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RESEARCH ARTICLE

Efficacy and safety of camrelizumab combined with chemotherapy in the treatment of advanced biliary malignancy and associations between peripheral blood lymphocyte subsets and clinical outcomes

Jian Zhao[1] · Hongxing Guo[1] · Chenxuan Wu[1] · Hongsheng Guo[1]

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Abstract

Background Biliary tract cancer (BTC) is a highly heterogeneous aggressive tumor, and advanced patients have poor prognosis. This work aimed to evaluate the efficacy and safety of camrelizumab combined with chemotherapy in treating advanced BTC, and to explore predictive biomarkers for distinguishing effective population.

Methods 183 advanced BTC patients admitted from September 2018 to September 2021 were retrospectively selected. 93 patients were treated with camrelizumab combined with chemotherapy (C+C group) and 90 patients were treated with chemotherapy alone (C group). Objective response rate (ORR), disease control rate (DCR), median progression-free survival (mPFS), and median overall survival (mOS) were analyzed between two groups. Peripheral blood lymphocyte subsets were assessed by flow cytometry pre- and post-treatment.

Results The mPFS (6.9 months) and mOS (12.1 months) in the C+C group were significantly longer than those in the C group, which were 5.2 months and 9.8 months respectively (HR 0.46, 95% CI 0.38–0.54, p=0.017; HR 0.39, 95% CI 0.32–0.47, p=0.033). The percentage of Total T, CD4+T, natural killer (NK) cells, lymphocyte, and CD4+/CD8+ cell ratios were significantly increased in effective patients after C+C treatment, but didn't increase in progressive disease (PD) patients. Higher percentage of Total T, CD4+T, and higher CD4+/CD8+ cell ratios post-treatment were associated with longer OS.

Conclusions Camrelizumab combining chemotherapy significantly prolonged the mPFS and mOS of advanced BTC patients. Immunotherapy may improve the immune status of advanced patients, and immunotherapy efficacy might be predicted based on the peripheral blood lymphocyte subsets.

Keywords Anti-PD-1 · Camrelizumab · Chemotherapy · Biliary tract cancer · Efficacy · Peripheral blood lymphocyte subsets

Abbreviations

BTCs	Biliary tract cancers
C + C group	Camrelizumab combined with chemotherapy
C group	Chemotherapy along
ORR	Objective response rate
DCR	Disease control rate
mPFS	Median progression-free survival
mOS	Median overall survival
NK	Natural killer
PD	Progressive disease
ICI	Immune checkpoint inhibitor
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
PD1	Programmed death-1
PD-L1	PD1 ligand

Introduction

Biliary tract cancers (BTCs) refer to a group of carcinomas defined by their primary original anatomical sites, including intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, and gallbladder cancer [1]. Despite accounting for only 15% of primary liver cancer, BTC has been the second most common liver tumor after hepatocellular carcinoma [2, 3]. Although the incidence of BTCs is

✉ Chenxuan Wu
chenxuanwu2022@126.com

[1] Department of Oncology, Tianjin Third Central Hospital, No.83 Jintang Road, Hedong District, Tianjin 300170, People's Republic of China

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relatively low, during the past several decades, the incident BTC cases have significantly increased 76.0%, and BTC-related deaths have been elevated 65% [4]. Moreover, substantial variation of BTC has been reported across different regions, and higher BTC incidence and mortality rates are observed in Asia and South America compared to Europe or North America [5]. Cancer statistics in China in 2015 showed even grimmer cases, with about 52,800 new cases of BTCs and 40,700 deaths (W. [6]). Owing to the asymptomatic feature at early stages of BTC, most BTC patients are often diagnosed at advanced stages [7]. Once the disease progresses to unresectable or metastatic status, the most common curative approach surgical resection is not applicable, and the BTC patients have to receive systemic therapy. For patients with unresectable BTC, the 5-year survival rate ranges from 5 to 10%, with median overall survival (mOS) of about 6 months. Hence, it is imperative to explore novel systemic treatment strategy and powerful biomarkers for BTC patients, in order to further improve their prognosis.

During the past years, quite limited innovation has been achieved in chemotherapy of BTC [8]. Up to now, combination of gemcitabine and platinum is still the predominant standard chemotherapy for BTC, which has been firstly proposed in a phase III trial in BTC in 2010 ([9]). Unfortunately, the prognoses of BTC patients received combination chemotherapy are not satisfactory, as chemotherapy-resistant events are found followed by initial beneficial effects [10]. It's objective response rate (ORR) is only 26% and its survival rate is poor, with mean progression-free survival (mPFS) of 8 months and mean overall survival (mOS) of 11.7 months only. Thus, traditional chemotherapy seems to reach a plateau with undesirable ORR and poor prognosis in advanced BTC, and novel treatment strategy is urgently needed.

More recently, immune checkpoint inhibitor (ICI) therapy has been established as an effective systemic therapy option in many solid tumors [11]. Typically, representative ICIs include monoclonal antibodies against cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed death-1 (PD1), and PD1 ligand (PD-L1). Of which, anti-PD-1/PD-L1 therapy has been evidenced to be effective against multiple tumors, thus attracts more attention in BTC as an alternative [12]. PD-1 is expressed on activated T cells, and PD-L1 is expressed on tumor cells and immunocytes. Monoclonal antibody against PD-1 inhibits PD-L1 and PD-1 binding, which would thereby enhance the tumor immune response [13, 14]. Several studies have reported that PD-L1/PD-1 is expressed in BTC cells and tumor-infiltrating leukocytes [15, 16], providing a logical basis for PD1/PD-L1 inhibitor immunotherapy in BTC patients. However, the current effectiveness of PD-1/PD-L1 inhibitors in BTC treatment remains controversial. Previous studies have provided initial assessment of nivolumab and pembrolizumab by combination chemotherapy or monotherapy in patients with advanced BTCs [17], [18, 19], [20]; [21], but ICI combined with chemotherapy in treating advanced BTC patients is still under exploratory stage. Moreover, there were quite fewer PD-1 related exploration in advanced BTC, of them camrelizumab-related work was even limited (X. [22]). Meanwhile, small sample size and rare predictive biomarkers further restrict the related evidence in guiding clinical investigation of immunotherapy combining chemotherapy.

Hence, in this work, based on a real-world advanced BTC cohort, we herein aimed to investigate the short-term and long-term efficacy of camrelizumab combined chemotherapy, meanwhile evaluating the predictive performance of peripheral blood lymphocyte subsets in clinical outcomes of BTC patients. Our findings are expected to provide novel reference information for a better treatment strategy of advanced BTC patients.

Materials and methods

Patients and ethic statement

In this work, an institutional review board-approved retrospective study was developed, basing on the clinical records of BTC patients in Tianjin Third Central Hospital from September 2018 to September 2021. Herein, we focused on the patients with unresectable or postoperatively recrudescent BTC, which were confirmed histologically or cytologically. All patients were treated with chemotherapy (gemcitabine plus cisplatin (GP) or gemcitabine plus oxaliplatin (GEMOX)) alone or in combination with camrelizumab (camrelizumab + GP or camrelizumab + GEMOX). None of the enrolled patients had received prior anticancer treatment.

Treatment strategy

Gemcitabine was given through intravenous drip at 1000 mg/m^2 on day 1 and 8. Cisplatin was injected intravenously at 25 mg/m^2 for consecutive 3 days or 75 mg/m^2 for one day. Oxaliplatin was given intravenously at 130 mg/m^2 on day 1. Camrelizumab was given via intravenous drip at 200 mg on day 0. Treatment was repeated every 3 weeks. All patients received at most 8 chemotherapy cycles until disease progression, unacceptable toxicity, or other rejections were found.

Computerized tomography (CT) was conducted every two cycles. Peripheral blood lymphocyte subsets were measured at baseline and after four cycles. Radiological response was assessed according to the *Response Evaluation Criteria in*

Solid Tumors (RECIST) v. 1.1: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). In this work, participants were then allocated to an effective group (including all patients with CR, PR, or SD) and an ineffective group (including all patients with PD). The PFS herein referred to the date of first instance of tumor progression according to RECIST (RECIST-PFS), date at last clinical examination, date of death of any cause before tumor progression, or the data at last contact.

Sample collection

Peripheral blood samples (2 mL) were collected from the participants who had provided written informed consents before their first treatment. Then, the percentage of total T, CD4 + T, CD8 + T, natural killer (NK) cells, lymphocytes and CD4 + /CD8 + cell ratios in peripheral blood were analyzed using flow cytometry.

Statistical analysis

In this study, SPSS 18.0 was adopted for statistical analysis. Baseline characteristics were counted by descriptive analysis. Difference significance among various groups was analyzed using Chi-square test. The PFS and OS curves were finished using the Kaplan–Meier method. The associations between peripheral blood lymphocyte subsets and PFS were estimated by multivariate Cox proportional hazards regression models. GraphPad Prism 5.0 was used to draw the survival curve. Paired sample t test was conducted to determine the difference significance between Pretherapy and Post-treatment groups. $P < 0.05$ was considered statistically significant difference.

Results

Baseline characteristics of enrolled BTC patients

After exclusion, totally 183 patients were finally enrolled in this work, and the flowchart of study population is displayed in Fig. 1. Among all patients included, there were 93 patients in observation group (patients treated with camrelizumab plus chemotherapy (C + C)) and 90 patients in control group (patients treated with chemotherapy only). Their basic characteristics were evenly distributed between the study groups. The median age of patients was 66 (51–79) years old. Male and female patients accounted for 62% and 38% of the samples, respectively. All patients had a ECOG PS (Eastern Cooperative Oncology Group Performance Status) score of 0–2. Moreover, the study population consisted of 53 cases of intrahepatic cholangiocarcinoma, 46 cases of hilarcholangiocarcinoma, 56 cases of distal cholangiocarcinoma, and 28 cases of gallbladder carcinoma. More detailed clinical information between observation group and control group are presented in Table 1.

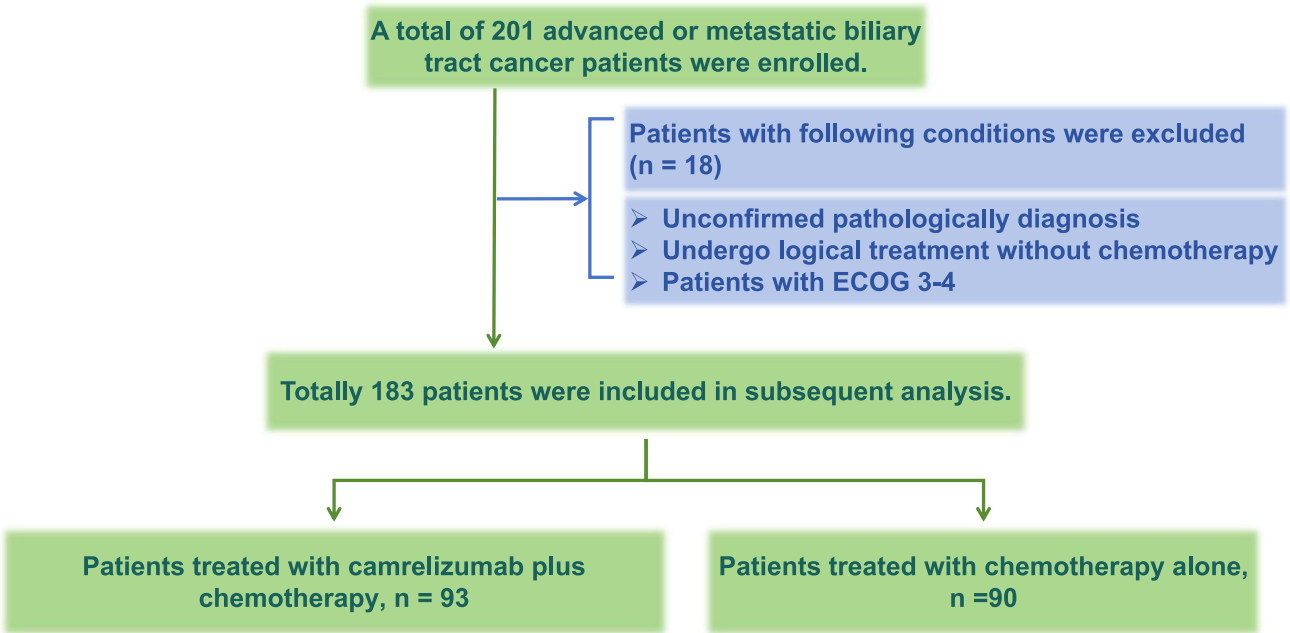

Fig. 1 Flowchart of study population in this work

Table 1 Baseline characteristics

Characteristic	Camrelizumab plus chemotherapy ($n=93$)	Chemotherapy ($n=90$)	*P* value
Age, n (%)			0.73
≤65	41(44.4%)	51(56.3%)	
>65	52(55.6%)	39(43.7%)	
Sex, *n* (%)			0.72
Male	62(66.7%)	50(56.2%)	
Female	31(33.3%)	40(43.8%)	
Primary tumor site, *n* (%)			0.35
Intrahepatic bile ducts	67(72.2%)	56(62.5%)	
Extrahepatic bile ducts	20(22.2%)	11((12.5%))	
Gall bladder	6(6.6%)	23((25%))	
ECOG performance status, *n* (%)			1.0
0–1	72(77.8%)	73(81.3%)	
≥2	21(22.2%)	17(18.7%)	
Histological grade, n (%)			1.0
Well differentiated (G1) or moderately differentiated (G2)	56(61.1%)	50(56.3%)	
Poorly differentiated (G3)	36(38.9%)	40(43.7%)	
Previous surgery, n (%)			0.27
Yes	73(77.8%)	51(56.3%)	
No	20(22.2%)	39(43.7%)	
Chemotherapy regimens, n (%)			0.29
GP	69(74.2%)	61(67.8%)	
GEMOX	24(25.8%)	29(32.2%)	
Biliary stent or drain, n (%)			0.31
Yes	32(34.4%)	27(30.0%)	
No	61(65.6%)	63(70.0%)	
HBV/HCV infection, n (%)			0.11
Yes	18(19.4%)	15(16.7%)	
No	75(80.6%)	75(83.3%)	
CA199, n (%)			0.08
≤400 ng/mL	43(46.2%)	46(51.1%)	
>400 ng/mL	50(53.8%)	44(48.9%)	
Maximum diameter of tumor			0.21
≤5 cm	35(37.6%)	32(35.6%)	
>5 cm	58(62.4%)	58(64.4%)	
Metastatic site, *n* (%)			0.51
Liver	67(72.2%)	78(87.5%)	
Lymph node	57(61.1%)	45(50%)	
Lung	26(27.8)	11(12.5%)	

ECOG: Eastern Cooperative Oncology Group; *HBV*: hepatitis B virus; *HCV*: hepatitis C virus

Efficacy and survival in BTC patients

As of March 31, 2022, 183 (100%) PD events and 168 (91.8%) deaths were observed. The mPFS of patients was 6.9 months and the mOS was 12.1 months in the observation group, which were significantly longer than those in control group [mPFS = 5.2 months (HR 0.46, 95% CI 0.38–0.54, $p=0.017$, Fig. 2), and mOS = 9.8 months (HR 0.39, 95% CI 0.32–0.47, $p=0.033$, Fig. 3)]. There were totally 41 patients (44.1%) with CR and PR in the observation group, and 37 SD patients (39.8%) and 15 PD patients (16.1%). In the control group, the cases of CR, PR, SD, and PD patients were 0, 23 (25.6%), 33 (36.7%), and 34 (37.8%), respectively. Moreover, we noticed that the ORR (41.1%) and DCR (83.9%) of the patients in observation group were both significantly higher than that of control group (ORR = 25.6%, DCR = 62.2%) ($p<0.05$, determined by Chi-Squared test,

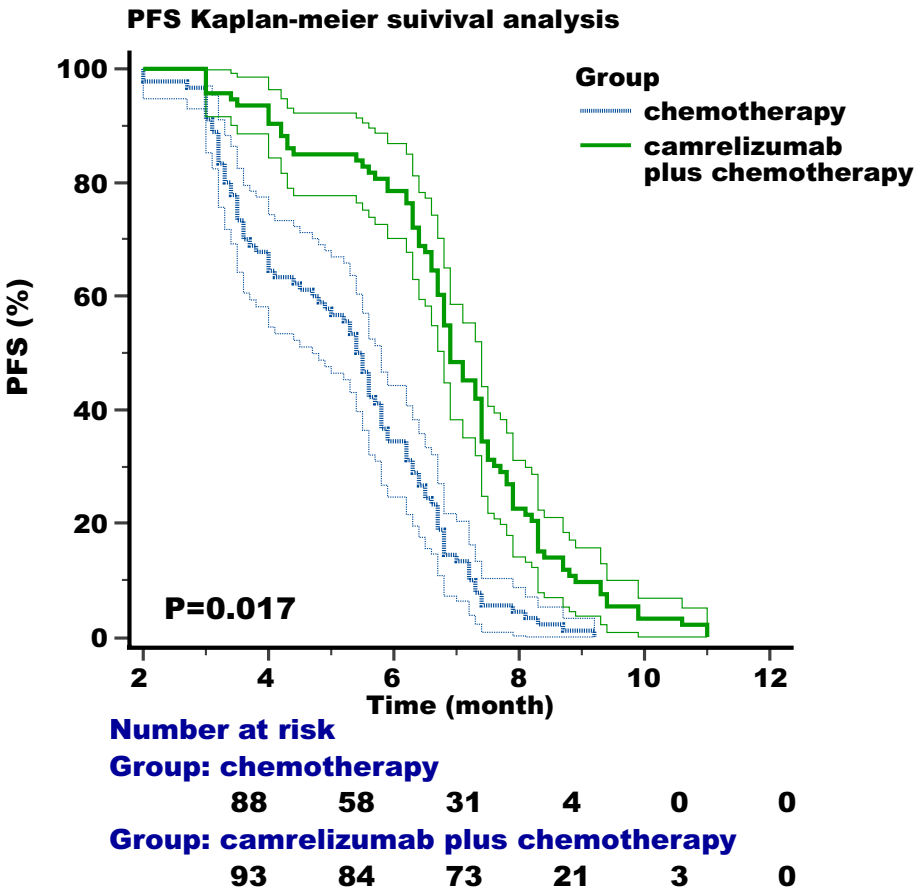

Fig. 2 Kaplan–Meier analysis of PFS outcomes of patients treated with camrelizumab plus chemotherapy compared to those of patients treated with chemotherapy alone. The mPFS in C + C group was 6.9 months and 5.2 months in C group (HR 0.46, 95% CI 0.38–0.54, $p = 0.017$). C + C group: camrelizumab plus chemotherapy group; C group: chemotherapy group; mPFS: median progression-free survival. P value was determined by log-rank test

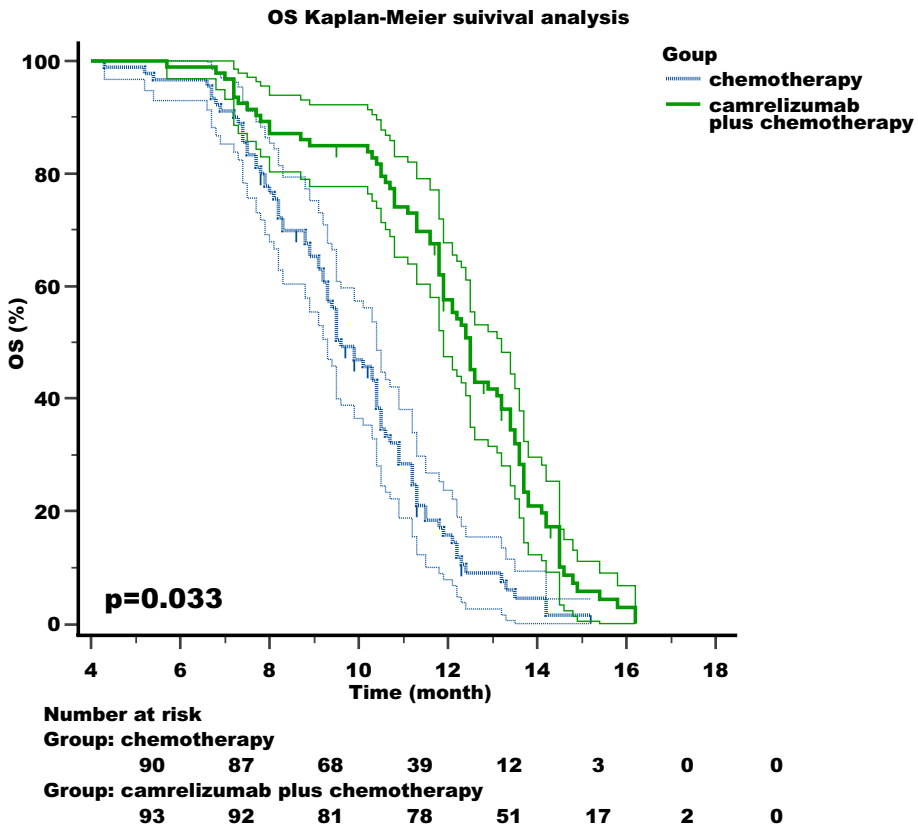

Fig. 3 Kaplan–Meier analysis of OS outcomes of patients treated with camrelizumab plus chemotherapy compared to those of patients treated with chemotherapy alone. The mOS in C + C group was 12.1 months and 9.8 months in C group (HR 0.39, 95% CI 0.32–0.47, $p = 0.033$). mOS: median overall survival. *P* value was determined by log-rank test

Table 2 Tumor response for camrelizumab plus chemotherapy group and chemotherapy group

Response	Camrelizumab plus chemotherapy group ($N=93$)	Chemotherapy group ($N=90$)	P value
CR	0	0	
PR	41	23	
SD	37	33	
PD	15	34	
ORR	41.1%	25.6%	0.007
DCR	83.9%	62.2%	0.001
Median PFS	6.9m	5.2m	0.017
Median OS	12.1m	9.8m	0.033

CR, complete response; *PR*, partial response; *SD*, stable disease; *PD*, progressive disease; *ORR*: objective response rate; *DCR*: disease-control rate. *P* value was determined by Chi-Squared test

Table 2), implying a better efficacy of camrelizumab plus chemotherapy.

Predictive value of lymphocyte subsets in BTC patients

Subsequently, to identify more predictive factors for BTC patients, totally 6 variables in peripheral blood lymphocytes subsets were evaluated in 93 advanced BTCs patients in the observation group, including lymphocyte, total T cells, NK cells, $CD8^+$ T cells, $CD4^+$ T cells, and $CD4^+/CD8^+$ ratio. Among the 93 advanced BTCs patients, patients with CR, PR, or SD were defined as effective patients. In effective patients, we noticed that the proportions of total T, CD4 + T, NK cells, lymphocyte, and CD4 + /CD8 + cell ratios after four cycles of treatment were significantly higher than that of pretherapy status (all $P<0.01$, determined by paired t test) (Table 3, Fig. 4). However, in PD patients, the proportions of all lymphocyte subsets showed no statistical difference before and after treatment (Table 3, Fig. 5).

Considering the important impacts before and after C + C treatment, the receiver-operating characteristic (ROC) analysis was conducted on these factors to evaluate their roles in predicting efficacy of C + C treatment. The area under curve (AUC) values of lymphocyte, NK cells, CD4 + T cells, and CD4 + /CD8 + ratio were all more than 0.8 (all $P<0.05$), implying that these factors could distinguish effective patients from ineffective patients (Fig. 6).

To further evaluate the prognostic value of these peripheral blood lymphocytes in BTC patients, multivariate Cox proportional hazards regression analysis was employed to determine their association with PFS. The results suggested that proportions of total T ($P=0.014$), CD4 + T ($P=0.001$), and CD4 + /CD8 + cells ratio ($P=0.019$) after four cycles of treatment were significantly correlated with PFS of BTC patients, exhibiting prognostic predictive potential (Fig. 7).

Safety

Treatment-related adverse events (TRAEs) were observed in 55.6% of the patients taking camrelizumab combined with chemotherapy, and 56.2% of patients in chemotherapy alone group. Regarding chemotherapy, whether it was used alone or in combination with camrelizumab, it showed similar safety performance (Table 4). The most frequently reported TRAEs in grade 3–4 were thrombocytopenia (22.2% in observation group and 25% in control group) and leukopenia (16.7% in observation group and 25% in control group) in two cohorts. Reactive cutaneous capillary endothelial proliferation (RCCEP) in grade 1–2 was observed in almost two-thirds of patients in the observation group, but it showed no significant effects on quality of life and medication. In both two groups, there was no drug-related deaths.

Table 3 Distribution of peripheral blood immune cell subsets before and after chemotherapy combined with camrelizumab in 78 effective patients with and 15 ineffective patients ($\bar{x}\pm$ s)

Lymphocyte subset	CR + PR + SD ($n=78$)			PD ($n=15$)		
	Pretherapy	Post-treatment	P value	Pretherapy	Post-treatment	P value
Lymphocyte (%)	26.6 ± 3.3	35.9 ± 4.0	< 0.01	25.6 ± 3.9	26.0 ± 4.0	0.16
Total T (%)	56.6 ± 3.7	74.7 ± 5.4	< 0.01	58.5 ± 4.0	59.0 ± 3.9	0.22
$CD4^+$T (%)	30.3 ± 5.3	44.5 ± 5.2	< 0.01	30.3 ± 2.4	30.9 ± 2.0	0.13
$CD8^+$T (%)	20.3 ± 4.2	21.9 ± 3.4	0.13	22.5 ± 3.7	23.5 ± 4.6	0.11
$CD4^+/CD8^+$	1.58 ± 0.52	2.09 ± 0.47	< 0.01	1.38 ± 0.28	1.36 ± 0.31	0.54
NKC (%)	2.2 ± 0.9	4.1 ± 1.2	< 0.01	2.2 ± 0.9	2.2 ± 0.8	0.57

CR, complete response; *PR*, partial response; *SD*, stable disease; *PD*, progressive disease; effective patients including patients with CR, PR, or SD; ineffective patients including patients with PD; Total T, Total T cell; $CD4^+$T, $CD4^+$T cell; $CD8^+$T, $CD8^+$T cell; $CD4^+/CD8^+$, $CD4^+/CD8^+$ radio; NKC, NK cell. P was determined by paired *t* test

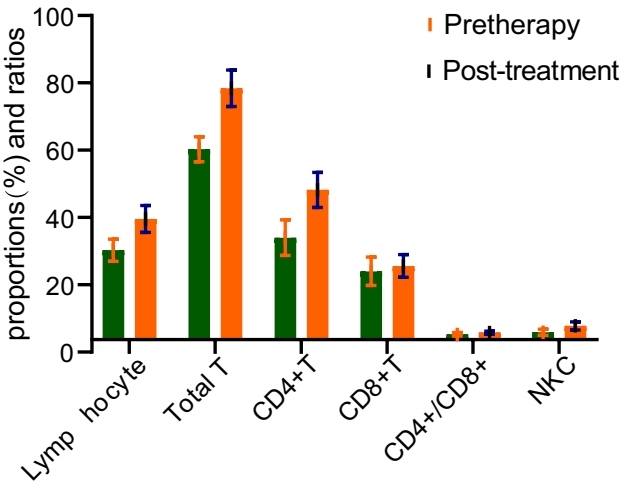

Fig. 4 Proportions of lymphocyte subsets before and after treatment in effective patients. Effective patients were defined as patients with CR, PR, or SD. The percentage of total T, CD4 + T, NK cells, lymphocyte, and CD4 + /CD8 + cell ratios after treatment for 4 cycles were significantly higher than that in effective patients before treatment (All $P < 0.01$, determined by paired t test). CR, complete response; PR, partial response; SD, stable disease. Total T, Total T cell; CD4 + T, CD4 + T cell; CD8 + T, CD8 + T cell; CD4 + /CD8 +, CD4 + /CD8 + radio; NKC, NK cell

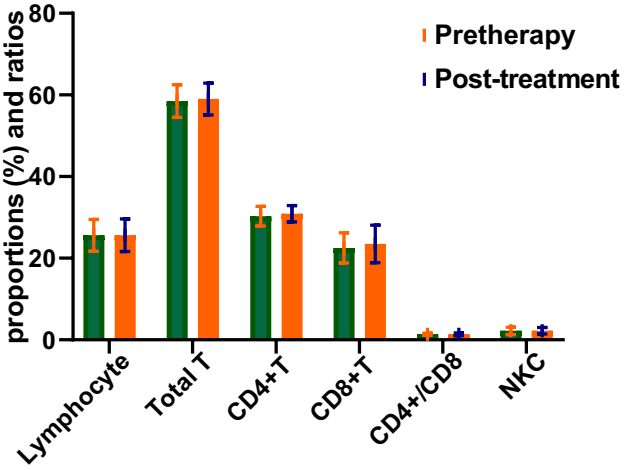

Fig. 5 Proportions of lymphocyte subsets before and after treatment in ineffective patients. Ineffective patients referred to patients with progressive disease (PD). The proportions of lymphocyte subsets showed no statistical difference before and after treatment (All $P > 0.05$, determined by paired t test)

Discussion

As a highly heterogeneous tumor, the BTC often progresses covertly. Despite only accounting for about 3% of gastrointestinal malignant tumors, the incidence and mortality rates of BTC are still increasing [23]. Whereas, during the past

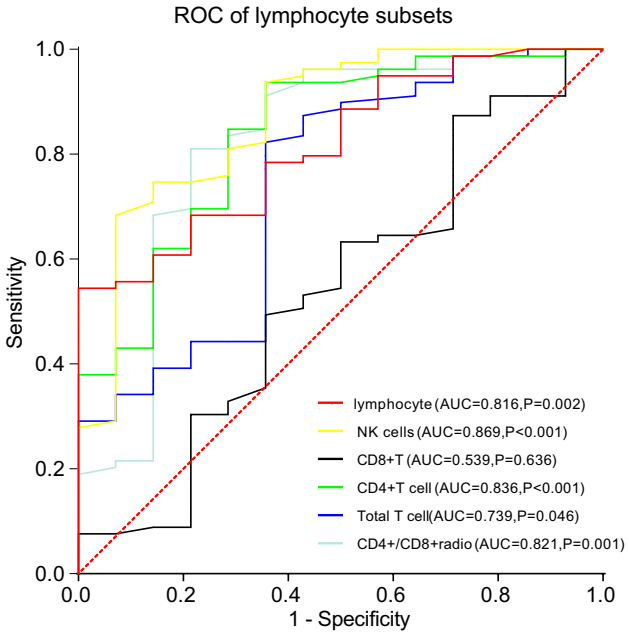

Fig. 6 The ROC curves of peripheral blood lymphocytes subsets in BTC patients. Area under curve (AUC) values were employed to evaluate the predictive value. P value was determined by paired t test

decade, GP-based chemotherapy has been the first-line treatment for advanced BTC, with no real breakthrough in treatment strategy (Harding, Khalil, Fabris, & Abou-Alfa, 2023). More recently, immunotherapy has brought new light for further improving prognosis of advanced BTC patients [24]. Hence, we retrospectively analyzed the efficacy of chemotherapy vs. chemotherapy plus immunotherapy in a real world cohort. Our data indicated a significantly better survival of BTC receiving chemotherapy plus immunotherapy.

Previous studies have documented that either immune monotherapy or PD-1 inhibitor combined with CTLA-4 inhibitor exhibited limited effects on patients with BTC [21, 25–27], and immunotherapy combining chemotherapy may be the hope of breaking through the existing treatment bottleneck. In our retrospective study, the data indicated that anti-PD-1 therapy combining chemotherapy significantly improved the PFS (6.9 vs. 5.2 months) and OS (12.1 vs. 9.8 months) of patients in observation group, implying a better efficacy. The survival of BTC patients receiving chemotherapy in our study was worse than the data from ABC-02 study (mPFS = 8.0 months, and mOS = 11.7 months) (J. [9]), which might be resulted from differential hematologic and biochemical functions, patient conditions, etc. Nevertheless, our data have evidenced that PD-1 inhibitor camrelizumab significantly enhanced the effects of chemotherapy, exhibiting short-term and long-term survival benefit. Recently, in a phase single arm, phase II study, they have evaluated the efficacy of camrelizumab combined with gemcitabine and oxaliplatin in advanced BTCs, revealing that the

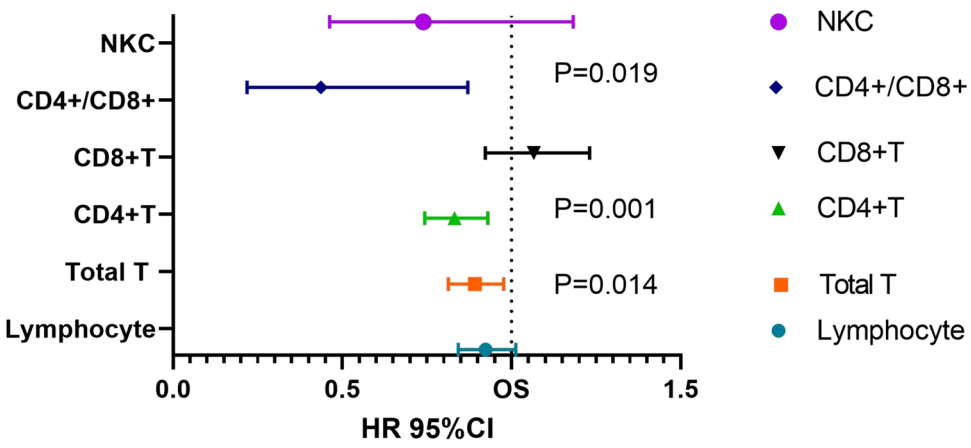

Fig. 7 The associations between peripheral blood lymphocytes subsets and PFS in camrelizumab + chemotherapy group. The percentage of total T ($p = 0.014$), CD4 + T ($p = 0.001$), and CD4 + /CD8 + cells ratio ($p = 0.019$) after treatment was significantly associated with PFS (analyzed using multivariate Cox proportional hazards regression model)

Table 4 Treatment-related adverse events

Adverse event	Camrelizumab + chemotherapy group		Chemotherapy group	
	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4
Patients with ≥ 1 events	52(55.6%)	31(33.3%)	51(56.2%)	23(25.5%)
Rash	10(11.1%)	6(6.3%)	6(6.7%)	0
Nausea and/or vomiting	26(27.8%)	0	28(31.2%)	0
Diarrhea	5(5.6%)	0	5(5.6%)	0
hepatitis	0	0	7(7.8%)	0
Fatigue	16(16.7%)	0	11(12.5%)	0
Peripheral sensory neuropathy	14(15.1%)	0	23(25%)	0
Fever	4(4.3%)	0	6(6.7%)	0
Hypothyroidism	0	3(3.2%)	0	0
Alopecia	5(5.6%)	0	7(7.8%)	0
Hematologic				
Anemia	0	0	0	0
Leukopenia	16(16.7%)	12(12.5%)	23(25%)	11(12.5%)
Thrombocytopenia	21(22.2%)	9 (9.6%)	23(25%)	13(14.4%)
RCCEP	67(72%)	0	0	0

RCCEP, reactive cutaneous capillary endothelial proliferation

combination treatment achieved a 6.1 months mPFS and 11.8 months mOS, which was consistent with our data (X. [22]). Moreover, studies from Sun et al.[19] and from Gou et al.[28] similarly suggested that PD-1 inhibitors combined with chemotherapy could significantly improve the antitumor activity compared to PD-1 inhibitor monotherapy or chemotherapy alone. Furthermore, the ORR and DCR for the camrelizumab plus chemotherapy group were both better than those for the chemotherapy alone group (41.1% vs 25.6% and 83.9% vs 62.2%, respectively). Previously, it has been reported that the ORR and DCR of nivolumab plus GP in a phase I study was 37% and 63%, respectively. Their data indirectly supported that efficacy of camrelizumab might be better than nivolumab in advanced BTC, which deserved further investigation in the future.

In clinical practice, to precisely distinguish potential benefit populations is an important and crucial step in making optimal treatment strategy. PD-L1 has been reported to be strongly related to the response to anti-PD-1 inhibitors in several tumors [29], while its predictive value in BTC remained to be further validated. Of the PD-L1-positive BTC patients enrolled in the KEYNOTE-028 study, 17% (4/23) responded to pembrolizumab monotherapy[30]. In addition, 46.2% (11/26) of the patients were PD-L1 positive and only one patient achieved an objective response in the JVDF trial of ramucirumab and pembrolizumab [25]. Meanwhile, except for MSI-H/dMMR, many predictive markers have been explored but not been validated [31]. Accordingly, more efficient biomarkers are urgently needed to identify effective patients who might respond to camrelizumab combined with chemotherapy.

Therefore, six peripheral blood lymphocyte subsets were then detected pre- and post-treatment. Notably, we found that some lymphocyte subsets were associated with the clinical outcomes of advanced BTC patients, besides some subsets were of great potential in distinguishing effective patients. After C + C treatment, the percentage of Total T, CD4 + T, NK cells, lymphocyte, and CD4 + /CD8 + cell ratios were significantly increased in effective patients, meanwhile their ROC results suggested a good predictive performance. More recently, Zhang et al. have documented that BTC could be classified into three subsets based on the characterization of infiltrating T cells, which would be helpful in making immunotherapeutic strategy [32]. Their findings might partly explained our results, involving the underlying mechanisms of these markers' roles. Regarding the correlation between Total T, $CD4^+$T, and $CD4^+/CD8^+$ cells ratio after treatment and PFS, it probably indicated that immunotherapy might improve the immune status of patients with advanced BTCs, and the efficacy may correlate with the degree of improvement in immune status.

Regarding the safety of combination treatment, hematologic adverse events were similar in the two groups. Rash and hypothyroidism in grade 3–4 were more frequent in patients receiving camrelizumab, possibly due to the addition of the PD-1 inhibitor, which was considered immune-related adverse event. Our data are consistent with the previous report from Michot et al. [33]. It has been indicated that immunotherapy might result in immune-related organ dysfunction, including in the lung, skin, thyroid, liver, and kidney [34]. Reactive cutaneous capillary endothelial proliferation was observed in almost two-thirds of patients and the incidence of RCCEP was similar to previous study, but all events were grade 1–2 and showed no significant effects on quality of life and medication. The adverse events in this work were controllable, and no death was caused by adverse events.

Although our study have evaluated the efficacy of chemotherapy plus immunotherapy and revealed six novel predictors for effective patients, there were several limitations in this work. Firstly, we have prospectively designed our study, while it was retrospective in nature, which might limit the interpretation of the results. Moreover, the clinical information has been collected for a long time in order to include more samples, while a larger sample size would further avoid selection bias and recall bias. Despite the above limitations, the real-world data were still helpful for the subsequent prospective study. Meanwhile, in our future work, it is vital to further investigate the prognostic value of peripheral blood lymphocyte subsets in BTC patients.

In conclusion, basing on a real world advanced BTC cohort, patients treated with camrelizumab combining chemotherapy have longer survival, implying a better efficacy. Furthermore, six peripheral blood lymphocytes exhibit great potential in predicting the effective population and clinical outcomes of BTC. Our findings give novel insights into the immunotherapy combining chemotherapy of BTC, providing a more inexpensive ICI inhibitor choice for patients.

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Authors' contributions Jian Zhao: conception, data entry, data statistics and analysis, drafting. Hongxing Guo: data statistics and analysis. Hongsheng Guo: data collection, data entry, data analysis. Chenxuan Wu: data collection, data analysis.

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Data availability All data generated or analyzed during this study are included in this article.

Declarations

Conflict of interests The authors declare that they have no competing interests.

Ethical approval and Informed consent This study has been approved by Institutional Ethics Committee of Tianjin Third Central Hospital (ethic code: IRB2019-010–01). Informed consent was obtained from all individual participants included in the study.

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