this mechanism has been widely regarded to aid in immunotherapy (4). High levels of PD-L1expression are inversely associated with long-term prognosis; a high rate of lymph node metastasis and worse overall survival (OS) has been observed in patients with positive PD-L1 expression (5). Numerous studies have demonstrated that the PD-1/PD-L1 blockade, combined with other therapy, facilitated objective responses in advanced BTC patients. In one study, a complete response was observed in two intrahepatic cholangiocarcinoma (ICC) patients treated with a combination of pembrolizumab and chemotherapy (6). Generally, these results show that immunotherapy with PD-1/PD-L1 blockers may be effective for the treatment of BTC.

Also, angiogenesis is one of the critical steps in tumor growth and hematogenous metastasis and plays a significant role in providing oxygen, nutrients, and growth factors to the tumor. In recent years, some studies have shown that vascular endothelial growth factor A (VEGF-A) is highly expressed in GBC and that angiogenic microvessel density and high expression of VEGF-A are correlated with a poor prognosis in GBC, suggesting that GBC is angiogenesis-dependent. Despite small sample numbers and various adverse events, these studies suggest that anti-vascular drugs might be promising for patients with advanced BTC (7,8). Until now, there has been only one retrospective analysis with a small sample size (21 patients) that have evaluated the efficacy and safety of Apatinib in advanced BTC (9). Thus, a combination of ICIs and antiangiogenics may be a promising regimen for the treatment of GBCa.

We report a case of GBCa with unexpected remission following the proactive application of a combination of ICIs and antiangiogenics. Also, we analyze potential biomarkers for these unexpected remission effects. The patient, in this case, achieved a complete response in GBCa with multiple hepatic metastases after treatment with a combination of immunotherapy and an antiangiogenic agent. We present the following article in accordance with the CARE reporting checklist (available at http://dx.doi.org/10.21037/atm-20-4420).

Case presentation

In July 2019, a 70-year-old female patient was admitted to the First Affiliated Hospital of Nanjing Medical University due to upper abdominal distension. She had a 10-year history of cholecystolithiasis and cholecystitis without any treatment owing to no obvious discomfort. She had no history or family history of cancer and no pathogenic germline mutation. *Figure 1* shows the process of development and treatment of the disease. Abdominal magnetic resonance imaging (MRI) (*Figure 2*) revealed significant enlargement of the gallbladder and the presence of unasserted lumps. A mass of approximately 4 cm was observed in the S4 hepatic segment, accompanied by porta hepatis, intermesentery, and retroperitoneum lymphadenopathy. The preoperative concentration of tumor marker CA19-9 was 102.2 U/mL (normal level <39 U/mL).

We performed radical surgery (*Figure 3A*) involving cholecystectomy, selective S4+S5 segment hepatectomy, and systemic lymph node dissection on July 18, 2019. The pathology report (*Figure 3B*) showed that the tumor

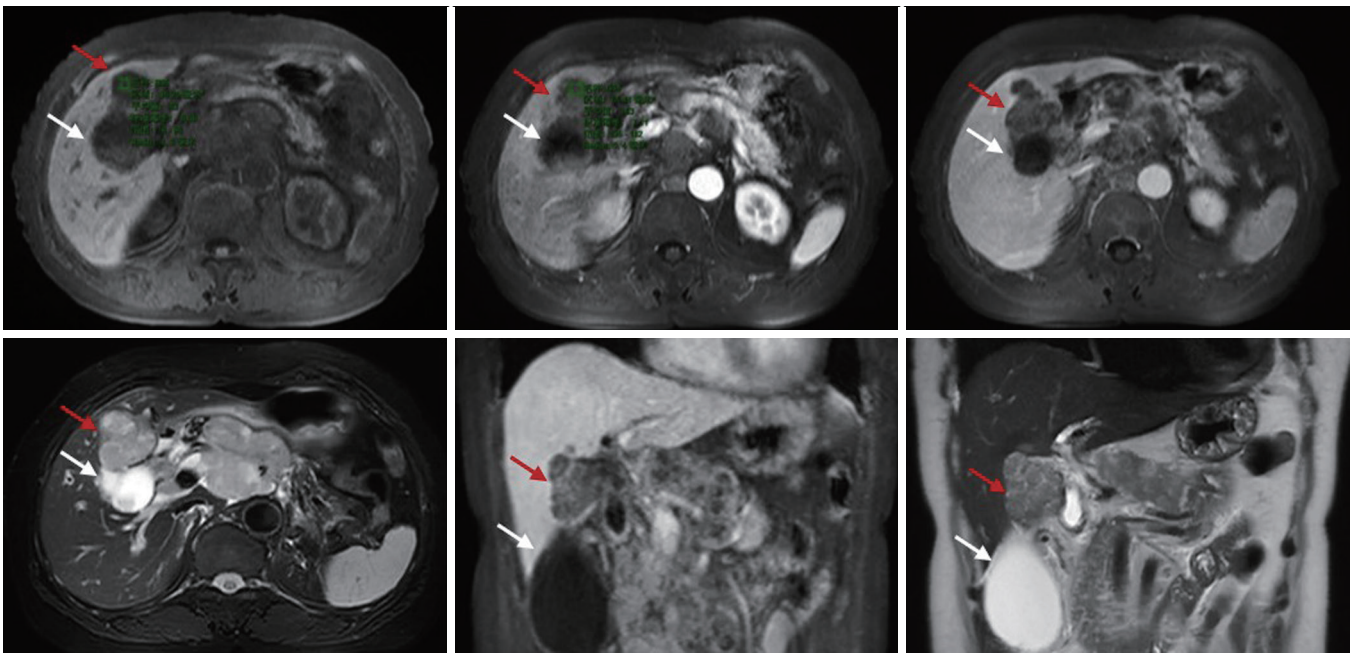

Figure 2 MRI imaging before operation (white arrow, gallbladder; red arrow, hepatic metastasis).

measured 3×2.5×1.4 cm in the gallbladder, and 5×3.2×4.6 cm in the liver and extensive lymph node metastases were found in group 8A, 8P, 9, 12A, 12B, 12P, and 13A. Pathological diagnosis confirmed poorly-differentiated GBC with liver and multiple lymph node metastases at stage IVB (T3N2M0). The patient recovered well after surgery and was recommended for further chemotherapy. However, the patient refused further chemotherapy in light of her age and toxicity.

Unfortunately, tumor recurrence and metastasis (stage IVB) occurred in the liver 1-month after the operation. Computed tomography (CT) revealed metastasis to the whole liver; the largest tumor measured approximately 3.7×3.0 cm. To identify an effective therapeutic regimen, the tumor sample was subjected to next generation sequencing (NGS) analysis using a 428-gene panel provided by Excellent Genetech Health, and immunohistochemistry (IHC) detection of PD-1 and PD-L1 expression. NGS results indicated that the tumor mutational burden (TMB) was 0.3 mutants/Mb, and the microsatellite state (MSS) was stable. Mutated genes included *HLA-A*, *MLH3*, *EPHA2*, *AXIN2*, *RAD51D*, and *SDHA* (*Table 1*). Except for *HLA-A* and *MLH3*, gene mutations were associated with the PD-1 antibody inhibited by Camrelizumab (10,11). The *EPHA2* mutation is targeted by the vascular endothelial growth factor receptor 2 (VEGFR2)-selective inhibitor Apatinib. The other gene mutations do not have any recommended clinical drugs.

IHC results indicated that PD-1 and PD-L1 expression was weakly positive (10%) (*Figure 3C*). Based on the above results, Camrelizumab (200 mg, every three weeks) and Apatinib (40 mg/d) were administered to the patient beginning August 28, 2019. After two cycles of this regimen, the patient achieved a partial response [85% depth of response (DpR)] based on CT and serum CA19-9 (*Figures 4,5*). Abdominal CT showed that the hepatic lesions were gone by January 13, 2020. At the time of writing, the patient was still alive, with a disease-free survival (DFS) exceeding five months (*Figures 4,5*), and no significant adverse events were observed. The patient expressed a positive and optimistic attitude toward future life.

Monitoring of peripheral blood biomarkers is a simple, accessible, and cost-effective method of assessing the prognostic value of ICI treatment in various cancers (12,13). However, the predictive effect of the lymphocyte subpopulation ratio, including the cluster of differentiation (CD)3^+CD4^+T, $CD3^+CD8^+$T cells, and $CD16^+CD56^+$natural killer (NK) cell ratios for this regimen has not yet been investigated. We analyzed the correlation between the lymphocyte above subsets and the efficiency of a PD-1 antibody combined with an antiangiogenic agent in this patient. As shown in *Figure 6*, the $CD3^+CD4^+$T and

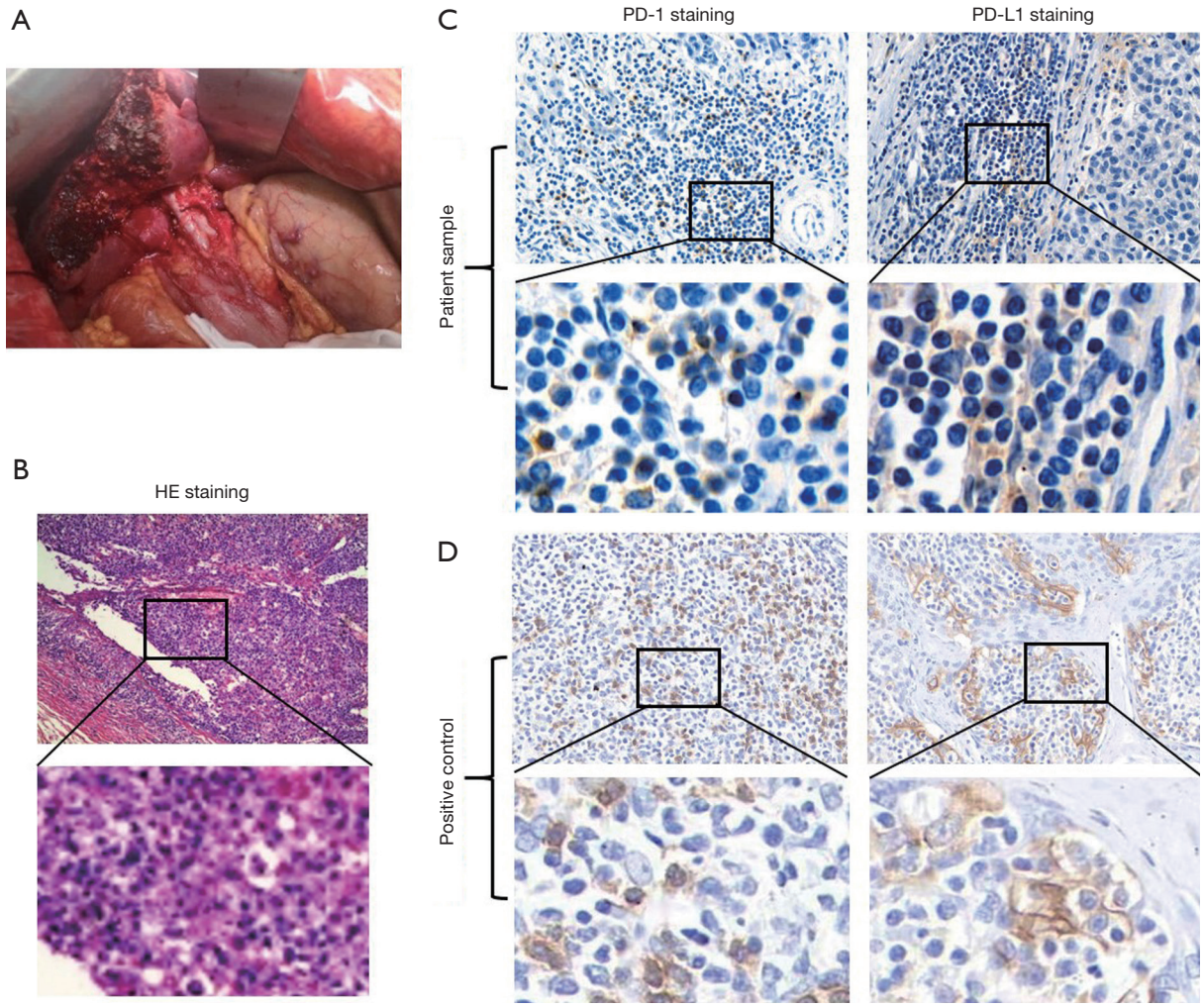

Figure 3 Surgical photo and pathological analysis: (A) operation photo; (B) HE staining; (C) PD-1 and (D) PD-L1 staining (×100).

Table 1 Gene mutation list

Gene name	Mutation
HLA-A	p.Q250fs*exon4; p.T202M*exon3
MLH3	p.R1232C*exon7
EPHA2	p.T457M*exon6
AXIN2	P.R394H*exon5
RAD51D	P.I199N*exon7
SDHA	p.T567fs*exon13

$CD3^+CD8^+$T cell ratios did not change considerably; however, the $CD16^+CD56^+$NK cell ratio was significantly increased.

All procedures performed in studies involving human participants were following the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient.

Discussion and conclusions

Patients with GBCa who do not manifest symptoms at the early stages of the disease tend to have poor prognoses. Traditionally, complete surgical resection is the only curative modality for GBCa, even patients who receive curative resection frequently develop recurrence of the disease. We report a relapsed GBCa patient who underwent treatment with an ICI combined with an antiangiogenic

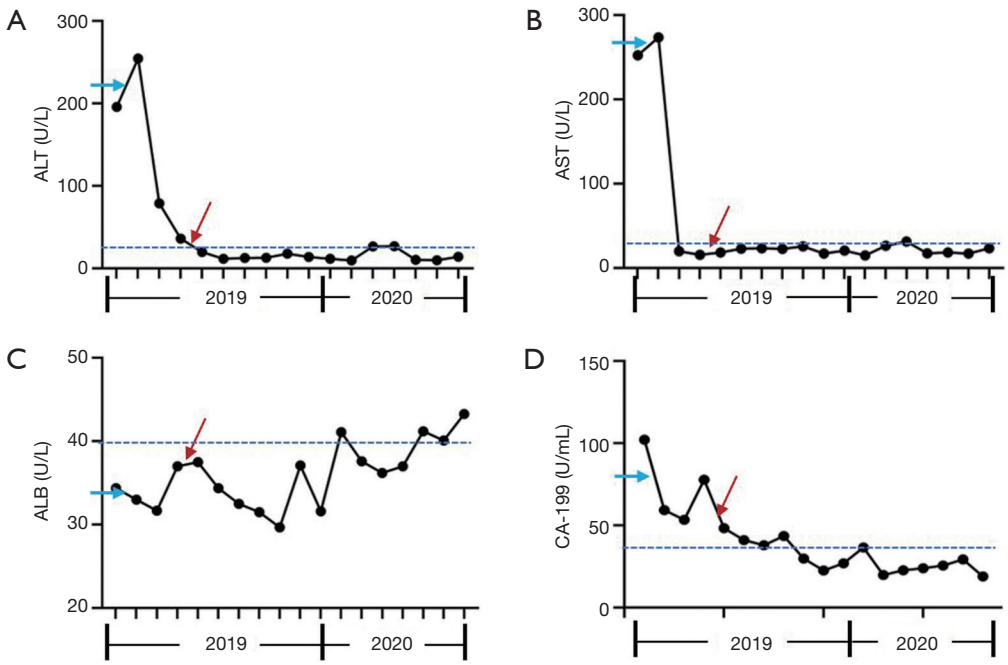

Figure 4 Serum biochemical parameters and tumor parameters (operation time, blue arrow; combined treatment time, red arrow); (A) ALT; (B) AST; (C) ALB; (D) CA-199.

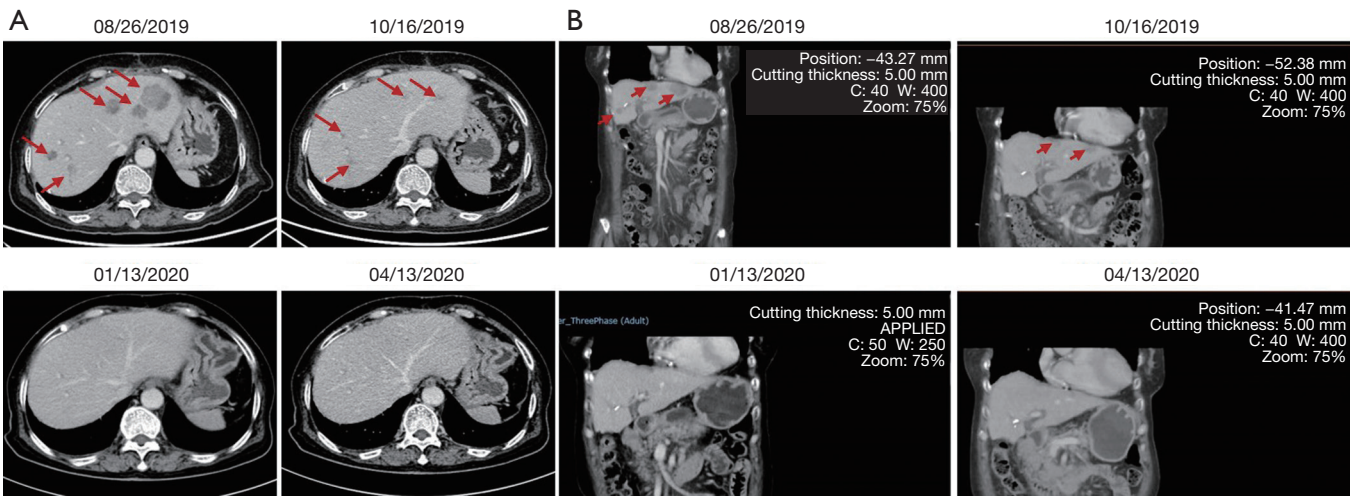

Figure 5 CT imaging showing change of lesion (arrow, tumor lesion); (A) anamorphic surface; (B) coronal plane.

drug, and had an unexpected complete response to treatment.

At present, there is no good method of evaluating the efficacy of this combination therapy in advance. Tumor PD-L1 expression may be one important criterion with which to evaluate the effect of immunotherapy with PD-1/PD-L1 inhibitors in GBC. Previous studies have shown that PD-L1 expression is the only predictive biomarker for PD-1/PD-L1 inhibitors in non-small-cell lung cancer, melanoma, and other tumors (14,15). Kong *et al.* also showed one case of partial response to GBCa with high PD-L1 expression following treatment with nivolumab combined with radiotherapy (16). However, this indicator has limitations. A study involving 101 patients with GBC showed that only

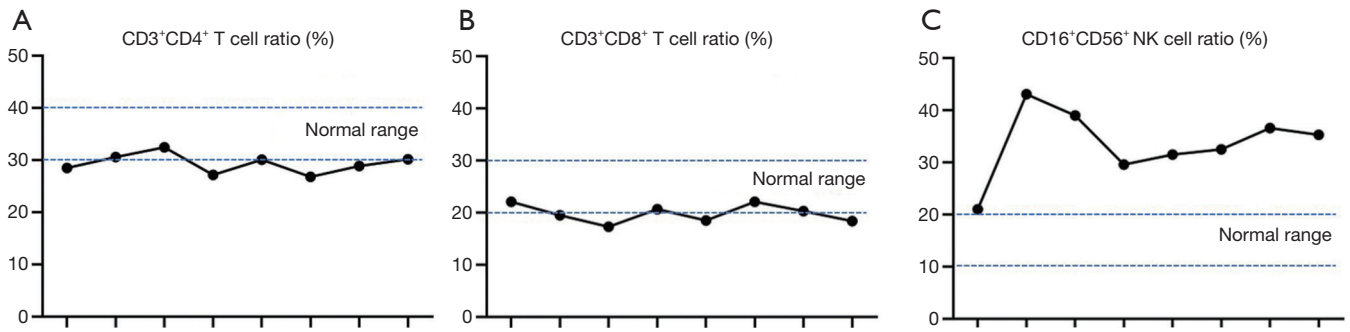

Figure 6 Lymphocyte subpopulation of peripheral blood: (A) $CD3^+CD4^+$ T cell ratio; (B) $CD3^+CD8^+$ T cell ratio; (C) $CD16^+CD56^+$ NK cell ratio.

18.8% of patients had PD-L1 expression with a cutoff level >1%, and 7.9% had high PD-L1 expression (>50%) (17). Also, PD-L1 expression varies greatly concerning the primary and metastatic sites of the tumor lesions. A dramatically different clinical response can also be observed in some tumors with similar PD-L1 expression (18). PD-L1 inhibitors are effective in some tumors which are negative for PD-L1 expression (18). In our case, the tumor tissue showed weak PD-L1 expression. Therefore, given the high level of variation in existing reports, information regarding the association between PD-L1 expression and the effect of immunomodulatory therapies is lacking.

We also investigated other potential biomarkers from our successfully treated patient. Previous studies reported that high TMB burden facilitated PD-L1 inhibitor therapy by contributing to neoantigen formation, and then improving tumor immunogenicity; microsatellite instability (MSI) represents the condition of deoxyribonucleic acid (DNA) mismatch repair, which is a system that maintains the integrity and stability of gene replication (19). However, both TMB and MSI were significantly low in our case, and no obvious correlation was found. In GBCa, a high monocyte-to-lymphocyte ratio (MLR) (>0.24) is inversely correlated with progression-free survival (PFS) and OS compared to a low MLR (<0.24) following chemotherapy (20). In this case, we observed for the first time that the lymphocyte subpopulation ratio in peripheral blood might have predictive value in ICI combined with antiangiogenic treatment. There was a significant increase in the proportion of $CD16^+CD56^+$NK cells in our case. Further clinical data are required to verify our findings.

To our knowledge, this is the first case describing a weakly PD-L1 positive GBC patient who achieved a complete response to Camrelizumab-Apatinib combination treatment. This case indicated that a combination of ICIs and antiangiogenic drugs might be a novel regimen for treating patients with recurrent metastatic GBC or GBCa. However, more studies are needed to validate this finding. The mechanisms underlying the pathological and physiological response of GBCa to the combination therapy are extremely complicated. Multiple indicators, such as TMB, MSI, PD-L1 expression, and peripheral blood lymphocyte subpopulation are effective biomarkers of the combination therapy. We used the combination preferentially in relapsed GBC or GBCa patients without considering the above markers, based on highly malignant biological activity and extremely poor prognosis.

Acknowledgments

Funding: The project was supported by the National Natural Science Foundation of China (81871259, 81530048, 81971495, and 81570562), the Foundation of Jiangsu Collaborative Innovation Center of Biomedical Functional Materials, Jiangsu Key Lab of Cancer Biomarkers, Prevention and Treatment, Collaborative Innovation Center for Personalized Cancer Medicine, Nanjing Medical University, The Six talent peaks project in Jiangsu Province (2017-WSW-019).

Footnote

Reporting Checklist: The authors have completed the CARE reporting checklist. Available at http://dx.doi.org/10.21037/atm-20-4420

Peer Review File: Available at http://dx.doi.org/10.21037/atm-20-4420

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm-20-4420). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to any part of the work are appropriately investigated and resolved. All procedures performed in studies involving human participants were following the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient.

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Cite this article as: Rao J, Xia J, Yang W, Wu C, Sha B, Zheng Q, Cheng F, Lu L. Complete response to immunotherapy combined with an antiangiogenic agent in multiple hepatic metastases after radical surgery for advanced gallbladder cancer: a case report. Ann Transl Med 2020;8(23):1609. doi: 10.21037/atm-20-4420

