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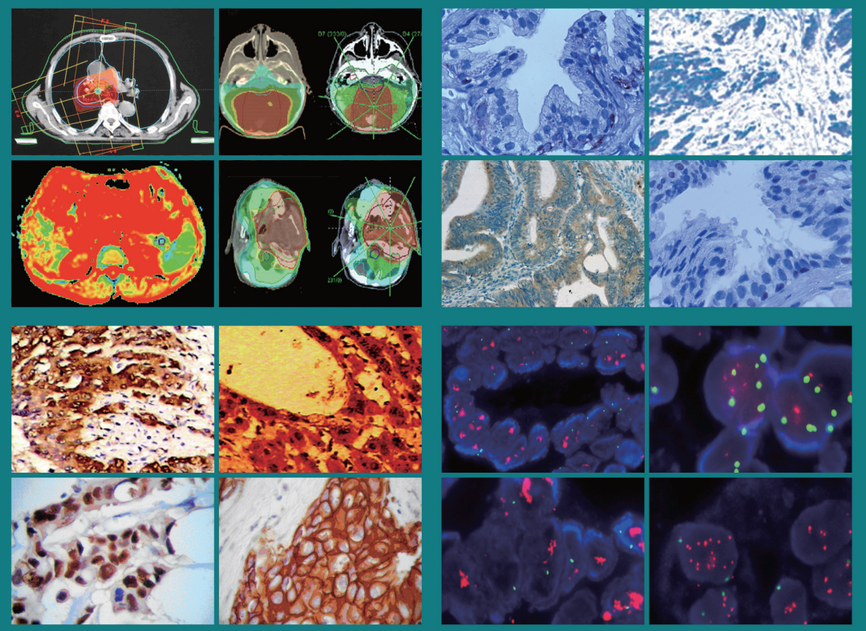

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Mailing address
Editorial office of
Oncology and Translational Medicine
Tongji Hospital
Tongji Medical College
Huazhong University of Science and Technology
Jie Fang Da Dao 1095
430030 Wuhan, China
Tel.: +86-27-83662395
Email: otm.office@tjh.tjmu.edu.cn

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EXPERT CONSENSUS

Chinese expert consensus on managing thrombocytopenia in patients with cancer and liver injury

Xianglin Yuan (✉), Committee of neoplastic supportive-care (CONS), China Anti-Cancer Association

Abstract

Thrombocytopenia and liver injury are serious clinical problems in patients with cancer. The etiology of thrombocytopenia in patients with cancer and liver injury (TCLI) is complicated. Managing cancer therapy-induced thrombocytopenia has gradually become standardized, and managing liver injury-associated thrombocytopenia has become more effective with the approval and marketing of relevant drugs. However, the optimal strategy for managing thrombocytopenia in patients with cancer and liver injury remains unclear, and the superposition of thrombocytopenia and liver injury further increases the difficulty of cancer treatment. Therefore, the Committee of Cancer Support Therapy of the Chinese Anti-Cancer Association has organized experts to analyze and discuss relevant literature to form a Chinese expert consensus on managing thrombocytopenia in patients with cancer and liver injury (2022 Edition) to guide clinical practice.

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Key words: cancer; liver disease; liver injury; thrombocytopenia; expert consensus

✉ Correspondence to: Xianglin Yuan. Email: yuanxianglin@hust.edu.cn

Thrombocytopenia, usually defined as a platelet count of $< 100 \times 10^9$/L in the peripheral blood [1], is a common complication in patients with cancer. Guidelines for cancer therapy-induced thrombocytopenia have been published. However, in clinical practice, when patients with cancer have concomitant liver injury, the risk and severity of thrombocytopenia are further increased because of secondary portal hypertension and hypersplenism; for such patients, doctors face more severe challenges in managing thrombocytopenia. Therefore, to bridge the gap in this field and scientifically manage thrombocytopenia in patients with cancer and liver injury, the Committee of Cancer Support Therapy of the Chinese Anti-Cancer Association has organized multidisciplinary expert discussions to formulate the first Chinese expert consensus on managing thrombocytopenia in patients with cancer and liver injury. The levels of evidence-based medical proof, recommendation grades, and their definitions are presented in Tables 1 and 2 [2].

Thrombocytopenia in patients with cancer and primary liver disease

Epidemiology of thrombocytopenia in patients with cancer and primary liver disease

In China, approximately 7 million people have liver cirrhosis, leading to 460,000 new liver cancer cases annually [3]. The prevalence of chronic liver disease-associated thrombocytopenia ranges from 6% among patients without cirrhosis to 85% among those with cirrhosis [4, 5]. Patients with hepatitis B and/or C have a higher incidence of thrombocytopenia than those with chronic liver disease caused by other etiologies (toxin/drug-induced hepatitis, alcoholic hepatitis, and non-alcoholic steatohepatitis) [6]. Initial thrombocytopenia is the most significant risk factor for hepatitis B virus-associated thrombocytopenia (HBV-TP) and hepatitis C virus-associated thrombocytopenia (HCV-TP). Splenomegaly and cirrhosis are significant risk factors for moderate HCV-TP. Hyperbilirubinemia is an important risk factor for moderate to severe HBV-TP. Anti-platelet antibodies are associated with HCV-TP severity, and anti-platelet autoantibody alone or in combination with splenomegaly may cause thrombocytopenia [7]. The prevalence of thrombocytopenia in patients with cancer and primary liver disease has not been reported. Given that multiple factors associated with tumors can also lead to thrombocytopenia, the risk of thrombocytopenia in patients with cancer and primary liver disease will be further increased.

Pathophysiology of thrombocytopenia in patients with cancer and primary liver disease

Thrombocytopenia in patients with liver disease was previously thought to be mainly caused by hypersplenism. However, thrombocytopenia in patients with liver disease is a complex and multifactorial process involving different mechanisms. It usually includes decreased platelet production, increased destruction, and splenic sequestration [8, 9].

Platelet production decreased

Thrombopoietin (TPO) is an important factor in promoting thrombopoiesis and is mainly synthesized in the liver parenchyma and sinusoidal endothelial cells. TPO regulates the proliferation, differentiation, and maturation of megakaryocytes by binding to c-mpl receptors on megakaryocytes and mediates thrombopoiesis [9]. As liver disease progresses and worsens in severity, the ability of the liver to synthesize TPO decreases [10]. In addition, the

Table 1 Levels of evidence-based medical proof and definitions

Level of Evidence	Definition
Level I (high quality)	Further research is very unlikely to change the confidence in the clinical efficacy assessment result. It is regarded as high-level evidence.
Level II (moderate quality)	Further research is likely to have an important impact on the confidence in the efficacy assessment result and may change the assessment result. It is regarded as medium-level evidence.
Level III (low quality)	Further research is very likely to have a significant impact on the confidence in the efficacy assessment results and is very likely to change the assessment result. It is regarded as low-level evidence.
Level IV (very low quality)	Any efficacy assessment is very uncertain. It is regarded as very low-level evidence.

Table 2 Recommendation grades and definitions

Recommended grade	Definition
Recommendation	Based on available evidence, recommendation is made if it is well established that the benefits outweigh the risks and burdens.
Weak recommendation	Based on available evidence, weak recommendation is made if the benefits, risks, and burdens are fairly balanced, or if there is significant uncertainty in the extent of the benefits and risks.

direct myelosuppressive effect of hepatitis virus is one of the causes of decreased platelet production [9, 10]. The toxic effects of alcohol on bone marrow megakaryocytes may also result in decreased platelet production [10].

Platelet destruction increased

The detection rate of platelet-related antibodies is significantly higher in patients with cirrhosis than in healthy individuals [11], and autoantibodies against platelet surface antigens can enhance platelet clearance by the splenic and hepatic reticuloendothelial systems [12]. Moreover, in patients with cirrhosis, decreased levels and activity of a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 drive the accumulation of von Willebrand factor multimers, which mediate the enhancement of shear stress-induced platelet aggregation [13, 14].

Splenic sequestration

A larger spleen volume suggests that hypersplenism is more pronounced, more blood cells are retained in the enlarged spleen, and macrophages in the spleen have stronger phagocytosis; hence, the spleen size is inversely proportional to the platelet count [10, 15]. In addition to the increased storage of platelets in the enlarged spleen, the mechanism by which hypersplenism results in decreased platelets involves increased vasoactive substances and cytokines released by the spleen [9, 10].

Diagnosis and evaluation of thrombocytopenia in patients with cancer and primary liver disease

Diagnostic criteria

The clinical diagnostic criteria for primary liver disease-associated thrombocytopenia include: (1) Peripheral blood platelet count < 100×10^9/L; (2) The liver disease meets relevant diagnostic criteria for viral liver disease, alcoholic liver disease, non-alcoholic fatty liver disease, or autoimmune liver disease due to primary etiologies [16-21] (Attached Table 1); (3) Excluding thrombocytopenia caused by other underlying diseases and/or comorbidities, such as aplastic anemia, leukemia, primary immune thrombocytopenia, and bone marrow tumor cell infiltration; (4) Excluding thrombocytopenia caused by anti-cancer therapy and/or other drugs, including radiotherapy, chemotherapy, targeted therapy, immunotherapy, antibiotics, antibody-drug conjugates, and heparin; (5) Excluding pseudothrombocytopenia caused by ethylenediaminetetraacetic acid anticoagulant.

Differential diagnosis

In patients with cancer therapy-induced thrombocytopenia, especially chemoradiotherapy-induced thrombocytopenia, platelet count fluctuations are often closely related to dose reduction or discontinuation of tumor therapy. For patients whose platelet count slowly declines over time compared with the baseline, which does not appear to be related to fluctuations in cancer treatment, tumor metastases to the bone or chronic liver disease should be considered. Patients with cancer are prone to co-infection due to iatrogenic reasons such as immunosuppression, indwelling catheters, and surgery, and infection can lead to thrombocytopenia through multiple mechanisms. In addition to cancer therapy, drugs such as heparin and antibiotics may also cause thrombocytopenia, and blood transfusion precipitation may lead to post-transfusion purpura [22]. Therefore, a detailed understanding of the clinical background of thrombocytopenia in patients and the completion of laboratory tests is helpful in identifying the complex etiology of thrombocytopenia. If

Table 3 Differential diagnosis of thrombocytopenia [1, 24–26]

Disorders		Examination items
Infection	Serious infections	Hematology: C-reactive protein; procalcitonin
	Viral infection	Epstein-Barr virus, cytomegalovirus, HIV
Hematological system disease	Hemolytic anemia; paroxysmal nocturnal hemoglobinuria	Lactate dehydrogenase
	Aplastic anemias, tumor metastases to bone marrow	Bone marrow smear, bone marrow biopsy
	Immune thrombocytopenia	Platelet glycoprotein-specific autoantibodies
Autoimmune disorders	Systemic lupus erythematosus, sicca syndrome, rheumatoid arthritis	Antinuclear antibodies, rheumatoid factor
Thrombotic disorders	Disseminated intravascular coagulation	Prothrombin time, partial activated prothrombin time, thrombin time, fibrinogen, D-dimer, fibrin degradation products
Renal impairment	Renal impairment-associated thrombocytopenia	Creatinine
Malnutrition	Malnutrition-associated thrombocytopenia	Vitamin B12, folic acid
Thrombocytopenia associated with anti-tumor therapy and/or other drug therapy	Chemotherapy (e.g., Platinum, Gemcitabine , Capecitabine, and Temozolomide), radiotherapy, checkpoint inhibitors, targeted therapy (e.g., Lenvatinib, Imatinib, Zanubrutinib, and Niraparib), antibody-drug conjugates (e.g., ADC-Trastuzumab Emtansine), antibiotics, and heparin	Hematology, a certain drug that can cause thrombocytopenia is administered exactly before the onset of thrombocytopenia, and platelet count returns to normal after discontinuation of the drug.

the diagnosis of thrombocytopenia cannot be confirmed by routine screening, laboratory tests to confirm the diagnosis can be selected based on medical history and clinical manifestations [1]. For example, anti-platelet-specific antibodies have important clinical significance in differentiating immune thrombocytopenia from non-immune thrombocytopenia [23] (Table 3).

Grading of thrombocytopenia

The most commonly used severity grading criteria for thrombocytopenia are the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE). According to CTCAE (v5.0) grading for adverse event "thrombocytopenia," platelet count below the lower limit of normal (75×10^9/L) is considered as Grade 1; platelet count < 75 to 50×10^9/L is considered as Grade 2; platelet count < 50 to 25×10^9/L is considered as Grade 3; platelet count < 25×10^9/L is considered as Grade 4 [27]. When the platelet count is > 50×10^9/L, bleeding symptoms are less common; when the platelet count is (30–50) $\times 10^9$/L, the patient may present with skin purpura and ecchymosis; when the platelet count is (10–30) $\times 10^9$/L, the patient will have difficulty stopping bleeding due to trauma; when the platelet count is < 10 $\times 10^9$/L, the patient may be at risk of life-threatening bleeding [28].

Grading of bleeding severity

Mild to moderate: No bleeding symptoms or skin bleeding spots/ecchymoses only [29].

Severe: Bleeding symptoms, including mucocutaneous bleeding, gastrointestinal, respiratory, genitourinary, and intracranial bleeding [29].

Treatment of thrombocytopenia in patients with cancer and primary liver disease

Treatment goals

The treatment goals are: (1) to increase the platelet count and reduce the risk of spontaneous bleeding caused by it; (2) to minimize dose reduction or delay in systemic treatment of cancer due to thrombocytopenia; and (3) to maintain the platelet count above the reference threshold for invasive procedures or surgery, and to reduce the risk of traumatic bleeding related to perioperative procedures or surgery.

Treatment principles

Treatment of thrombocytopenia in patients with cancer and primary liver disease begins with the treatment of etiology, with graded management based on the bleeding status and treatment goals. Comprehensive treatment with platelet transfusion, drugs (e.g., thrombopoietic drugs and immunosuppressants), or surgery (e.g., splenectomy and partial splenic artery embolization) should be considered in a timely manner to avoid fatal bleeding caused by low platelets. Treatment measures should be selected based on the etiology and pathophysiological mechanism of thrombocytopenia in patients. Considering the management of liver injury, drugs with approved indications without hepatotoxicity should be preferred. Platelet count should be closely monitored during treatment to avoid excessive elevation, which can increase the risk of thrombosis.

Treatment measures

Platelet transfusion: *In vitro* studies have suggested that platelet counts above 56×10^9/L improve thrombin production in patients with cirrhosis [30], but platelet transfusion has not been shown to significantly improve thrombin generation or normalize thromboelastometry tests in patients with cirrhosis [31]. The American Gastroenterological Association Institute recommends risk stratification based on the type of surgery and surgical site, with no safety margin for routine laboratory tests related to preoperative bleeding and coagulation in patients with cirrhosis. For managing bleeding and coagulation, individualized treatment can be guided by thromboelastography while avoiding unnecessary blood transfusions and volume overload, and routine use of blood products (e.g., fresh frozen plasma or platelets) is not recommended to prevent bleeding [32, 33]. The American Society of Clinical Oncology believes that the threshold

Table 4 TPO-RAs approved for marketing in China [35, 36]

	Romiplostim	Eltrombopag	Avatrombopag	Hetrombopag
TPO receptor binding	Extracellular	Transmembrane	Transmembrane	Transmembrane
Route of administration	Subcutaneous injection	Oral	Oral	Oral
Dietary effects	No	Yes	No	Yes
Interaction with cations	No	++	No	++
Dose reduction required for East Asian population	No	Yes	No	Yes
Dose reduction required for patients with hepatic insufficiency	No	Yes	No	Yes
Additional liver function tests	No	+	No	?
Use in patients with renal failure	Yes	May be used	May be used	No data
Use during pregnancy	No	No	No	No
Approved indications in China	ITP	ITP	Liver disease-associated thrombocytopenia	ITP\SAA

for platelet transfusion should be adjusted according to the patient's diagnosis, clinical condition, and treatment modality. The risk of bleeding during chemotherapy-induced thrombocytopenia in patients with solid tumors is related to the duration of the nadir platelet count. The panel recommends a prophylactic platelet transfusion threshold of 10×10^9/L, whereas the platelet transfusion threshold is higher than 10×10^9/L for patients with active bleeding. In the absence of relevant coagulation abnormalities, the platelet transfusion threshold is 40×10^9/L–50×10^9/L for high-risk invasive procedures, and it is critical that platelet count meets the safety threshold required for invasive procedures or surgery if platelets are transfused preoperatively [34].

Thrombopoietin receptor agonists: TPO receptor agonists (TPO-RAs) have improved immunogenicity compared with first-generation TPO analogs. Currently, TPO-RAs approved for marketing in China include romiplostim, eltrombopag, avatrombopag, and hetrombopag, mainly for treating immune thrombocytopenia (ITP), severe aplastic anemia (SAA), and chronic liver disease (CLD)-associated thrombocytopenia [22], of which only avatrombopag is approved for CLD-associated thrombocytopenia (Table 4).

Two Phase III studies with avatrombopag showed that the proportion of patients who did not require platelet transfusion or any rescue for bleeding was significantly higher in the avatrombopag group than in the placebo group, and the proportion of patients with platelet counts $\geq 50 \times 10^9$/L on the day of invasive procedures was also higher in the avatrombopag group than in the placebo group [37]. Eltrombopag significantly reduced the proportion of patients treated with platelet transfusions from pre-procedure to 7 days after the procedure. However, portal vein thrombotic events occurred in six patients treated with eltrombopag, leading to premature termination of the study [38]. A small sample size research with romiplostim suggested that the preoperative use of romiplostim for HCV infection-associated thrombocytopenia (platelet count $< 50 \times 10^9$/L) could result in a platelet count of $\geq 70 \times 10^9$/L and surgery in most patients [39]. A meta-analysis showed that TPO-RA significantly increased the proportion of patients with a preoperative platelet count of $> 50 \times 10^9$/L and decreased the platelet transfusion rate, without increasing the risk of thrombosis [40].

Recombinant human thrombopoietin: Recombinant human thrombopoietin (rhTPO) was first developed abroad, but its development was terminated because of concerns about the generation of neutralizing antibodies [41]. In 2005, rhTPO was approved for marketing in China, mainly for adjuvant treatment of thrombocytopenia caused by chemotherapy for solid tumors and primary immune thrombocytopenia. A retrospective analysis showed that for patients with liver disease-associated thrombocytopenia treated with rhTPO before surgery, the platelet count was significantly higher on day 8 than at baseline and peaked on day 12 [42]. In addition, another study found that rhTPO was more effective in patients with cirrhosis but without splenomegaly [43].

Recombinant human interleukin-11: In 1997, recombinant human interleukin-11 (rhIL-11) was formally approved by the United States Food and Drug Administration (FDA) and became the first specific platelet-elevating drug to be used in chemotherapy or radiotherapy-induced thrombocytopenia [44]. In 2003, the first rhIL-11 was approved for marketing in China,

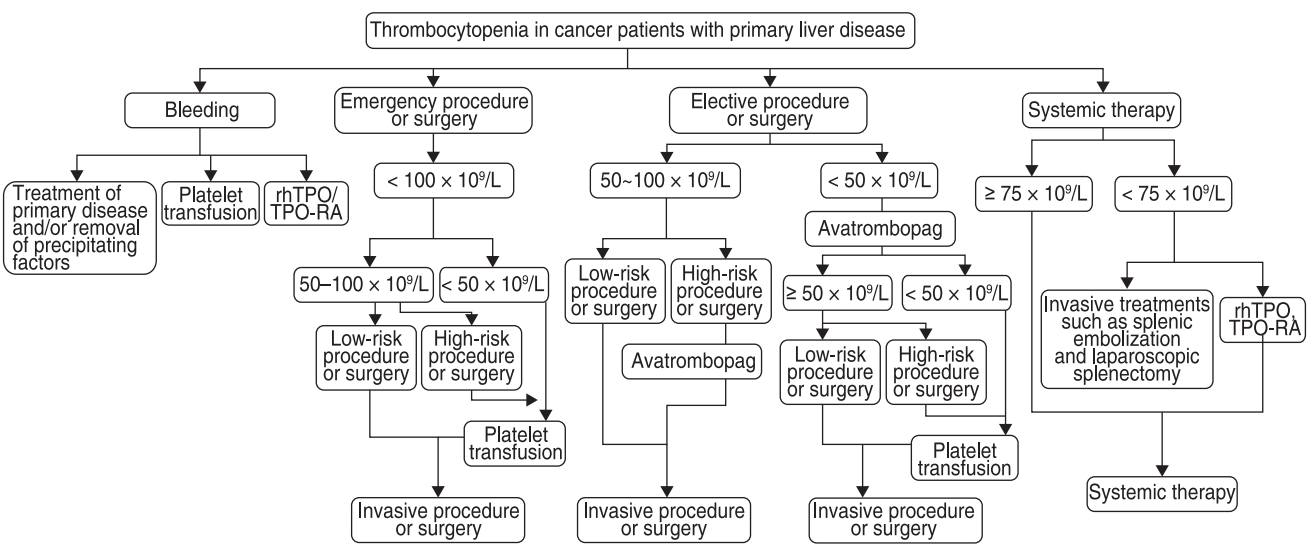

Fig. 1 Algorithm for managing thrombocytopenia in patients with cancer and primary liver disease

mainly for treating grade 3 or 4 thrombocytopenia after chemotherapy for solid tumors and non-myeloid leukemia [45]. RhIL-11 was used in patients with thrombocytopenia caused by cirrhosis and hypersplenism (platelet count ≤ 75×10^9/L), while the mean increase in platelet count was only $(5.95 \pm 12.31) \times 10^9$/L after treatment [46].

Surgical treatment: Splenectomy, partial splenic artery embolization (PSAE), and radiofrequency ablation (RFA) are the main invasive treatments for splenomegaly/hypersplenism-associated thrombocytopenia [47]. Invasive treatment measures can achieve relatively long-term sustained improvement in platelet count in patients with splenomegaly/hypersplenism-associated thrombocytopenia [48-50], but the duration of sustained platelet improvement after PSAE or RFA treatment tends to correlate closely with embolized or ablated spleen volume [50]. In addition, patients undergoing invasive treatment have a worse short-term prognosis, with a significantly increased incidence of postoperative complications, such as venous thrombosis, but no significant improvement in long-term prognosis [51-54].

Management of thrombocytopenia in patients with cancer and primary liver disease

PSAE and splenectomy are effective treatment options for splenomegaly/hypersplenism-induced thrombocytopenia; however, these invasive treatments often increase the risk of complications and even death. TPO-RAs have been approved for treating CLD-associated thrombocytopenia and can elevate platelet count with a low adverse event rate. However, the selection of specific treatment measures should be based on the pathophysiological mechanism of thrombocytopenia in patients, such as the severity of hypersplenism/splenomegaly, treatment purpose, and physical status. Invasive treatment may even need to be conditionally applied to specific populations after multidisciplinary discussion.

Recommendation 1: For patients with thrombocytopenia and cancer complicated by primary liver disease who develop bleeding, platelet transfusion, rhTPO, or TPO-RAs may be considered if the primary disease and/or precipitating factors are actively managed (Level of Evidence: III, Recommendation Grade: weak recommendation).

Recommendation 2: For patients with thrombocytopenia and cancer complicated by primary liver disease who are scheduled to undergo invasive procedures or surgery, platelet transfusion may be administered in emergency situations. TPO-RAs (e.g., avatrombopag) or rhTPO, with less effect on liver function, may be considered in non-emergency situations to increase the platelet count to a safe threshold required for invasive procedures or surgery. (Level of Evidence: II, Recommendation Grade: Recommendation).

Recommendation 3: Among patients with thrombocytopenia and cancer complicated by primary liver disease who intend to undergo systemic therapy, invasive treatments such as splenic embolization and splenectomy may be considered for patients with severe hypersplenism/splenomegaly because of the long-term nature of systemic therapy. RhTPO or TPO-RA may be considered for patients with contraindications to invasive treatment (Level of Evidence: III, Recommendation Grade: weak recommendation).

Managing thrombocytopenia in patients with cancer therapy-induced thrombocytopenia complicated by primary liver disease

For managing thrombocytopenia in patients with cancer therapy-induced thrombocytopenia complicated by primary liver disease, please refer to the Guidelines for the Diagnosis and Treatment of Cancer Therapy-Induced Thrombocytopenia (2022 edition) [29]. The Guidelines recommend prophylactic platelet-stimulating growth factor for patients with a nadir platelet count of < 50×10^9/L in the last chemotherapy cycle or patients with a nadir platelet count of ≥ 50×10^9/L and < 75×10^9/L in the last chemotherapy cycle combined with at least one high-risk factor for bleeding to ensure that the chemotherapy is conducted smoothly [29]. There is a lack of evidence-based secondary prevention for patients with cancer therapy-induced thrombocytopenia complicated by primary liver disease. Since liver disease affects the production, destruction, and distribution of platelets, it will increase the risk of cancer therapy-induced thrombocytopenia and the difficulty of its recovery. Therefore, more aggressive secondary prevention strategies should be adopted.

Recommendation 4: For patients with cancer therapy-induced thrombocytopenia complicated by primary liver disease, drugs with less effect on liver function (e.g., avatrombopag) should be selected when developing a management scheme for thrombocytopenia. In addition, the timing of treatment should be differentiated, such as active secondary prevention (Level of Evidence: III, Recommendation Grade: weak recommendation).

Managing adverse reactions

Overview of adverse drug reactions

Adverse drug reactions (ADRs) caused by TPO-RAs mostly occur within 6 months of treatment, mainly affecting the circulatory and hematological systems, with clinical manifestations of arteriovenous thrombosis, myelofibrosis, and hepatotoxicity. The dose and duration of such drugs were not significantly correlated with the time and severity of thrombosis [55]. However, if patients have concomitant thrombotic diseases, such as coronary atherosclerotic disease, myocardial infarction, and stroke, the control of the primary disease should be

closely monitored during treatment with TPO-RAs, and regular reexamination should be performed. Ghanima et al. summarized the clinical application progress of TPO-RAs in the past decade of marketing, and moderate myelofibrosis was observed in few patients; however, the correlation between this adverse event and the type, dose, or duration of the therapeutic agent has not been clearly established [56]. The literature reported that two pediatric patients developed acute liver failure after treatment with eltrombopag [55, 57], and the FDA and National Medical Products Administration gave a black box warning regarding the hepatotoxicity for eltrombopag, suggesting it may increase the risk of serious and potentially life-threatening hepatotoxicity. Hence, patients should be monitored for liver function and the medication should be adjusted appropriately during use [58].

Adverse reactions to rhTPO mainly include fever, chills, general malaise, and asthenia, which are generally mild [59-61]. A nonrandomized, parallel-controlled study showed that the incidence of adverse reactions after treatment with rhTPO was 11.4%, whereas that after treatment with rhIL-11 was 78.4% [62].

As an inflammatory factor, rhIL-11 is prone to systemic adverse reactions, including asthenia, slight fever, vomiting, joint soreness, and thrombosis. In addition, rhIL-11 causes damage to the heart and kidney, which can cause tachycardia, vasodilatation, atrial fibrillation, atrial flutter, and body fluid retention. It can also affect the digestive, nervous, and respiratory systems, causing serious allergic reactions [63].

Recommendation 5: Most adverse reactions to TPO-RAs, rhTPO, and rhIL-11 are mild to moderate and can resolve rapidly after drug withdrawal. In cases of suspected adverse reactions, appropriate symptomatic management should be provided after differential diagnosis (Level of Evidence: III, Recommendation Grade: recommendation).

Managing thrombosis/thromboembolism

Portal vein thrombosis (PVT) is a common complication in patients with cirrhosis, with a prevalence of 10–25% [64, 65]. Malignancies are the most important risk factors for venous thromboembolism (VTE), and 20%–30% of cases with the first occurrence of VTE are associated with tumors [66]. Venous thrombosis has been reported with drugs and invasive treatment of liver disease-associated thrombocytopenia [38, 52, 53]. The Expert Consensus on the Management of Portal Vein Thrombosis in Cirrhosis (2020) recommends endoscopic and hematologic examinations to fully assess the risk of bleeding before anticoagulant therapy in patients with cirrhosis and PVT. The main indications for anticoagulant therapy are acute symptomatic PVT, waiting for liver transplantation, and concomitant mesenteric vein thrombosis. Anticoagulant therapy should be suspended in patients with cirrhosis and PVT with a recent history of bleeding, severe gastroesophageal varices, and severe thrombocytopenia [67]. The 2022 European Hematology Association Guidelines on the Management of Antithrombotic Therapy in Patients with Cancer and Thrombocytopenia recommend that patients with grade 1/2 thrombocytopenia can continue oral or parenteral anticoagulant therapy if the platelet count is stable or use low-molecular-weight heparin for anticoagulation if the platelet count is unstable. For patients with grade 3 thrombocytopenia, anticoagulant therapy with a halved dose of low-molecular weight heparin may be considered if the risk of thrombosis is high, thrombocytopenia is expected to remain stable for weeks to months, and platelet count should be closely monitored. Anticoagulant therapy should be discontinued in patients with grade 4 thrombocytopenia [68]. The Guidelines for the Prevention and Treatment of Venous Thromboembolism in Patients with Cancer (2020 Edition) recommend that platelet count should be closely monitored during the treatment of patients with cancer and VTE or those at high risk of VTE, and prophylaxis or treatment should be performed according to the assessed risk of venous thromboembolism [69].

Recommendation 6: Platelet count should be closely monitored during the treatment of thrombocytopenia in patients with cancer and primary liver disease to prevent excessive elevations in platelet count and an increased risk of thrombosis. Anticoagulant therapy should be considered in cases of grade 1/2 thrombocytopenia with thrombosis. In cases of grade 3/4 thrombocytopenia with thrombosis, caution should be exercised with anticoagulant therapy (Level of Evidence: II, Recommendation Grade: recommendation).

Table 5 Common anti-cancer drugs causing liver injury in clinical practice [71-77]

Classification of anti-cancer drug	Typical drugs
Platinum	Oxaliplatin, and Carboplatin
Antimetabolites	Methotrexate, Cytarabine, and Gemcitabine
Alkylating agents	Cyclophosphamide, Ifosfamide, and Busulfan
Molecular targeted drugs	Imatinib, Dasatinib, Lapatinib, Erlotinib, and Lenvatinib, Sorafenib, Bevacizumab, Gemtuzumab, and Trastuzumab
Immune checkpoint inhibitors	Nivolizumab , Pembrolizumab, and Ipilimumab
Others	Irinotecan, and antibody-drug conjugates (ADCs)

Thrombocytopenia with liver injury secondary to tumor therapy

Overview

Anti-cancer drugs or immunomodulators account for 8.34% of patients with drug-induced liver injury (DILI) [70]. Hepatic sinusoidal obstruction syndrome (HSOS) occurs in up to 50% of patients treated with oxaliplatin for colon adenocarcinoma [71]. Anti-cancer drugs such as 5-fluorouracil, irinotecan, azathioprine, carmustine, cyclophosphamide, and dacarbazine also increase the risk of HSOS [72, 73]. Immune checkpoint inhibitor-induced hepatitis occurs in 9%–20% of patients treated with programmed cell death protein 1/programmed cell death ligand 1 inhibitors [74]. Increased serum transaminase levels were observed in 52% of patients treated with lenvatinib [75]. The incidence of DILI after treatment with antibody-drug conjugates (ADCs) was 7.9% [76]. In addition, radiation therapy, especially for thoracic and abdominal tumors, can lead to clinical subacute and chronic liver injuries [77].

Thrombocytopenia is a common hematologic toxicity associated with anticancer therapies. Studies have reported an incidence of chemotherapy-induced thrombocytopenia of 9.7% [78], an incidence of thrombocytopenia associated with different targeted therapies ranging from 2% to 78% [79, 80], and an incidence of immune checkpoint inhibitor-associated thrombocytopenia ≥ grade 3 of 1.73% [81]. Therefore, secondary liver injury and thrombocytopenia often coexist during cancer treatment (Table 5).

Diagnosis

The clinical diagnosis of DILI relies on exclusion methods. The diagnosis of DILI is inferred by collecting complete medical history, comprehensive blood tests, hepatobiliary imaging, and liver biopsy to exclude other etiologies. To reduce the subjective tendency of clinical diagnosis, there are currently some relatively objective causality scoring systems, including the Roussel-Uclaf Causality Assessment Method (RUCAM) scale (Attached Table 4) [82]. The etiological diagnosis of thrombocytopenia is often complicated because cancer therapy can directly lead to hematological toxicity and may also be associated with liver injury secondary to cancer therapy. Therefore, accurate knowledge of the history of drug exposure and the course of hepatic dysfunction and thrombocytopenia is important.

Treatment principles and schemes

The principles for treating thrombocytopenia with liver injury secondary to cancer therapy [83] are as follows: (1) Suspected drugs that can injure the liver should be discontinued promptly, and reuse of suspected or similar drugs should be avoided whenever possible; (2) The risk of primary disease progression caused by drug withdrawal and aggravation of liver injury caused by continued medication should be fully weighed; (3) Appropriate drug therapy should be selected according to the clinical type of DILI; (4) Thrombocytopenia should be managed with reference to primary liver disease-associated thrombocytopenia and cancer therapy-induced thrombocytopenia.

Clinical treatment measures for oxaliplatin immune-induced syndrome include permanent discontinuation of oxaliplatin. The use of corticosteroids and intravenous immunoglobulin has not been demonstrated, but it can be considered [84]. Jácome et al. retrospectively analyzed the use of PSAE for chemotherapy-induced hypersplenism. After PSAE, 80% of patients had a platelet count of ≥ 100 × 10^9/L, 81% of patients resumed systemic treatment, and the proportion of splenic infarction was linearly correlated with increased platelet count [85]. A cohort study by Overman et al. showed that the addition of bevacizumab to oxaliplatin-based chemotherapy reduced the risk of splenomegaly and thrombocytopenia in patients with metastatic colorectal cancer [86].

Recommendation 7: For patients with thrombocytopenia and liver injury secondary to cancer therapy, the selection of a treatment regimen for thrombocytopenia can refer to the management of primary liver disease-associated thrombocytopenia and cancer therapy-induced thrombocytopenia. Drugs with less effect on liver function (e.g., avatrombopag) are recommended as platelet-elevating drugs (Level of Evidence: III, Recommendation Grade: weak recommendation).

Multidisciplinary management and health education of thrombocytopenia

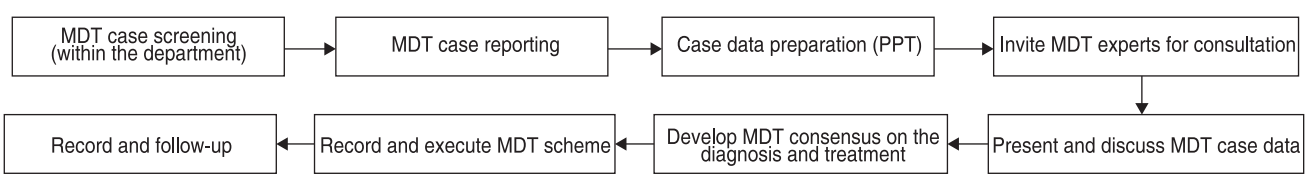

Fig. 2 MDT execution process

in patients with cancer and liver injury

Multidisciplinary management of thrombocytopenia in patients with cancer and liver injury

The etiology and pathogenesis of thrombocytopenia in patients with cancer and liver injury are complex, and its diagnosis and treatment involve multidisciplines, such as oncology, hepatology, hematology, intervention, and surgery. Through multidisciplinary collaborative diagnosis and treatment, the professional advantages of each discipline can be maximized for patient benefit. For patients with unknown etiology of thrombocytopenia, patients with severe thrombocytopenia and critically ill condition, and patients who experience poor recovery of platelet count after conventional treatment, which affects further diagnosis and treatment of cancer, it is recommended to report them to the intra- or inter-hospital multidisciplinary team (MDT), invite relevant experts of MDT to perform consultation, formulate the diagnosis and treatment plan, and conduct follow-up.

Recommendation 8: Multidisciplinary collaborative diagnosis and treatment are recommended for patients with unknown etiology of thrombocytopenia, severe thrombocytopenia and critically ill condition, and poor recovery of platelet count after conventional treatment (Level of Evidence: IV, Recommendation Grade: recommendation).

Health education of thrombocytopenia in patients with cancer and liver injury

Hemorrhage caused by severe thrombocytopenia in patients with cancer and liver injury is one of the main causes of death. Patients with concomitant ruptured esophagogastric varices, gastric and duodenal peptic ulcers, or esophageal/gastric/duodenal mucosal erosions

Appendix Table 1 Diagnostic criteria for primary liver disease [16-21]

Type of primary liver disease	Clinical diagnostic criteria
Viral liver disease	The etiological diagnosis of viral liver disease should be made based on the results of serology and etiology tests. Infection with hepatitis viruses includes hepatitis A, B, C, and E. Clinically, the diagnosis is usually further subdivided based on the results of serology, virology, biochemistry, radiography, pathology, and other ancillary examinations in infected patients.
Alcoholic liver disease	There is no specific clinical diagnostic method for alcoholic liver disease. Careful inquiry about long-term alcohol consumption history is very important. For patients who meet Item 1, exclude other causes of liver disease, and if they meet Items 3 and 4, the diagnosis of alcoholic liver disease can be confirmed; for patients who meet Items 1, 3, and 4, with evidence of viral hepatitis infection, the diagnosis of alcoholic liver disease with viral hepatitis can be confirmed: 1. A history of long-term alcohol consumption for more than 5 years, equivalent to ethanol ≥ 40 g/d for men and ≥ 20 g/d for women; or a history of heavy alcohol consumption within 2 weeks, equivalent to ethanol > 80 g/d. However, attention should be paid to the influences of factors such as sex and genetic susceptibility. The conversion formula of ethanol amount (g) = alcohol consumption (mL) × ethanol content (%) × 0.8. The Alcohol Use Disorders Identification Test, the Michigan Alcoholism Screening Test, CAGE questionnaire and other scales can be used to screen for ethanol (alcohol) abuse and ethanol (alcohol) dependence. 2. The clinical symptoms are nonspecific and may be asymptomatic or accompanied by right upper abdominal distending pain, inappetence, asthenia, body weight loss, and jaundice; with the aggravation of the disease, there may be neuropsychiatric symptoms, spider naevus, liver palms, and other manifestations. 3. The parameters such as serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), total bilirubin (TBil), prothrombin time (PT), mean corpuscular volume (MCV), and carbohydrate-deficient transferrin (CDT) are increased. Among these parameters, AST/ALT > 2, increased GGT, and increased MCV are characteristic of alcoholic liver disease. CDT test is specific; however, it is not routinely conducted in clinical practice. These parameters can decrease significantly after abstinence and usually return to normal within 4 weeks (but GGT recovers more slowly), which is helpful in diagnosis. 4. There are typical findings in B-mode ultrasound, X-ray computed tomography, magnetic resonance imaging or transient elastography of the liver. 5. Current infection with hepatotropic virus, drug-induced and toxic liver injuries, and autoimmune liver disease are excluded.
Non-alcoholic fatty liver disease	The diagnosis of non-alcoholic fatty liver disease (NAFLD) requires radiographic or histologic evidence of diffuse hepatocellular steatosis, and other causes of hepatic steatosis such as ethanol (alcohol) abuse should be ruled out. Considering the absence of specific symptoms and signs, NAFLD is suspected in most patients owing to incidental findings of elevated serum ALT and GGT or diffuse fatty liver on imaging examination. NAFLD is assessed by quantifying the degree of hepatic steatosis and fibrosis, determining the presence or absence of metabolic and cardiovascular risk factors and complications, the presence or absence of hepatic inflammatory injury, and the presence or absence of other causes of liver disease.
Autoimmune liver disease	The International Autoimmune Hepatitis Group (IAIHG) developed descriptive diagnostic criteria and diagnostic scoring systems for AIH in 1993 and revised them in 1999 (Attached Table 2). In 2008, IAIHG proposed a simplified diagnostic scoring system for AIH (Attached Table 3). The simplified scoring system is prone to missed diagnosis of some atypical patients, such as those with low or negative autoantibody titers and/or low or even normal serum IgG levels. Therefore, for patients with suspected AIH or undiagnosed by simplified diagnostic scoring system, it is recommended that the integrated diagnostic scoring system be used for comprehensive evaluation to avoid missed diagnosis.

Appendix Table 2 Integrated diagnostic scoring system for AIH (1999) [17]

Parameter/Clinical Feature	Score	Parameter/Clinical feature	Score
Female	+2	Drug history	
Ratio of ALP (fold upper limit of normal) to AST (or ALT) (fold upper limit of normal)		Positive	–4
< 1.5	+2	Negative	+1
1.5–3.0	0	Average alcohol intake daily (g/day)	
> 3.0	–2	< 25	+2
Ratio of serum gamma-globulin or IgG to normal value		> 60	-2
> 2.0	+3	Liver histology	
1.5–2.0	+2	Interface hepatitis	+3
1.0–1.5	+1	Lymphoplasmacytic infiltrate	+1
< 1.0	0	Rosette-like changes in hepatocytes	+1
ANA, ASMA or LKM-1 titer		None of the above	–5
> 1:80	+3	Bile duct changes	–3
1:80	+2	Other changes	–3
1:40	+1	Other immune diseases	+2
< 1:40	0	Other available parameters	
AMA positivity	–4	Positive for other specific autoantibodies (SLA/LP, LC-1, ASGPR, pANCA)	+2
Hepatitis virus markers		HLA-DR3 or DR4	+1
Positive	–3	Response to treatment	
Negative	+3	Complete	+2
		Relapse	+3
Total Score Interpretation			
Before treatment		After treatment	
Unequivocal AIH	≥ 16	Unequivocal AIH	≥ 18
Possible AIH	10–15	Possible AIH	12–17

Appendix Table 3 Simplified diagnosis criteria for AIH in IAIHG [17]

Variable	Criteria	Score	Remark
ANA or SMA	1:40	1	Equivalent to the lowest titer of ANA 1:100 commonly used in China
ANA or SMA	1:80	2	Maximum score of 2 for multiple simultaneous occurrences
LKM-1	1:40	2	
SLA	Positive	2	
IgG	> upper limit of normal	1	
	> 1.1 times upper limit of normal	2	
Liver histology	Compatible with AIH	1	Interface hepatitis, lymphoplasmacytic infiltrate in portal tracts and lobules, rosette-like changes in hepatocytes, and penetration phenomena are characteristic of liver histological changes. When three of the four items are met, it is considered as typical.
	Typical of AIH	2	
Exclude viral hepatitis	Yes	2	

score = 6: possible AIH; score ≥ 7: confirmed AIH

have a higher risk of acute upper gastrointestinal bleeding, which is the most common emergency of the digestive system. Qdaisat et al. analyzed the characteristics and outcomes of intracranial hemorrhage in patients with cancer in the emergency department and found that low platelet count was significantly associated with in-hospital and 30-day mortality [87]. Therefore, it is particularly important to strengthen the risk management and health education for thrombocytopenia in patients with cancer and liver injury.

Risk management and health education

(1) Carefully inquire about medical history, menstrual history, medication history, especially medical history related to liver disease, bleeding history, and concomitant medications, such as anticoagulant and anti-platelet drugs.

(2) Monitor blood chemistry and hematology regularly during tumor treatment. Graded management

Appendix Table 4 RUCAM table [82]

	Hepatocyte type		Cholestasis or mixed		Evaluation
1. Time of onset (d)	Initial treatment	Retreatment	Initial treatment	Retreatment	Score
1a: Time from drug initiation to disease onset					
Suggestive	5–90	1–15	5–90	1–90	+2
Suspected	< 5 or > 90	>15	< 5 or > 90	>90	+1
1b: Time from drug discontinuation to disease onset					
Related	≤ 15	≤ 15	≤ 30	≤ 30	+1
2. Course	Difference between peak ALT and ALT upper limit of normal		Difference between peak ALP (or TBIL) and ALP upper limit of normal		
2a: Changes after drug discontinuation					
Highly suggestive	Decrease > 50% within 8 days		Not applicable		+3
Suggestive of being related	Decrease ≥ 50% within 30 days		Decrease ≥ 50% within 180 days		+2
No conclusion	Not applicable after 30 days		Decrease < 50% within 180 days		+1
Contrary to drug effect	No relevant data or decrease ≥ 50% after another 30 days		No change, increase, or no information		0
2b: If the drug is still being used					
	Decrease < 50% or re-increase after 30 days		Not applicable		–2
No conclusion	All of the above		All of the above		0
3. Risk factors	Alcohol		Alcohol or pregnancy		
Yes					+1
No					0
Age ≥ 55 years					+1
Age < 55 years					0
4. Concomitant medications					
None, relevant information is absent, or concomitant medications are inconsistent with the time of disease onset					0
Concomitant medications are appropriate to the time of disease onset, or suggestive					–1
Concomitant medications have known hepatotoxicity, and are consistent with the time of disease onset, or suggestive					–2
Evidence of liver injury with concomitant medications (re-challenge reaction or valuable testing)					–3
5. Exclusion of other reasons					
(1) Recent HAV infection (anti-HAV-IgM), HBV infection (anti-HBc-IgM), or HCV infection (anti-HCV and HCV RNA); biliary obstruction (B-ultrasonography); alcoholism (AST/ALT ≥ 2), recent history of acute hypotension (especially underlying cardiac disease)			Exclude all reasons in (1) and (2)		+2
			Exclude all reasons in (1)		+1
			Exclude 4–5 reasons in (1)		0
			Exclude less than 4 reasons in (1)		–2
			High suspicion of non-drug reasons		–3
(2) Important disease complications; clinical and/or biological indicators suggest CMV, EBV, or herpesvirus infection					
6. Reports of previous drug-induced liver injury					
Drug-induced liver injury is labeled in the Instructions for Use					+2
Previously reported but not labeled in the Instructions for Use					+1
Not reported					0
7. Re-challenge reactions					
Positive	ALT increased to ≥ 2 ULN after re-administration of the drug alone		Other conditions, ALP (or TBIL) increased to ≥ 2 ULN after re-administration of the drug alone		+3
Suspected	ALT increased to ≥ 2 ULN after re-administration of the same drugs		ALP or TBIL increased to ≥ 2 ULN after re-administration of the same drugs		+1
Negative	ALT increased after re-administration with the same drug		ALP (or TBIL) still within normal range after re-administration with the same drug		–2
Not done or not judged	Still within normal range		Other conditions		0

Final judgment: > 8, highly probable; 6-8, probable; 3-5, possible; 1-2, impossible; ≤ 0, unrelated

should also be performed according to the degree of thrombocytopenia.

(3) Follow the instructions, clinical guidelines, and expert consensus for rational drug use. Strictly follow the indications for medication, avoid the use of non-standard, inappropriate, and unusual prescriptions, and avoid drug

abuse.

(4) Strengthen the remote health management. Realizing remote care and management during treatment intermissions can improve the accessibility of medical support to patients.

(5) Perform multidisciplinary and dynamic management of patients with cancer and liver injury as recommended owing to their pathophysiological characteristics and the complexity of anticancer therapy.

(6) Strengthen the risk awareness management of patients, inform patients of the risk of thrombocytopenia and possible symptoms, avoid excessive exertion and trauma during thrombocytopenia, monitor platelet count, pay attention to symptoms such as skin ecchymosis, mucocutaneous bleeding, gingival bleeding, and gastrointestinal and urinary tract bleeding, and urge patients to remain alert to thrombocytopenia.

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REVIEW ARTICLE

Advances in pharmacotherapies in cancer-related cachexia*

Ze Ouyang, Weili Tao, Shiying Yu (✉), Man Zou (✉)

Department of Oncology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Abstract

Cancer-related cachexia is highly prevalent in patients with advanced cancer, affecting approximately 50%–80% of patients and seriously interfering with active therapy, quality of life, and survival time. There are currently no effective treatments for cachexia. Therefore, new therapeutic strategies are required. In recent years, advances in understanding the mechanisms underlying cachexia have been made, and new drugs have been developed to combat cachexia muscle wasting and weight loss due to cancer. In this systematic review, we discuss these novel targets and drug treatments.

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Cancer cachexia is a multifactorial and irreversible syndrome often associated with dysfunction, such as decreased appetite, loss of body mass, muscle wasting, and deterioration of nutritional status. It is characterized by loss of skeletal and visceral muscle mass, with or without body fat loss, which cannot be entirely reversed by conventional nutritional support [1]. The pathogenesis of cancer-related cachexia is complex, involving endocrine metabolic disorders caused by tumors, muscle wasting, abnormal energy metabolism mediated by cytokines, and anorexia regulated by the hypothalamus [2–3]. Approximately 50%–80% of patients with tumor progression suffer from cachexia, accounting for up to 20% of patients dying from cachexia instead of cancer [4]. Diagnosis, staging, and treatment of cachexia are challenging.

An international consensus on the criteria for cachexia diagnosis and staging was proposed in 2011, which divided patients with cachexia into precachexia, cachexia, and refractory cachexia [1]. American Society of Clinical Oncology (ASCO) provided evidence-based guidance for adult patients with cancer cachexia in 2020. Progesterone analogs and corticosteroids have been recognized clinically as short-term appetite enhancers. Dietary counseling allows patients and caregivers to obtain more scientific guidance on managing cachexia [5]. Personalized exercise plus nutritional support or multimodal intervention have been demonstrated to be feasible in clinical trials; however, no study has monitored these interventions for symptom improvement or survival benefits [6–7]. Currently, no effective therapeutic strategy can improve the outcomes of cancer cachexia. In the future, pharmacological interventions will continue to be the main target of cachexia treatment. In recent years, anamorelin, TCMCB07, and GDF15-targeting drugs have been demonstrated to prevent weight loss and alleviate skeletal muscle wasting *in vivo* and *in vitro*. In this review, we summarize the progress in the development of drugs for cachexia treatment.

ASCO-recommended pharmacological intervention

Megestrol acetate/medroxyprogesterone

Megestrol acetate (MA) was initially used for contraception and palliative care of advanced breast and endometrial cancers. Owing to its weight-gaining effects, MA is used for cachexia treatment. In 1993, MA was approved for the treatment of cachexia in many European and American countries [8]. The mechanism by which MA stimulates appetite and food intake is unknown and may be related to the concentration of neuropeptide Y, a central

✉ Correspondence to: Shiying Yu. Email: syyu@tjh.tjmu.edu.cn; Man Zou. Email: skyfountain@163.com
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appetite stimulant located in the hypothalamus [9]. The ASCO recommended dosage of MA for cancer cachexia is 200–600 mg/day, with adverse effects, including edema, thromboembolism, and adrenal insufficiency.

It is unclear whether MA, alone or in combination with other drugs, has a therapeutic effect [5]. In 2018, Ruiz-García *et al* [10] found that oral MA resulted in weight gain but did not improve the patient's quality of life. A recent meta-analysis by Lim *et al* [11] highlighted the therapeutic effect of MA in cancer cachexia and showed that MA was generally well tolerated but did not improve the patient's weight or quality of life; the overall pooled mean change in weight gain was 0.75 kg, and patients using doses above 320 mg/day suffered weight loss, which may be associated with severe cachexia symptoms. These results do not support the use of MA in improving anorexia/cachexia symptoms [11]. The efficacy and optimal dose of MA for cachexia are controversial and need to be further observed in more extensive studies with long follow-up periods.

Corticosteroids

Corticosteroids can remarkably improve cancer-related fatigue and can be used as short-term appetite enhancers; however, they do not affect weight gain in patients. Appetite regulation mechanisms are associated with the expression of genes encoding hypothalamic neuropeptide Y, corticotropin-releasing hormone, and agouti-related peptide [12]. ASCO does not recommend a particular corticosteroid type but only recommends a 3–4 mg dexamethasone equivalent dose/day for cancer cachexia. The European Society for Medical Oncology (ESMO) clinical guidelines state that corticosteroids should not be used for more than 2–3 weeks and that their prolonged use has no increased clinical benefit [5, 13–14]. Long-term or high-dose use of corticosteroids can cause side effects, such as insulin resistance, muscle wasting, osteoporosis, and high blood sugar levels [15–16].

The Chinese Society of Clinical Oncology-recommended pharmacological intervention

Long-chain omega-3 polyunsaturated fatty acids (ω-3 LC-PUFAs), such as eicosapentaenoic and docosapentaenoic acids, are effective in maintaining body weight and lean body mass, improving appetite, and sensitizing chemotherapy and are recommended by the Chinese Society of Clinical Oncology for patients with cancer cachexia [17]. A recent case-control study conducted by Abe *et al* [18] examined the effects of ω-3 LC-PUFAs in patients with unresectable or recurrent biliary tract cancer or pancreatic cancer undergoing chemotherapy. The study enrolled 39 patients, and enteric nutritional supplements containing ω-3 LC-PUFAs were administered for 56 days. The results showed that patients had an increased skeletal muscle mass compared to the baseline (median 17.3 kg *vs.* 14.8 kg, $P < 0.01$) and increased chemotherapy tolerance [18]. This is consistent with the conclusions of some previous studies [19–20]. However, other clinical trials have shown that ω-3 LC-PUFAs do not provide any benefits [21–22]. The Food and Drug Administration (FDA)-mandated maximum daily intake dose of ω-3 LC-PUFAs is 5 g [23]. In these clinical trials, the daily intake of ω-3 LC-PUFAs and number of treatment days were heterogeneous. How omega-3 PUFAs exert their pharmacological effects in patients with cachexia deserves further exploration.

Prospective therapeutic drugs

Appetite-stimulating drugs

Anorexia is commonly observed in patients with cancer who have cachexia. If left untreated, cancer-related anorexia (CA) eventually develops into anorexia-cachexia syndrome (CACS). The occurrence of CACS is closely related to energy intake and regulation. Contributing factors to CACS involve inflammatory factors, ghrelin, leptin, and melanocortin, which enable the brain to receive early satiety and anorexia information and, at the same time, change the normal energy metabolism in the body [24–25]. The treatment of cancer cachexia is mainly based on these appetite-regulating targets.

Anamorelin

Anamorelin is an orally active, highly selective ghrelin receptor agonist that increases lean body mass by modulating growth hormone and insulin-like growth factor 1 [26]. In 2016, Temel *et al* [27] published the results of two phase III multicenter randomized clinical trials, ROMANA1 and ROMANA2, which included a total of 979 patients with stage III or IV non-small cell lung cancer cachexia. After 12 weeks of administration, an increase in median lean body mass was observed in the 100 mg/day anamorelin group compared with that in the placebo group (ROMANA1: anamorelin 0.9 kg versus placebo –0.47 kg, $P < 0.0001$; ROMANA2: anamorelin 0.65 kg versus placebo –0.98 kg, $P < 0.0001$). After the completion of ROMANA1 and ROMANA2, 513 patients entered ROMANA3, a 12-week anamorelin phase III safety extension study. In total, 221 patients completed 24 weeks of anamorelin treatment (100 mg/day). Compared to those in the placebo group, patients in the treatment group experienced increased significantly weight from the baseline over the entire 0–24-week period, and their anorexia-cachexia symptoms improved; however, there was no significant change in handgrip strength. Anamorelin has demonstrated good tolerability, safety, and effective therapeutic effects with long-term

administration [28].

Increased lean body mass and improvement in cachexia symptoms were observed in a double-blind phase II clinical trial in patients with advanced non-small cell lung cancer and cachexia and an open-label, single-arm open trial in patients with advanced gastrointestinal tumors with cachexia [29–30].

Anamorelin also showed a significant increase in dose-related hunger scores and caloric intake in healthy male subjects [31]. Anamorelin was approved for the first time in Japan on December 11, 2020, and is indicated for patients with non-small cell lung cancer, gastric cancer, pancreatic cancer, and colon cancer cachexia. It is currently the only cancer cachexia treatment drug approved for marketing [32]. At present, the Clinical Pharmacology Research Center of Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, is conducting a phase I clinical trial to evaluate the safety, pharmacokinetics, and pharmacodynamics of anamorelin hydrochloride (CTR20171102). Anamorelin is ineffective in improving handgrip strength, walking ability, and other active functions, suggesting that cachexia cannot be entirely treated with a single drug [33]. This evidence indicated that anamorelin is currently the most promising drug for cancer cachexia treatment.

Ruxolitinib

Ruxolitinib, the first Janus kinase 1 and 2 inhibitor approved by the FDA in 2011, is used to treat patients with intermediate- or high-risk myelofibrosis [34]. In the COMFORT-I trial for myelofibrosis, a significant weight gain was observed in the ruxolitinib-treated group. Weight gain was not solely due to the therapeutic effect of the drug on myelofibrosis-linked symptoms. Ruxolitinib significantly increases body weight by blocking the production of leptin, an early satiety feedback signal in the Janus kinase 2/signal transducer and activator of tranions 3 (JAK2/STAT3) pathway. [35–36]. A phase I clinical trial (NCT04906746) exploring the dose-limiting toxicity of ruxolitinib in non-small cell lung cancer with cachexia is ongoing.

TCMCB07

TCMCB07, a melanocortin-4 receptor antagonist, reduces metabolism and energy expenditure by inhibiting hypothalamic melanocortin signaling and brain inflammatory factors. TCMCB07 significantly increased the body weight and fat mass of mice with cachexia and effectively crossed the blood-brain barrier when administered peripherally [37]. TCMCB07 exhibited not only good safety and tolerability at both high and low doses but also showed concomitant dose-related weight gain in a trial using dogs [38]. A phase I clinical trial (NCT04906746) to explore the safety, tolerability, and pharmacokinetics of TCMCB07 in healthy individuals is ongoing.

Inhibition of muscle wasting pathways

Since the discovery that blocking the ActRII receptor downstream of transforming growth factor-β (TGF-β) superfamily signaling pathways can alleviate muscle wasting and prolong the survival time of mice with cachexia, the biological role of the TGF-β superfamily in skeletal muscle has attracted extensive attention [39]. Activin, myostatin, growth and differentiation factors (GDFs), follistatin (FST), bone morphogenetic proteins, and STAT and Smad protein families are all components of the TGF-β superfamily and play a vital role in muscle wasting. Activin, myostatin, and FST promote muscle wasting by interacting with the ActRII receptors [40–41]. GDF15, also known as macrophage inhibitory cytokine 1, is the key factor regulating cachexia symptoms via glial-derived neurotrophic factor receptor α-like (GFRAL). Elevated GDF15 serum levels are associated with anorexia, muscle wasting, weight loss, regulation of body energy balance, and poor prognosis, indicating it might be a promising therapeutic target for cachexia [42–43].

Ponsegromab

Ponsegromab (PF-06946860) is a selective humanized anti-GDF15 monoclonal antibody that blocks GDF15/GFRAL-mediated signaling. A randomized, double-blind, phase I clinical trial of PF-06946860 in patients with cancer cachexia was completed on August 9, 2022; it explored the improvement in cachexia symptoms and appetite scores (NCT05546476) [44]. Two clinical trials comparing ponsegromab to placebo in patients with cancer cachexia are ongoing: one to evaluate its efficacy, safety, and tolerability (NCT05546476), and the other to assess its effect on appetite (NCT04803305).

3P10

3P10, an anti-mouse monoclonal antibody derived from a library of hybridoma clones generated from mice immunized with the GFRAL extracellular domain, can bind to GFRAL and prevent the GDF15-driven interaction between Ret proto-oncogene (RET) and GFRAL on the cell surface. 3P10 reversed skeletal muscle wasting in mice with cachexia by blocking GDF15/GFRAL/RET signaling, the mechanism of which is likely related to the inhibition of lipid mobilization and oxidation pathway. Surprisingly, researchers found that 3P10 also improved motor function and increased forelimb grip strength; the expression of muscle atrophy genes, including *Bnip3*, *Fbx032*, and *Gadd45a*, was completely prevented in mice with cachexia treated with 3P10 [45]. The weight regulatory mechanism of the GDF15/GFRAL/RET signaling pathway may be related to the sympathetic nervous system, independent of leptin, ghrelin, and other food intake-related factors, and weight increase does not depend on increasing caloric intake. GDF15 not only regulates body weight but also has anti-inflammatory effects, the mechanism of which has not been fully

elucidated. GDF15 has shown remarkable effects in the treatment of obesity, diabetes, and cancer cachexia [46].

Tilorone

Tilorone, a broad-spectrum antiviral drug with a history of more than 50 years, induces interferon production *in vivo* after oral administration. Tilorone and its analogs have anticancer activity [47–48]. Sartori *et al* [49] found that the BMP signaling pathway is one of the mechanisms regulating muscle wasting. In cachexia-related mouse models, elevated interleukin 6 (IL-6) activates STAT3, and the activated IL-6/STAT3 signaling pathway promotes the transcription of Noggin, a BMP signaling pathway inhibitor in mouse muscle tissue. High Noggin expression inhibits the BMP-Smad1/5/8 signaling pathway, resulting in abnormal neuromuscular junctions, which can promote tumor-related muscle wasting and dysfunction. By enhancing the activity of the BMP signaling pathway, tilorone can significantly improve muscle mass, improve motor neuron transmission dysfunction, and reduce the expression of key muscle atrophy-related genes, including *Fbxo32*, *Trim63*, and *Fbxo30*. Moreover, tilorone has no effect on tumor growth in mice with cachexia; however, it can prolong their survival by approximately 58% and is expected to be developed as a cachexia treatment drug.

mRNA therapy

Since 2020, mRNA therapeutics have undergone rapid development in the field of vaccines for infectious diseases, and mRNA therapeutics have been developed for cancer treatment [50]. Recently, Korzun *et al* [41] first reported a method for treating metastatic ovarian cancer and cachexia-induced muscle wasting using FST messenger RNA (mRNA) delivered by lipid nanoparticles (LNPs). Intraperitoneal injection of LNP-containing FST mRNA into metastatic ovarian cancer model mice triggered FST production in cancer clusters. FST is a highly targeted and regulated member of the TGF-β superfamily. FST binds to ActA with high affinity and irreversibility, thereby inhibiting the binding of ActA to ActRIIB receptors and reversing muscle wasting. FST mRNA LNP therapy has good tolerability and safety and avoids immunogenicity and dose-related toxicity that may be caused by recombinant protein supplementation. Both mRNA therapeutics and vaccines are promising cancer treatments [51].

Inflammatory factor-inhibiting drugs

A recent meta-analysis showed that inflammatory cytokines, including IL-6, IL-8, and TNF-α, were significantly elevated in the serum of patients with cancer cachexia and that elevated levels of IL-6 and TNF-α were associated with weight loss [52]. MABp1 is a human-derived monoclonal antibody that neutralizes IL-1α. Hickish *et al* [53] randomized patients with advanced colorectal cancer to receive MABp1 or a placebo treatment at a ratio of 2:1. In this phase III clinical trial, a composite endpoint was innovatively designed as the primary endpoint: patients with stable or increased lean body mass and stability or improvement in two of the three symptoms, including pain, fatigue, and anorexia. When administered for eight weeks, patients who met the primary endpoint in the MABp1 group had better clinical treatment response rates and longer median survival times than those in the placebo group.

In 2010, clazakizumab (ALD518), a humanized IL-6 antibody, was found to improve anemia symptoms in patients with stage II non-small cell lung cancer cachexia. Nonetheless, no follow-up phase III clinical trials have been conducted [54].

TNF-α-related drugs, including pentoxifylline, etanercept, infliximab, and melatonin, have not shown therapeutic efficacy in the treatment of cachexia [55]; therefore, existing targeted drugs against TNF-α and IL-6 have limited benefits in cachexia treatment. However, many new drugs that inhibit the secretion of TNF-α and IL-6 from macrophages and tumor cells are being developed, and clinical trials are ongoing.

Other therapeutic drugs

The following targets and drugs have been observed to significantly alleviate cancer cachexia muscle wasting in animal experiments: Calore *et al* [56] found that IMO-8503 can inhibit cancer-induced cachexia by antagonizing Toll-like receptor 7/8/9 (TLR7/8/9). Chiappalupi *et al* [57] targeted receptor for advanced glycation end-products (RAGE) prevents muscle wasting and prolongs survival in cancer cachexia, which was expressed only in cachexia atrophic myofibers. Murphy *et al* [58] demonstrated that mitochondrial assembly receptor (MasR) agonist AVE 0991 can alleviate weight loss and muscle wasting in cancer cachexia mice via substituting the angiotensin-coverting enzyme 2/angiotensin-(1-7)/MasR [ACE2/Ang-(1-7)/MasR] axis activated by AVE 0991 for the ACE/Angiotensin II/angiotensin type 1 (ACE/Ang II/AT1) axis.

Androgens and antidepressants have been tested in clinical trials to treat cachexia. Izumi *et al* [59] performed a prospective, randomized, non-placebo-controlled trial (the ARTFORM study) in male patients with advanced cancer and low serum testosterone levels. Unfortunately, androgen replacement therapy did not improve the quality of life [59]. Mirtazapine is a controversial drug that improves appetite and frequently causes adverse effects, such as drowsiness and hallucinations, during routine use. Hunter *et al* [60] recently studied 120 patients with incurable solid tumors with cachexia and anorexia who were randomly allocated to receive mirtazapine 15 mg/day at night *vs.* placebo for four weeks. The primary endpoint of appetite and secondary endpoints, including quality

Table 1 Clinical trials currently underway to treat cancer cachexia

Status	Start date	Study design	Estimated enrolment	Enrolled patients	Phase	Clinical trial identifier
Not yet recruiting	9-Sep-2022	1. Ponsegromab 2. Placebo	168	Patients with cachexia and elevated GDF15 levels in NSCLC, PAAD, and CRC	Phase II	NCT05546476
Recruiting	25-Dec-2021	1. Olanzapine 2. Placebo	164	Incurable solid cancer patients with anorexia and cachexia	Phase III	NCT05243251
Recruiting	1-Jun-2022	1. Mirtazapine 2. Megestrol acetate	80	Patients with advanced tumours and anorexia-cachexia	Phase II	NCT05380479
Recruiting	17-Dec-2020	Pancrelipase	30	Borderline resectable, locally advanced, and advanced PAAD patients with cachexia and exocrine pancreatic insufficiency	Phase II	NCT04098237

of life, fatigue, depressive symptoms, body weight, lean body mass, handgrip strength, and inflammatory markers, were not significantly different between the mirtazapine and placebo groups. These results do not support the use of mirtazapine tablets as appetite stimulants in patients with non-depressive cancer-related cachexia or anorexia [60]. National Comprehensive Cancer Network (NCCN) palliative care clinical guidelines recommend oral mirtazapine of 7.5–30.0 mg/day for patients with depression or anorexia when the life expectancy is several days to weeks, whereas, for patients with a life expectancy of months to years, oral olanzapine of 5.0 mg/day is recommended [61]. We summarize currently underway clinical trials of pharmacological treatments for cancer cachexia in Table 1.

Conclusion

Many studies have shown that the mechanism of cancer-related cachexia is complex and requires further research. Current research on drugs for cachexia treatment mainly focuses on blocking the muscle protein degradation pathway and stimulating an increase in appetite in patients. Multimodal combination therapies, including appetite-enhancing drugs, exercise interventions, and dietary supplements, have been used in many clinical trials. However, no ideal benefit has been observed. In addition, there is no uniform standard for assessing the therapeutic effects of clinical trial interventions, which makes it difficult to compare results across trials. Although cachexia muscle wasting cannot be completely alleviated, improving patients' appetite, weight, and muscle mass can undoubtedly buy time to perform the antitumor treatment. Developing new drugs or exploring multidimensional combinations to improve treatment tolerance and quality of life is the main direction of cachexia treatment.

In addition to the early detection of precachexia and early intervention, clinicians should also pay attention to the mental health status of patients, as depression and anxiety can also aggravate anorexia and weight loss.

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Conflicts of interest

The authors declared no conflict of interest.

Author contributions

Ze Ouyang: Conceptualization, writing, and original draft preparation. Weili Tao: Writing, reviewing, and editing. Corresponding authors: Supervision and funding acquisition. All the authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

The application study of dual-energy CT nonlinear blending technique in pulmonary angiography*

Siqi Yi, Peng Zhou, Yakun He, Changjiu He, Shibei Hu (✉)

Department of Radiology, Sichuan Cancer Hospital & Institute, Sichuan Cancer Center, University of Electronic Science and Technology of China, Chengdu 610041, China

Abstract

Objective This study aimed to explore the feasibility of enhancing image quality in computed tomography (CT) pulmonary angiography (CTPA) and reducing radiation dose using the nonlinear blending (NLB) technique of dual-energy CT.

Methods A total of 61 patients scheduled for CTPA were enrolled, and 30 patients underwent dual-energy scanning. Nonlinear blending images (NLB group) and three groups of linear blending images (LB group, 80 kV group, and 140 kV group) were reconstructed after scanning; 31 patients underwent single-energy scanning (120 kV group). The CT values and standard deviations of the pulmonary trunk, left and right pulmonary arteries, and ipsilateral back muscle at the bifurcation level of the left and right pulmonary arteries were measured. The signal-to-noise ratio (SNR) and contrast-to-noise ratio (CNR) of the five groups were calculated. The subjective image quality of the five groups was assessed. The radiation doses of the dual- and single-energy groups were recorded and calculated.

Results The CNR and SNR values of blood vessels in the NLB group were significantly higher than those in the LB, 140 kV, and 80 kV groups (CNR of pulmonary artery trunk: $t = 3.50, 4.06, 7.17$, all $P < 0.05$; SNR of pulmonary trunk: $t = 3.76, 4.71, 6.92$, all $P < 0.05$). There were no statistical differences in the CNR and SNR values between the NLB group and 120 kV group ($P > 0.05$). The effective radiation dose of the dual-energy group was lower than that of the single-energy group ($t = –4.52$, $P < 0.05$). The subjective scores of images in the NLB group were the highest (4.28 ± 0.74).

Conclusion The NLB technique of dual-energy CT can improve the image quality of CTPA and reduce the radiation dose, providing more reliable imaging data for the clinical diagnosis of pulmonary embolism.

Key words: dual-energy computed tomography (CT); CT pulmonary angiography (CTPA); non-linear blending (NLB); image quality; radiation dose

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Computed tomography pulmonary angiography (CTPA) is an accurate and reliable noninvasive imaging method for the diagnosis of pulmonary embolism (PE) [1]. In this study, an intelligent tracking method of segmented injection of contrast agent is adopted, which can not only avoid the poor image quality caused by the difference in individual cycles when using the empirical value method but also reduce the radiation dose and contrast agent dosage by the low-dose test method. The image quality can also be improved using reconstruction technology [2].

The non-linear blending (NLB) technique of dual-energy computed tomography (CT) can improve image quality by changing the calculation method of the CT value, preserving the enhancement degree of blood vessels, and reducing soft tissue noise [3]. The NLB technique has been used to improve the image quality of cranial vessels [4–5], abdominal vessels [6], and pulmonary nodules [7]. However, there are few studies on the application of this technique to pulmonary arteries. Patients with PE often require multiple examinations with short intervals, and the quality of CTPA images is easily affected by circulatory differences in contrast agents in different individuals. Therefore, the study of NLB technology is of great clinical significance in improving the CTPA image quality and reducing the radiation dose.

✉ Correspondence to: Shibei Hu. Email: h13438823608@163.com
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Materials and methods

Clinical data

We enrolled 65 patients who were suspected of having PE or lung aspiration system-related diseases and underwent CTPA examination with dual-energy CT in our hospital (Sichuan Tumor Hospital, Chengdu, China) from July 2020 to March 2021. All patients signed an informed consent form before participating. Patients were considered ineligible for the study if they had a history of an iodine allergy reaction, cardiac or renal failure (glomerular filtration rate < 30 mL/min), untreated hyperthyroidism, or are pregnant or lactating. Four patients were excluded after examination because of inability to hold their breath or have poor breath holding. Finally, 61 cases were included in the study and randomly divided into experimental and control groups. Dual-energy scanning was performed in the experimental group, including 14 males and 16 females [mean age (51.7 ± 10.2) years old, 31–72 years old; mean body mass index (BMI) (23.0 ± 2.7) kg/m^2, range 19.10–27.34 kg/m^2]. There were 19 males and 11 females in the control group (mean age (55.5 ± 7.4) years, 43–75 years old; mean BMI (23.1 ± 2.9) kg/m^2, range 18.11–26.67 kg/m^2).

Instruments and methods

CT scanning protocol

All CT scans were acquired using a second-generation dual-energy CT device (Somatom Definition Flash, Siemens Healthcare, Forchheim, Germany), using automatic tube current; a pitch of 1.2; 128 × 0.66-mm collimator width; 0.28-msec tube rotation time; and 350 mm × 350 mm visual field of view (FOV). The voltage of the single-energy tube was 120 kV and the dual-energy was 80/Sn140 kVp.

All patients were positioned supine, with their feet on the scanning table. After acquiring the scout radiograph, the monitoring layer was placed in the right ventricle. A scan was triggered automatically when the CT threshold reached 50 HU after a 5-msec fixed delay with the contrast agent intelligent tracking technology and real-time dynamic exposure dose adjustment CARE Dose 4D technology. Each research was obtained after the participant was instructed to hold his or her breath following inhalation.

Injection scheme

CT images were taken after a 25-mL non-ionic contrast agent (370 mg/mL iodine) was administered through the right antecubital venous catheter at a flow rate of 5 mL/s or 4 mL/s. The injection rate was based on the patency of the patient's blood vessels using a dual-syringe injector. First, 20-mL saline was injected, followed by a combination of 30% contrast agent and 70% saline in 20-mL volume, and finally 30 mL of saline flushing [8–9].

Image postprocessing and grouping

All image data were transmitted to the image workstation of the CT equipment (Syngo Via VB20, Siemens Healthcare) for postprocessing. After the experimental group acquired the dual-energy mode, four different series of images were reconstructed: 80 kV images, 140 kV images, LB images, and NLB images. NLB images were obtained by nonlinear blending with the equipment's own postprocessing Optimum Contrast software. The control group used single-energy scanning mode to obtain and reconstruct the 120-kV images.

When LB images are reconstructed with a default linear blending ratio of 0.6, the low-kVp information is multiplied by 0.6 and added to the high-kVp component, which is multiplied by 0.4. Unlike the LB technology, which uses a constant weighted contribution, the NLB algorithm establishes a smooth transition area that gradually increases the weighted contribution of the low-kVp image from 60% to 100% as a function of the CT number. The blending width (BW) is defined as the extent of the transition zone for image blending, and the blending center (BC) is defined as the center of the BW relative to the full CT number scale. In this study, BW was set at 200 and BC was at 150 [10].

After each scan, the dose-length product (DLP) and volume CT dose index ($CTDI_{vol}$) were recorded according to the patient protocol. The individual effective radiation dose (ED) was calculated using DLP values and a suitable standard conversion factor. ED = K × DLP (K = 0.059).

Image quality analysis

Qualitative analysis

After all images were processed, the subjective image quality was evaluated by two radiologists with more than 5 years of working experience. The experimental group images (NLB, LB, 80 kV, and 140 kV) and the control group images (120 kV) were displayed side-by-side, and the readers were blinded to the reconstruction methods. The 5-point scale method was adopted, and the specific scoring criteria are listed in Table 1 [11]. The readers were allowed to change their windows or level settings according to their personal preferences.

Quantitative analysis

Image contrast was evaluated using region of interest (ROI) measurements to calculate the contrast-to-noise ratio (CNR) and signal-to-noise ratio (SNR) values. Four ROIs were placed in the left, right, and trunk of pulmonary arteries, as well as the ipsilateral dorsal muscles, to measure CT values and standard deviation (SD) at the bifurcation level of left and right pulmonary arteries. The SD values of the ipsilateral muscles were used to represent background noise. [11]. SNR and CNR of the three consecutive layers were calculated as follows: $SNR = HU_{vessel} / StdDev_{muscle}$, $CNR = (HU_{vessel} - HU_{muscle}) /$

Table 1 The subjective evaluation criterion for image quality

Scores	The evaluation criterion
5	The pulmonary artery and its branches are shown clearly, and there is no obvious background noise.
4	The pulmonary aorta and its branches are shown clearly, and the background noise is slight.
3	The pulmonary aorta and its branches are shown clearly, and the background noise is obvious.
2	The pulmonary artery and its branches are shown clearly, and there is too much background noise.
1	The CT value at the beginning of pulmonary aorta is less than 150 HU, or the vascular branches are blurred and the background noise is large.

$StdDev_{muscle}$.

Furthermore, the ROI should be paced in the position of the distal 1 cm at the bifurcation of the blood vessels to avoid areas with severe calcification or vascular stenosis and should be kept consistent in size, shape, and location using a copy-and-paste function [12].

$CTDI_{vol}$, DLP, and ED values were compared between the experimental and control groups. The percentage dose reduction between these protocols was also calculated.

Statistical analysis

All statistical analyses were performed using SPSS 25.0 software. Results were presented as mean ± SD ($\bar{\chi} \pm s$) for parametric data. After logarithmic transformation, the CNR and SNR values of each group of images accorded with normal distribution and the variance was uniform. The values of ln(CNR) and ln(SNR) of five groups were analyzed using single-factor analysis of variance. If the difference was statistically significant, the least significant difference method was used to compare the mean of the samples. Comparison of ED, $CTDI_{vol}$, and DLP values between the dual-energy scan and single-energy scan was performed using independent-sample *t*-test. The Mann-Whitney *U* test was used for the subjective score of dual-energy and single-energy scanning. Inter-observer agreement of subjective image analysis was assessed using Cohen's kappa (values of 0–0.20, 0.21–0.40, 0.41–0.60, 0.61–0.80, and 0.81–1.00 represent slight, fair, moderate, substantial, and almost perfect agreement, respectively). A *P*-value of less than 0.05 was considered statistically significant.

Results

Image quality

Subjective image quality

Substantial agreement was found between the two readers for subjective image quality scores (kappa = 0.79). The NLB group had the highest score (mean point: 4.28 ± 0.74, range: 3–5, H = 215.724, $P < 0.05$). The scores of the five group images are shown in Table 2, and Fig. 1 depicts the pulmonary artery images of the two patients. As shown in Fig. 2, the CNR and SNR values of the NLB group at the pulmonary trunk were the highest in all images (10.69 and 12.00, respectively). The image contrast at the embolus was 296 HU in the 80-kV group, followed by the NLB group, 120-kV group, LB group, and 140-kV group, while the background noise in the 80-kV

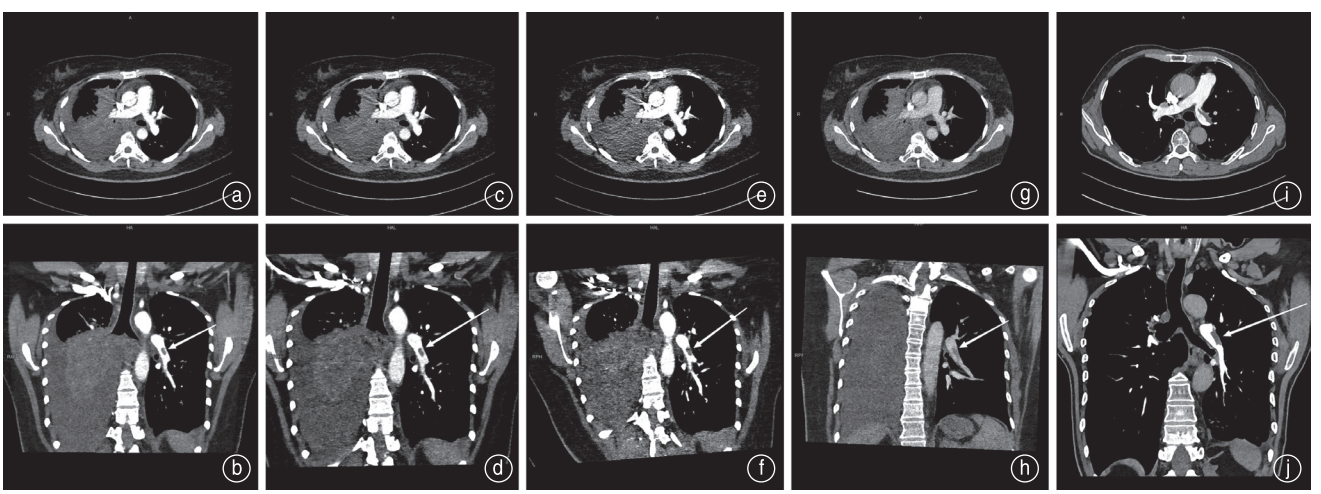

Fig. 1 (a–h) A 43-year-old woman with pulmonary embolism of the left lung artery segment and massive pleural effusion in right lung underwent dual-energy scanning with 80/Sn140 kVp. (i–j) A 63-year-old man with PE of the left lung artery trunk segment was scheduled to undergo single-energy scanning with 120 kV. NLB images are (a) and (b); LB images are (c) and (d); 80-kV images are (e) and (f); 140 kV images are (g) and (h); and 120kV images are (i) and (j). Pulmonary embolisms were demonstrated by the arrow in the images

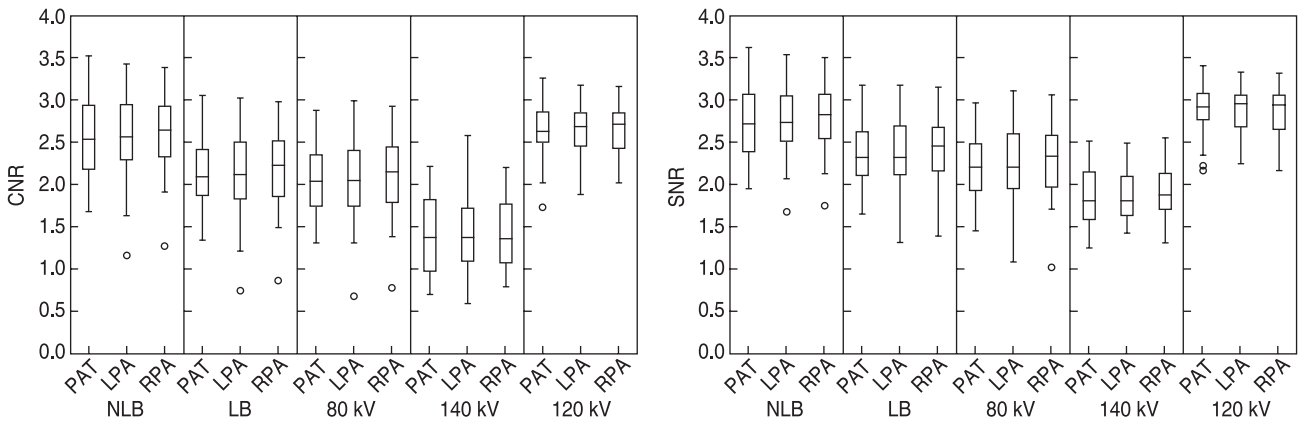

Fig. 2 Box-and-whisker plots show the median, interquartile spacing, and discrete values of CNR and SNR at the bifurcation level of the pulmonary artery at the pulmonary artery trunk (PAT), left pulmonary artery trunk (LPA), and right pulmonary artery trunk (RPA) in five sets of images

Table 2 Comparisons of ln(CNR), ln(SNR) and subjective image qualities between five groups ($\bar{\chi} \pm s$)

Groups	Pulmonary trunk		Left pulmonary artery		Right pulmonary artery		The score of image quality
	ln(CNR)	ln(SNR)	ln(CNR)	ln(SNR)	ln(CNR)	ln(SNR)	
NLB	2.56 ± 0.50	2.74 ± 0.43	2.58 ± 0.51	2.78 ± 0.42	2.60 ± 0.48	2.79 ± 0.41	4.28 ± 0.74
LB	2.13 ± 0.43	2.36 ± 0.37	2.12 ± 0.50	2.36 ± 0.42	2.18 ± 0.46	2.41 ± 0.39	3.67 ± 0.82
80 kV	2.05 ± 0.42	2.22 ± 0.36	2.05 ± 0.50	2.23 ± 0.44	2.11 ± 0.47	2.27 ± 0.43	3.74 ± 0.50
140 kV	1.41 ± 0.48	1.87 ± 0.37	1.37 ± 0.57	1.87 ± 0.30	1.40 ± 0.43	1.88 ± 0.31	1.47 ± 0.50
120 kV	2.65 ± 0.35	2.89 ± 0.30	2.64 ± 0.34	2.87 ± 0.30	2.63 ± 0.33	2.86 ± 0.29	3.89 ± 0.75
F/H value	37.902	36.2338	32.600	34.860	38.919	34.796	215.724
P value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 3 Radiation dose evaluation index of dual-energy and single-energy scanning ($\bar{\chi} \pm s$)

	Dual-energy	Single-energy	*t*	*P*
ED (mSv)	1.16 ± 0.14	1.43 ± 0.29	–4.529	0.002
DLP (mGy/cm)	198.63 ± 23.78	243.71 ± 48.99	–4.484	0.002
$CTDI_{vol}$ (mGy)	5.15 ± 0.60	7.15 ± 1.62	–6.249	< 0.001

group was the highest, followed by the LB group, the 120-kV group, NLB group, and the 140-kV group. Finally, the NLB group displayed better blood vessels and emboli than the other groups.

Objective image quality

There were significant differences in ln(CNR) and ln(SNR) among the five groups ($P < 0.05$; Table 2 and Fig. 2). The values of CNR and SNR in the NLB group were both higher than those in the LB, 140-kV, and 80-kV groups [CNR of the NLB group *vs.* LB group: pulmonary artery trunk (PAT), left pulmonary artery trunk (LPA), and right pulmonary artery trunk (RPA), $t = 3.50$, 3.58, and 3.61, all $P < 0.05$; 140-kV group: PAT, LPA, and RPA, $t = 4.06$, 4.09, and 4.14, all $P < 0.05$; 80-kV group: PAT, LPA, and RPA, $t = 7.17$, 7.60, and 8.29, all $P < 0.05$. SNR of the NLB group *vs.* LB group: PAT, LPA, and RPA, $t = 3.76$, 3.68, and 3.74, all $P < 0.05$; 140-kV group: PAT, LPA, and RPA, $t = 4.71$, 4.70, and 4.82, all $P < 0.05$; 80-kV group: PAT, LPA, and RPA, $t = 6.92$, 7.74, and 8.07, all $P < 0.05$]. There were no significant differences in CNR and SNR between the NLB and 120-kV groups (CNR of the NLB group *vs.* LB group: PAT, LPA, and RPA, $t = –0.38$, 0.01, and 0.195, $P = 0.707$, 0.992, and 0.846, respectively). SNR of the NLB group *vs.* LB group: PAT, LPA, and RPA, $t = –1.003$, –0.612, and –0.395, $P = 0.320$, 0.543, and 0.694, respectively).

Radiation dosage

There were significant differences in the $CTDI_{vol}$, DLP, and ED between dual-energy and single-energy scanning ($CTDI_{vol}$: $t = –6.249$; DLP: $t = –4.484$; ED: $t = –4.529$; $P < 0.05$). Compared with the single-energy group, the values of $CTDI_{vol}$, DLP, and ED in the dual-energy group decreased by 30.0%, 18.5%, and 19.9%, respectively (Table 3).

Discussion

We evaluated the potential value of nonlinear blending postprocessing algorithm for improving the contrast resolution of CTPA images and reducing the radiation dose. A higher detection rate of PE was associated with

better SNR and CNR values in CT images. The results show that NLB technology can significantly improve the objective image quality of pulmonary artery CT images in dual-energy, and the image quality can be consistent with that of conventional single-energy scanning ($P > 0.05$). The subjective image quality score was the highest in the NLB group. Therefore, NLB technology can also be used to remedy the poor image quality of the pulmonary artery. In addition, the $CTDI_{vol}$, DLP, and ED values of the dual-energy group were lower than those of the single-energy group ($P < 0.05$), and there was a definite 19.9% decrease in the ED radiation dose in the dual-energy group. Compared with conventional 120-kV scanning, dual-energy NLB technology can reduce the radiation dose to ensure image quality.

Because CTPA has become the first choice for the diagnosis of PE, better and more reliable image quality is required. The pulmonary circulation time was very short (approximately 2–4 s). Early scanning of the distal pulmonary artery branch did not show adequate filling of the contrast agent, while the ray beam sclerosis artifact formed by the contrast agent in the superior vena cava and right ventricle will interfere with the display of emboli in the large pulmonary artery branch. When the scan is triggered too late, the contrast agent will fully fill the pulmonary vein, affecting the display of the segmental and subsegmental branches. Owing to these CTPA features, we used an intelligent tracking method to determine the most appropriate triggering time and found a suitable postprocessing method, NLB technology, to improve image quality.

Dual-energy CT collects high-kVp and low-kVp images in a single scan, with the low-kVp images increasing the CT value of the intravascular iodine contrast agent owing to the photoelectric effect and K-edge effect, which is useful for detecting emboli in pulmonary veins but has a high background noise. The noise of the high-kVp image is small, but due to the low intravascular CT value, the contrast between the blood vessel and the focus is poor, and the focus is easy to miss [3]. Dual-energy CT image blending technology is divided into LB and NLB. The LB fuses high and low energies according to a fixed linear ratio. NLB is integrated according to the best proportion, using low-kVp images to reconstruct pixels with high CT values and high-kVp images to reconstruct pixels with low CT values, which not only retains high iodine contrast, but also reduces noise in a set of fused images [3, 13]. Among the many NLB methods, the most commonly used method (Moidal) is an improved S-shaped function curve fusion technique, which includes two parameters: BC and BW. The setting of different fusion parameters affects the image fusion. The BC value should be lower than the CT value of the blood vessel and higher than the CT value of the soft tissue around the blood vessel [14].

Conventional scanning methods place the monitoring layer on the trunk of the pulmonary artery, ascending aorta, or descending aorta, and a 90-mL contrast agent is used. This study used a 25-mL contrast agent and placed the monitoring layer on the right ventricle at the pulmonary artery entrance. Whether this study can improve the success rate and repeatability of scanning and has the same diagnostic value is worthy of further study and discussion.

This study has some limitations. First, there were few experimental participants. Second, only the large pulmonary vessels were highlighted; however, clinically, most PEs are likely to occur in the twigs of the pulmonary arteries. Finally, diagnostic accuracy was not compared.

In summary, the NLB technique of dual-energy CT can improve the contrast of the pulmonary arteries, improve the image quality, and minimize the radiation dose, which is worthy of clinical application in practice.

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Conflicts of interest

The authors declare no conflict of interest.

Author contributions

All authors contributed to data acquisition and interpretation, and have reviewed and approved the final version of this manuscript.

Data availability statement

Not applicable.

Ethical approval

Not applicable.

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ORIGINAL ARTICLE

Analysis of the intestinal flora in patients with primary liver cancer*

Chengcong Liu[1], Guoxin Sun[2], Huizhe Wang[2], Gaishuang Shang[3], Xiong Yan[4], Xiao Zou[5] (✉)

[1] Department of Gastrointestinal Surgery, Qingdao Central Hospital, Qingdao 266000, China
[2] School of Clinical Medicine, Qingdao University, Qingdao 266071, China
[3] Donghai Pharmaceutical, Qingdao 266431, China
[4] Department of Pathology, Qingdao Central Hospital, Qingdao 266000, China
[5] Department of Breast Surgery, Qingdao Sixth People's Hospital, Qingdao 266000, China

Abstract

Objective To investigate the differences in intestinal flora of patients with primary liver cancer and of healthy individuals and to investigate the effect of the differential flora on the development of liver cancer.

Methods Overall, 67 patients with primary liver cancer who received systematic and complete treatment between January 2019 and December 2020 at the Sixth People's Hospital of Qingdao and had complete clinical data were enrolled in this study, and 26 individuals who were healthy on physical examination in the same period were used as healthy controls. Macro genome and 16s ribosome Deoxyribo Nucleic Acid (rDNA) high-throughput sequencing were performed on the stool flora of the enrolled patients and controls, and the differences in the intestinal flora were analyzed using the LEfSe bioinformatics software.

Results Compared with the control samples, all the tested patient samples showed statistically significant differences in the number of colonies of 5 bacterial phyla, 5 orders, 8 families, 11 genera, and 14 species ($P < 0.05$).

Conclusion Compared with healthy people, patients with primary liver cancer have significant differences in the intestinal flora composition. The alteration of the intestinal flora may be correlated with the occurrence of primary liver cancer, and the intestinal flora may become a novel target for the prevention and treatment of primary liver cancer.

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Key words: liver cancer; intestinal flora; genome sequencing; 16s rDNA

Introduction flora is abundant and diverse, and it has an indispensable role in human digestion, metabolism, defense, and immunity. The intestinal flora is involved in the development and progression of many diseases [1]. Inflammatory diseases (e.g., enteritis and pancreatitis), metabolic diseases (e.g., type 2 diabetes mellitus and obesity), and psychiatric diseases (e.g., depression and Alzheimer's disease) are closely related to changes in the intestinal flora [2–4]. Primary hepatocellular carcinoma (HCC) is one of the most common malignancies and poses a serious risk to human health. Evidence suggests that intestinal flora composition is strongly associated with primary HCC, and that the intestinal microbiota plays a key role in promoting the progression of liver disease and HCC development. At present, in the diagnosis and treatment of liver-related diseases, most clinicians do not know enough about the correlation between the intestinal flora and liver diseases and do not consider treating liver diseases by regulating intestinal flora dysbiosis; therefore, a systematic and detailed exploration of intestinal flora changes in liver cancer patients can help us find new ways to prevent and treat liver cancer, achieve early diagnosis, and target treatment to improve prognosis.

Materials and methods

General information

Overall, 26 individuals who were healthy on physical examination at the Sixth People's Hospital of Qingdao between January 2019 and December 2020 were recruited as healthy controls (Group A), and 77 patients with liver cancer who received systematic and complete treatment

✉ Correspondence to: Xiao Zou. Email: 84285161@163.com
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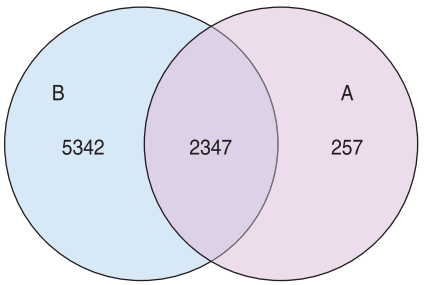

Fig. 1 Number of OTUs for both groups

Table 1 Differences in the phylum-level flora between the two groups

Group	A	B	*t*/*Z*	*P*
Bacteroidetes	50.52 ± 17.71	41.26 ± 14.36	2.75	< 0.01
Frimicutes	37.28 ± 17.49	43.87 ± 14.49	–1.85	0.07
Proteobacteria	5.80 (2.27, 11.96)	5.91 (3.50, 10.14)	–0.547	0.585
Actinobacteria	0.65 (0.41, 2.37)	1.59 (0.60, 3.20)	–1.549	0.121
Tenericutes	0.26 (0.05, 1.07)	0.14 (0.06, 0.39)	–1.253	0.210

during the same period (Group B) with complete clinical data were included in this study. The inclusion criteria for patients with liver cancer were as follows: disease diagnosis in accordance with the diagnostic criteria of the Diagnostic Code for Primary Liver Cancer (2017 version) [5]; normal function of vital organs such as the heart, brain, and kidney; no previous history of liver surgery; no liver transplantation; and no use of antibacterial or other drugs affecting the intestinal flora in the 3 months prior to enrollment. The inclusion criteria for the controls were as follows: no intestinal disease within the past 3 months and no use of antibiotics or other drugs that affect the intestinal flora within the past 3 months. The exclusion criteria for liver cancer patient group were as follows: autoimmune, drug-induced, or parasitic liver disease, human immunodeficiency virus infection, malnutrition, and a history of psychosis or psychiatric disorders. There was no statistical difference between the groups in terms of gender, age, disease duration, and other general information, and they were comparable. The study was ethically reviewed, and all patients included in this study were informed and agreed to participate voluntarily.

Sample collection and index testing

Stool samples (≥10 g) were collected from participants with sterile swabs, placed in sterile containers containing cache solution, and stored at -80°C within 1 h. The stool flora of both the patients and controls were subjected to 16s rDNA high-throughput and macro genome sequencing. Thereafter, the flora diversity and the flora differences were analyzed using LEfSe bioinformatics software.

Table 2 Differences in the class-level flora between the two groups

Group	A	B	*t*/*Z*	*P*
Bacteroidia	50.72 ± 17.36	41.19 ± 14.37	2.77	< 0.01
Bacilli	0.70 (0.32, 0.90)	1.87 (0.86, 3.22)	–4.21	< 0.01
Betaproteobacteria	2.12 (1.26, 4.34)	1.48 (1.05, 2.02)	–2.29	0.02
Erysipelotrichi	0.35 (0.18, 0.48)	0.82 (0.46, 1.51)	–4.73	< 0.01
Coriobacteriia	0.15 (0.10, 0.33)	0.37 (0.18, 0.84)	–2.63	< 0.01

Statistical methods

The data were processed and analyzed using SPSS software SPSS25.0, and the data are expressed as means ± standard deviations and medians (quartiles). Data were analyzed using the independent samples *t*-test and rank sum test. Statistical significance was set at $P < 0.05$.

Results

Differential analysis of Operational Taxonomic Unit (OTU) expression numbers

Overall, 7,689 OTUs were detected in the patient with liver cancer group and 2,604 OTUs were in the control group. Of these OTUs, 2,347 were common to both groups, 5,342 were unique to the patients with liver cancer, and 257 OTUs were unique to the controls (Fig.1).

Comparison of the relative abundance of the intestinal flora at the phylum level

Comparison of the relative abundance of liver cancer patients and healthy individuals at the phylum level showed that the top five significantly different phyla were Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, and Tenericutes. Only one microbial phylum, Bacteroidetes, showed significant statistical differences, with its abundance in the gut microbiota of the liver cancer group significantly lower than that of the healthy control group ($P < 0.05$; Table 1).

Comparison of the relative abundance of the intestinal flora at the class level

When comparing the relative abundance of the intestinal flora at the class level between the patients with liver cancer and controls, the following were the top five classes with a significantly different abundance: Bacteroidia and Betaproteobacteria had a decreased abundance, whereas Bacilli, Coriobacteriia, and Erysipelotrichia had an increased abundance. All differences were statistically significant ($P < 0.05$; Table 2).

Table 3 Differences in the order-level flora between the two groups

Group	A	B	*t*/*Z*	*P*
Bacteroidales	50.72 ± 17.36	41.19 ± 14.37	2.77	< 0.01
Burkholderiales	2.09 (1.23, 4.33)	1.37 (0.84, 1.98)	–2.60	< 0.01
Lactobacillales	0.46 (0.20, 0.74)	1.45 (0.63, 2.50)	–3.89	< 0.01
Erysipelotrichales	0.35 (0.17, 0.48)	0.82 (0.45, 1.51)	–4.73	< 0.01
coriobacteriales	0.14 (0.10, 0.33)	0.38 (0.18, 0.84)	–2.62	< 0.01

Comparison of the relative abundance of the intestinal flora at the order level

When comparing the relative abundance of the intestinal flora at the order level in the patients with liver cancer and controls, the following were the top five orders with a significantly different abundance: Bacteroidales had a decreased abundance, whereas Burkholderiales, Lactobacillales, Erysipelotrichales, and Coriobacteriales had an increased abundance. All differences were statistically significant ($P < 0.05$; Table 3).

Comparison of the relative abundance of the intestinal flora at the family level

When comparing the relative abundance of the intestinal flora at the family level in the patients with liver cancer and controls, the following were the top eight families that showed a significantly different abundance: *Alcaligenaceae*, *S247*, *Barnesiellaceae*, and *Christensenellaceae* had a decreased abundance, whereas *Coriobacteriaceae*, *Streptococcaceae*, *Erysipelotrichaceae*, and *Enterococcaceae* had an increased abundance. All differences were statistically significant ($P < 0.05$; Table 4).

Comparison of the relative abundance of the intestinal flora at the genus level

When comparing the relative abundance of the intestinal flora at the genus level between patients with liver cancer and controls population, the following were the top 11 genera with a significantly different abundance: *Roseburia*, *Ruminococcus*, *Clostridium*, *Streptococcus*, *Collinsella*, *Megamonas*, Veillonella, *Eubacterium*, *Enterococcus*, and *Pseudobutyrivibrio* had an increased abundance, *whereas Sutterella* had a decreased abundance. All differences were statistically significant ($P < 0.05$; Table 5).

Comparison of the relative abundance of the intestinal flora at the species level

When comparing the relative abundance at the species level between patients with liver cancer and controls, the following were the top 14 species with a significantly difference abundance: *Ruminococcus gnavus*, *Dorea formicigenerans*, *Collinsella aerofaciens*, *Veillonella dispar*, *Bacteroides plebeius*, *Streptococcus infantis*, *Ruminococcus torques*, *Eubacterium dolichum*, *Veillonella parvula*, and *Clostridium ramosum* had an increased abundance, whereas *Bacteroides coprophilus*, *Bacteroides eggerthii*, *Bifidobacterium bifidum*, and *Lactococcus garvieae* had a decreased abundance. All differences were statistically significant ($P < 0.05$; Table 6). Genus dendrogram of mycorrhizal species (Fig. 2).

Discussion

Primary liver cancer is usually associated with chronic liver disease (e.g., hepatitis, cirrhosis, and steatohepatitis), and its development is typically accompanied by an inflammatory response. Microecological studies are receiving increasing attention from both researchers and clinicians. Currently, patients with an early diagnosis of HCC can be treated with transcatheter arterial chemoembolization and radiotherapy; however, many patients are diagnosed at an intermediate to advanced stage and often present with a poor prognosis. Therefore, early prevention and diagnosis are the key to treating liver cancer. The intestinal flora is involved in the maintenance of homeostasis in the body and is associated with a variety of pathophysiological processes; the liver is one of the organs that is most susceptible to the effects of the intestinal flora [6]. Previous studies have confirmed a strong association between the intestinal flora and liver cancer. Therefore, alteration of the intestinal flora may be one of the underlying mechanisms of liver cancer development. Exploring the unique characteristics of the intestinal flora in patients with primary liver cancer is expected to enable early prevention and diagnosis of liver

Table 4 Differences in the family-level flora between the two groups

Group	Alcaligenaceae	S247	Barnesiellaceae	coriobacteriaceae	streptococcaceae	Erysipelotrichaceae	Enterococcaceae	Christensenellaceae
A	2.09 (1.20, 3.80)	0.47 (0.24, 1.48)	035 (0.15, 0.59)	0.15 (0.10, 0.33)	0.18 (0.09, 0.30)	0.35 (0.17, 0.47)	0.02 (0.01, 0.04)	0.17 (0.03, 0.39)
B	1.21 (0.68, 1.70)	0.14 (0.08, 0.29)	0.14 (0.06, 0.29)	0.38 (0.18, 0.84)	0.66 (0.21, 1.65)	0.82 (0.46, 1.51)	0.04 (0.02, 0.14)	0.04 (0.02, 0.11)
Z	–3.12	–3.81	–2.90	–2.62	–3.94	–4.73	–3.32	–2.87
P	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

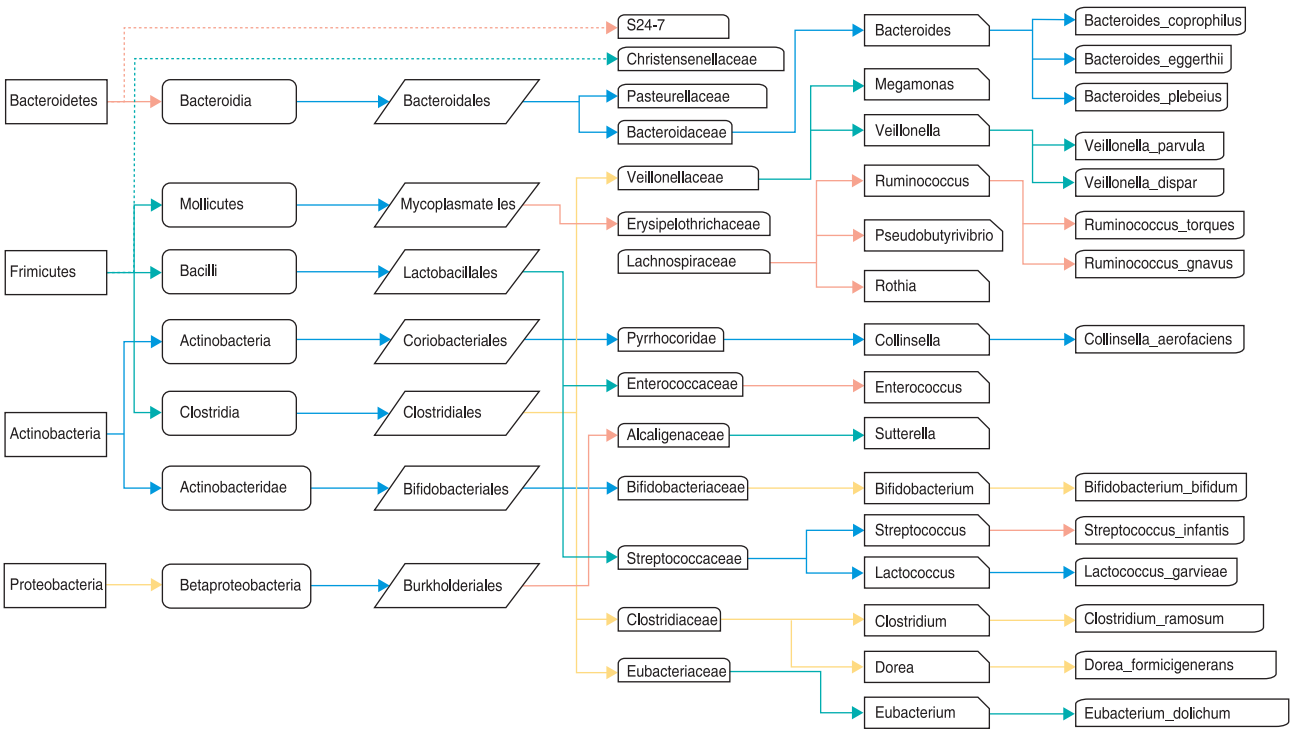

Fig. 2 Species relationship

Table 5 Differences in the genus-level flora between the two groups

Group	A	B	Z	P
Sutterella	2.09 (1.20, 3.79)	1.21 (0.68, 1.69)	–3.16	< 0.01
Roseburia	3.01 (1.89, 5.39)	5.24 (2.59, 7.43)	–2.44	0.01
Ruminococcus	1.81 (1.17, 3.45)	3.41 (2.12, 4.85)	–3.11	0.01
Clostridium	0.64 (0.41, 1.09)	0.87 (0.46, 2.05)	–2.07	0.04
Streptococcus	0.16 (0.09, 0.25)	0.63 (0.20, 1.47)	–4.30	< 0.01
Collinsella	0.08 (0.04, 0.22)	0.21 (0.07, 0.56)	–2.37	0.02
Megamonas	0.05 (0.01, 0.19)	0.16 (0.06, 0.42)	–2.38	0.02
Veillonella	0.04 (0.02, 0.05)	0.25 (0.06, 0.61)	–4.904	< 0.01
Eubacterium	0.06 (0.02, 0.11)	0.14 (0.07, 0.28)	–2.991	< 0.01
Enterococcus	0.01 (0.01, 0.03)	0.04 (0.02, 0.14)	–3.826	< 0.01
Pseudobutyrivibrio	0.00 (0.00, 0.01)	0.02 (0.01, 0.05)	–4.334	< 0.01

cancer, and early intervention regarding an abnormal flora may delay the disease process and provide new insights for the prevention and treatment of primary liver cancer.

Gut-liver axis and HCC correlation

The concept of the "gut-liver axis" has received much attention in recent years [7], and the relationship between the intestinal flora and liver disease has been redefined. The liver receives blood from the portal vein and nutrients absorbed by the intestine, as well as pathogenic bacteria and metabolites. An imbalance in the intestinal flora impairs the intestinal barrier and immune status, and pathogenic bacteria and flora metabolites are more likely to enter the liver through the portal vein and participate in pathophysiological processes in the liver, causing or promoting the development of liver disease [8]. *Veillonella parvula* is an anaerobic opportunistic pathogenic bacterium that is parasitic in the oral cavity and intestine and has been reported to cause bacteremia, meningitis, endocarditis, prosthetic joint infections, pulmonary infections, and vertebral osteomyelitis after spreading to other parts of the body [9, 10]. A previously published article demonstrated that *Bacteroides plebeius* plays a role in the degradation of porphyrins [11]. In this study, the abundance of common Bacteroides in the gut of liver cancer patients was significantly increased, which may indicate excessive degradation of porphyrins. *Bifidobacterium bifidum* produces biologically active interleukin 10 [12] and can inhibit the development of inflammation by targeting toll-like receptors via NF-κB [13]. This has also been shown to improve symptoms in patients with Alzheimer's disease [14] and to inhibit tumor growth by acting in concert with programmed cell death protein 1 (PD-1) inhibitors to induce host antitumor immune responses [15]. On sequencing the flora of patients with HCC, a decreased abundance of *Bifidobacterium bifidum* was observed, leading to decreased nutrient absorption and impaired immune function. *Ruminococcus gnavus* has been found to be enriched in patients with inflammatory bowel disease; its capsular polysaccharide promotes local inflammatory immune responses while activating hepatic oxidative

Table 6 Differences in the species-level flora between the two groups

Group	A	B	*Z*	*P*
Bacteroides_coprophilus	0.08 (0.04, 0.16)	0.01 (0.00, 0.14)	–2.67	< 0.01
Ruminococcus_gnavus	0.31 (0.22, 0.65)	0.68 (0.31, 1.27)	–2.97	< 0.01
Bacteroides_eggerthii	0.15 (0.06, 0.50)	0.06 (0.02, 0.25)	–2.69	< 0.01
Dorea_formicigenerans	0.04 (0.02, 0.12)	0.09 (0.05, 0.13)	–2.26	0.02
Collinsella_aerofaciens	0.10 (0.04, 0.20)	0.20 (0.06, 0.55)	–2.01	0.04
veillonella_dispar	0.02 (0.01, 0.03)	0.18 (0.05, 0.51)	–5.09	< 0.01
Bifidobacterium_bifidum	0.01 (0.00, 0.07)	0.00 (0.00, 0.01)	–3.95	< 0.01
Bacteroides_plebeius	0.43 (0.08, 2.51)	2.08 (0.59, 9.35)	–2.99	< 0.01
streptococcus _infantis	0.02 (0.01, 0.03)	0.06 (0.03, 0.11)	–4.66	< 0.01
Ruminococcus_torques	0.02 (0.01, 0.06)	0.05 (0.02, 0.10)	–2.49	0.01
Eubacterium_dolichum	0.01 (0.00, 0.03)	0.05 (0.01, 0.10)	–3.60	< 0.01
veillonella_parvula	0.01 (0.00, 0.02)	0.06 (0.02, 0.15)	–4.39	< 0.01
Lactococcus_garvieae	0.02 (0.01, 0.06)	0.00 (0.00, 0.01)	–3.96	< 0.01
Clostridium_ramosum	0.01 (0.00, 0.01)	0.03 (0.01, 0.05)	–4.10	< 0.01

stress [16, 17] and can even lead to bacteremia in patients with hematological malignancies [18]. *Veillonella dispar* is commonly found in the oral cavity, but there are reports indicating that bladder cancer patients may develop bacteremia due to intestinal Veillonella_dispa infection [19, 20]. All of the differentially abundant bacterial taxa mentioned above have a direct or indirect relationship with liver function.

Effect of metabolic diseases on liver cancer

In recent years, with changes in living standards and diet structure, disorders of glucose metabolism and fatty liver disease, apart from hepatitis and cirrhosis, have become high-risk factors for the development of HCC. In addition, disorders of hepatic lipid metabolism and the interruption of dynamic balance lead to the accumulation of lipids in hepatocytes and to hepatocellular steatosis. The incidence of fatty liver disease in China is gradually increasing and has become the second most common liver disease after viral hepatitis. Many scholars believe that fatty liver disease is closely related to HCC [21]. *Clostridium ramosum* has been shown to cause obesity and affect liver metabolism in mouse models [22]; *Ruminococcus torques* is significantly more abundant in obese populations [23]; and *Dorea formicigenerans* is positively correlated with obesity and can be used as an indicator of obesity [24]. Moreover, the abundance of *Eubacterium dolichum* has been demonstrated to be increased when rats are fed a high-sugar and high-fat diet decreased with the addition of flaxseed to the diet, indicating that *Eubacterium dolichum* is enriched in the intestine when an unhealthy dietary structure is present [25]. The sequencing results showed that the abundance of all four of these obesity-related bacteria was significantly increased in the intestinal flora of patients with liver cancer. In recent years numerous studies have shown that *Bifidobacterium bifidum* has an important contribution to physical health and can promote the digestion of food and the absorption of nutrients [26]. In addition, the results of animal experiments have shown that the addition of *Bifidobacterium bifidum* can effectively relieve constipation [27]. Despite these health advantages, the abundance of *Bifidobacterium bifidum* is significantly reduced in patients with HCC, which is detrimental to the health of these patients.

The relationship between the intestinal flora and cancer

Some studies have reported that the structure of the intestinal flora is closely associated with pancreatic, colon, thyroid, and liver cancers, and by reviewing the literature, 3 of the top 14 flora with significant differences in the results of this experiment were correlated with malignancy. Notably, *Bacteroides eggerthii* can produce antitumor compounds using quercetin [28]. The results suggest that its abundance was reduced in patients with HCC, which is similar to the findings of other studies on patients with colon cancer, where the bacterium was also significantly reduced [29]. *Streptococcus infantis* was shown to be closely associated with oral cancer when its abundance increased [30]. In recent years, anti-PD-1 therapy for tumors has become a hot research topic, but not all patients can benefit from it. The higher abundance of *Collinsellaaerofaciens* in the intestinal flora of patients in whom anti-PD-1 therapy was effective was verified in animal experiments, and transplantation of PD-1-sensitive patient flora enhanced T cell responses and improved the efficacy of anti-PD-L1 therapy [31]. Furthermore, the

increased abundance of this bacterium in the intestinal flora of patients with liver cancer suggests that they could benefit more from PD-L1 blockade therapy.

Intestinal flora is regulated by genetic factors

The structure of the intestinal flora is influenced by many factors, including dietary habits, diseases, and the application of antibiotics. Genetics also plays an important role in shaping the structure of the flora. As early as 2001, Zoetendal et al. demonstrated a high degree of similarity in the gut flora of twins using DNA techniques [32]. In 2014, Goodrich *et al.* [33] found that the abundance of many bacteria in the intestinal flora was influenced by the genetic background of the host, and that the gut flora of identical twins was more similar than that of heterozygous twins. Christensenellaceae was also shown to be the most heritable bacterium and to form symbiotic networks with other heritable bacteria, thereby influencing host metabolism. Yatsunenko later verified Goodrich's experimental results using more samples and found that bacteria of the phylum Synechococcus were non-heritable and were mainly influenced by environmental factors, whereas the thick-walled Actinomycetes and soft-walled Archaea phyla were heritable [34, 35]. The abundance of *Christensenellaceae* in patients with liver cancer in this experimental study was significantly lower than that in controls. This indicates that there may be a correlation between this flora and liver cancer susceptibility genes, which can be assessed by screening the intestinal flora of people at high risk of developing liver cancer.

In summary, compared with healthy individuals, patients with primary liver cancer have significantly altered intestinal flora, and patients with liver cancer can be evaluated by monitoring changes in the intestinal flora.

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Conflicts of interest

The authors declare no conflict of interest.

Author contributions

Chengcong Liu wrote this case report and analyzed all the data. Guoxin Sun collected the literature for this paper. Chengcong Liu, Guoxin Sun, Huizhe Wang, and Gaishuang Shang completed the operation. Xiong Yan and Xiao Zou validated modifications to the paper. Xiao Zou provided financial support for the article's writing and publication. All the authors have read and approved the final manuscript.

Data availability statement

Not applicable.

Ethical approval

The study was ethically reviewed, and all study participants provided informed consent and participated voluntarily.

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ORIGINAL ARTICLE

Xiaoaiping injection affects the invasion and metastasis of hepatocellular carcinoma by controlling AFP expression

Shu Huang[1], Ganxin Wang[2, 3] (✉)

[1] Department of Hepatology of Integrated Traditional Chinese and Western Medicine, The Third People's Hospital of Hubei Province affiliated with Jianghan University, Wuhan 430056, China
[2] Division of Oncology, Liyuan Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430077, China
[3] Cancer Center, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

Abstract

Objective Xiaoaiping (XAP) is a traditional Chinese medicine that is a commonly used as an anticancer drug in clinical practice owing to its high efficiency and low toxicity. Specifically, XAP can effectively inhibit the growth of hepatocellular carcinoma (HCC). Alpha-fetoprotein (AFP) is a key HCC diagnostic marker and is closely related to certain malignant cytological behaviors of HCC. However, whether AFP expression and XAP treatment are related to the invasion and metastasis of HCC remains unclear. In the present study, we aimed to evaluate the effects and underlying mechanism of XAP on the invasion and metastasis of HCC..

Methods Using a cell scratch assay, Transwell technology, and western blotting we detected the different invasion and metastatic abilities of Hep3B cells in XAP treatment and blank control groups. This allowed comparison of the invasion and metastatic abilities of Hep3B cells with differing levels of AFP expression. AFP mRNA sequencing technology was used to analyze the mechanism of tumor invasion and metastasis associated with AFP and XAP treatment.

Results Cell invasion and metastasis abilities in the XAP group were significantly lower than those in the control group ($P < 0.05$). Additionally, compared to the control group, the expression of AFP significantly decreased after XAP treatment ($P < 0.05$). The ability of Hep3B cells to invade and metastasize was promoted when AFP expression was up-regulated, whereas it was inhibited when AFP was silenced. XAP injection and AFP regulate the invasion and metastatic ability of HCC by affecting matrix metalloproteinases (MMPs).

Conclusion XAP injection inhibits the invasion and metastatic ability of HCC by influencing the expression of AFP; additionally, this inhibition of AFP is achieved by affecting MMPs.

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Key words: Xiaoaiping injection; Alpha-fetoprotein (AFP); hepatocellular carcinoma (HCC); invasion, metastasis

Cancer is a globally prevalent malignant disease that threatens quality of life, with a corresponding increase in cancer-related incidence and mortality every year [1]. Primary hepatocellular carcinoma (HCC), which is the second most common cancer worldwide in terms of tumor mortality, is one of the ten most malignant tumors in the world. There are more than 500,000 new HCC-related cases and deaths worldwide every year, of which approximately 51% occur in China [2, 3]. At present, HCC treatments have been developed from surgical and radiotherapy techniques, interventional chemoembolization, and novel molecular targeted drugs [4]. However, due to the complexity of the occurrence and development of HCC, singular or traditional treatment methods cannot satisfy clinical needs. Surgical treatment and radiotherapy aim to avoid the complications

✉ Correspondence to: Ganxin Wang. Email: Medicine_wgx@163.com

associated with chemotherapy, such as physical trauma and adverse side effects [5, 6]. In fact, the primary reason why HCC has become a long-term problem in humans is that it is a vascular-rich tumor with high vascular invasiveness with tendency for distant metastasis [7]. AFP is a specific diagnostic marker of HCC. It has been widely established in clinical data that patients with high levels of AFP protein and/or gene have significantly higher postoperative tumor metastasis and recurrence rates than those with lower AFP expression [8]. Moreover, AFP has the ability to promote HCC cell invasion *in vitro* by activating the PI3K/Akt/mTOR signaling pathway [9].

At present, traditional Chinese medicine (TCM) are widely researched in the study of anti-tumor therapeutics because of their unique advantages, such as no obvious side effects, high efficiency, and low toxicity [10]. Therefore, an increasing number of people have turned their attention to TCM, which can regulate the immune function of the body while demonstrating anti-tumor characteristics. Moreover, TCM does not simply kill tumor cells, but can strongly influence their metastatic and invasion abilities [11, 12]. Xiaoaiping (XAP) injection, a TCM preparation extracted from *Marsdenia tenacissima* (MT), can improve quality of life, strengthen immune function, and effectively prolong the survival of tumor patients [13]. Previous studies have indicated that XAP has remarkable curative effects on gastric cancer, lung cancer, esophageal cancer, osteosarcoma, and many types of malignant tumors, including HCC; in particular, XAP inhibits cellular proliferation and induces cell apoptosis in these tumors [14, 15]. Dai et al. showed that after treatment of A20 mouse lymphoma with XAP, the production of angiogenesis-related molecules, such as vascular endothelial growth factor and matrix metalloproteinases (MMPs), was significantly reduced, and angiogenesis in tumor tissues consequently decreased [16]. It has been confirmed by *in vitro* cell experiments that XAP can inhibit growth and promote apoptosis of HepG2 and Bel7402 cells in human HCC, thus achieving potential efficacy in clinical treatment of HCC [17]. However, its corresponding effectiveness and mechanism of action in HCC metastasis remains unclear.

The present study was designed to investigate the effects of XAP on the migration of human HCC Hep3B cells and to explore the role of AFP in the anti-metastatic effect of XAP.

Materials and methods

Chemicals and regents

Hep3B Cell lines were purchased from China typical species Conservation Center (Wuhan, China); the cells were cultured in medium consisting of Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin in an incubator at 37 °C with 95% air, and 5% CO_2. XAP injection was purchased from Nanjing Shenghe Pharmaceutical Co., Ltd. (No. Z20025868). DMEM medium and FBS were purchased from Hyclone Corporation, USA, Trizol was purchased from Invitrogen, and a reverse transcription kit was purchased from Fermentas.

RNA interference

Hep3B cells in the logarithmic growth phase were cultured in DMEM containing 5% FBS; these cells were seeded in a 6-well plate. We selected anti-AFP-specific siRNA-expressing vectors (AFP-siRNA) directed at the 923–944 region of the AFP gene [18]. Transfection of AFP-siRNA vectors and the control virus vector into Hep3B cells was induced using Lipofectamine 2000 (Invitrogen, USA) once the confluency of the cells reached approximately 65%. The cells were cultured for five days and AFP expression was detected using western blotting.

Generation of an AFP-expressing construct

AFP-expressing cells (pcDNA3.1-AFP) [18] were constructed by lentiviral infection as previously described. Hep3B cells were transfected with the control virus vector and packaging plasmids (pcDNA3.1-AFP) using Lipofectamine 2000 (Invitrogen, USA). The cells were incubated with the plasmid-lipid complex for 24 h and western blot analysis was performed to detect AFP levels.

Western blotting

Western blotting was performed as previously described. Briefly, A549 cells were washed twice with phosphate-buffered saline and lysed in radioimmunoprecipitation assay (RIPA) buffer. Cell lysates were incubated at 4 °C for 30 min, and cellular debris were pelleted by centrifugation at 15000 × g for 15 min at 4°C. Total protein was quantified using the BCA Protein Assay Reagent Kit with bovine serum albumin as a standard. Equal amounts (50 μg) of protein were loaded into each lane of a 12% SDS-PAGE gel, followed by transfer to a polyvinylidene fluoride membrane (Bio-Rad, USA) on a semi-dry transfer apparatus (Bio-Rad). After blocking with 5% non-fat milk for 1 h at room temperature, the membrane was incubated with specific antibodies overnight at 4°C. After washing, horseradish peroxidase–linked anti-mouse IgG was used as the secondary antibody and incubated with the membrane for a further 1 h at room temperature. Protein bands were detected using ECL Western blotting detection reagents (Amersham Biosciences, USA). All antibodies used in this experiment were purchased from Santa Cruz Biotech.

Scratch test

Cell migration was evaluated using a scratch test. The cells were grown to 80% confluency in 12-well microplates before scratching. Cell images were captured using a light microscope at 0, 24, and 48 h after treatment. The scratch area was calculated using ImageJ software.

Transwell assay

The migration assay for Hep3B cells was performed using Transwell membranes (Corning, USA) coated with Matrigel (BD Biosciences, USA). DMEM containing 10% FBS was placed in a 24-well chamber. The invading cells were removed from the top well using a cotton swab. After washing three times with PBS, the cells were fixed for 30 min and stained with 0.1% crystal violet for 20 min. The cells were incubated at 37 °C for 24 h. Migrating cells attached to the subventricular surface were observed under a microscope (×200 magnification). The number of cells entering the chamber were counted in five random fields to evaluate cell invasion and migration. The experiment was repeated thrice. Invasion rate was determined as follows: Invasion rate (%) = number of invasive cells in the experimental group/number of invasive cells in the control group × 100%.

Statistical analysis

Statistical analysis was performed using SPSS software (version 22.0; IBM Corp., Armonk, NY, USA), and the results were expressed as mean ± standard deviation ($\bar{x}$ ± s); P<0.05 was considered statistically significant. Image-pro plus 6.0 (Media Cybernetics, Inc., USA) was used to count the number of cells and calculate the scratch areas.

Results

XAP can inhibit the invasion and metastasis of HCC

The migration abilities of Hep3B cells injected with XAP (80 μL/mL) and the control group were detected by the cell scratch test and Transwell metastasis assay. The corresponding results showed that the cell migration and invasion abilities of the XAP and control groups were significantly different (P< 0.05). Compared to that in the control group, the migration distance length and the number of invading cells in the XAP-treated group were significantly lower (both P<0.05). Therefore, these results revealed that XAP injection impaired HCC cell migration and invasion abilities (Fig. 1).

XAP can inhibit the expression of AFP

Next, we detected the influence of XAP injection on the expression of AFP protein using western blotting. The XAP-treated Hep3B/HepG2 cells exhibited a downregulated expression of AFP protein (Fig. 2).

AFP overexpression promotes invasion and metastatic ability of Hep3B cells

The migration distances of the two groups of cells were observed under a microscope at 0, 24, and 48 h after transfection of Hep3B cells with the AFP-expressing lentivirus vector (pcDNA3.1-AFP). The migration distance in the AFP overexpression group was significantly smaller than that in the control group (P < 0.05). The corresponding Transwell assay results were consistent with those of the scratch test in that the number of cells penetrating the membrane in the AFP overexpression group was significantly higher than that in the control group. Overall, these results indicated that cell invasion and metastasis were enhanced when AFP expression was upregulated (Fig. 3).

Silencing AFP expression reduces the invasion and metastasis ability of Hep3B cells

Compared with that in the control group, the migration distance of cells in the silencing shRNA-AFP group was significantly higher (P < 0.05) and the number of cells in shRNA-AFP group was significantly decreased (P < 0.05). Therefore, these results demonstrated that the metastatic ability of Hep3B cells decreased with silencing of AFP expression (Fig. 4).

XAP and AFP control HCC invasion and metastasis by affecting the expression of MMPs

Using Western Blotting, we determined that the expression levels of MMP-2 and MMP-7 in the two cell lines treated with XAP were significantly decreased compared to those in the controls (P<0. 05) (Fig. 5).

Discussion

HCC accounts for approximately 90% of primary liver cancers and is prone to intrahepatic and extrahepatic metastasis. Radical resection of HCC is currently the most effective treatment; however, several studies have determined that even with radical surgery, 50% of patients still develop metastases within 5 years, which has been attributed to the vascular-enrichment characteristics of HCC [7, 19]. Therefore, exploring the mechanisms of HCC invasion and metastasis has important clinical significance in the prevention and treatment of this cancer. XAP, one of the most popular TCM drugs for HCC treatment, has been shown to inhibit the growth and proliferation of many types of tumor cells, including HCC, and possesses unique clinical anti-tumor effects [14, 15]. Although XAP injection has shown promising cytotoxicity against primary tumors, its effect on cancer metastasis remains unknown. The results from the current study demonstrated that the invasion and metastasis ability of Hep3B cells treated

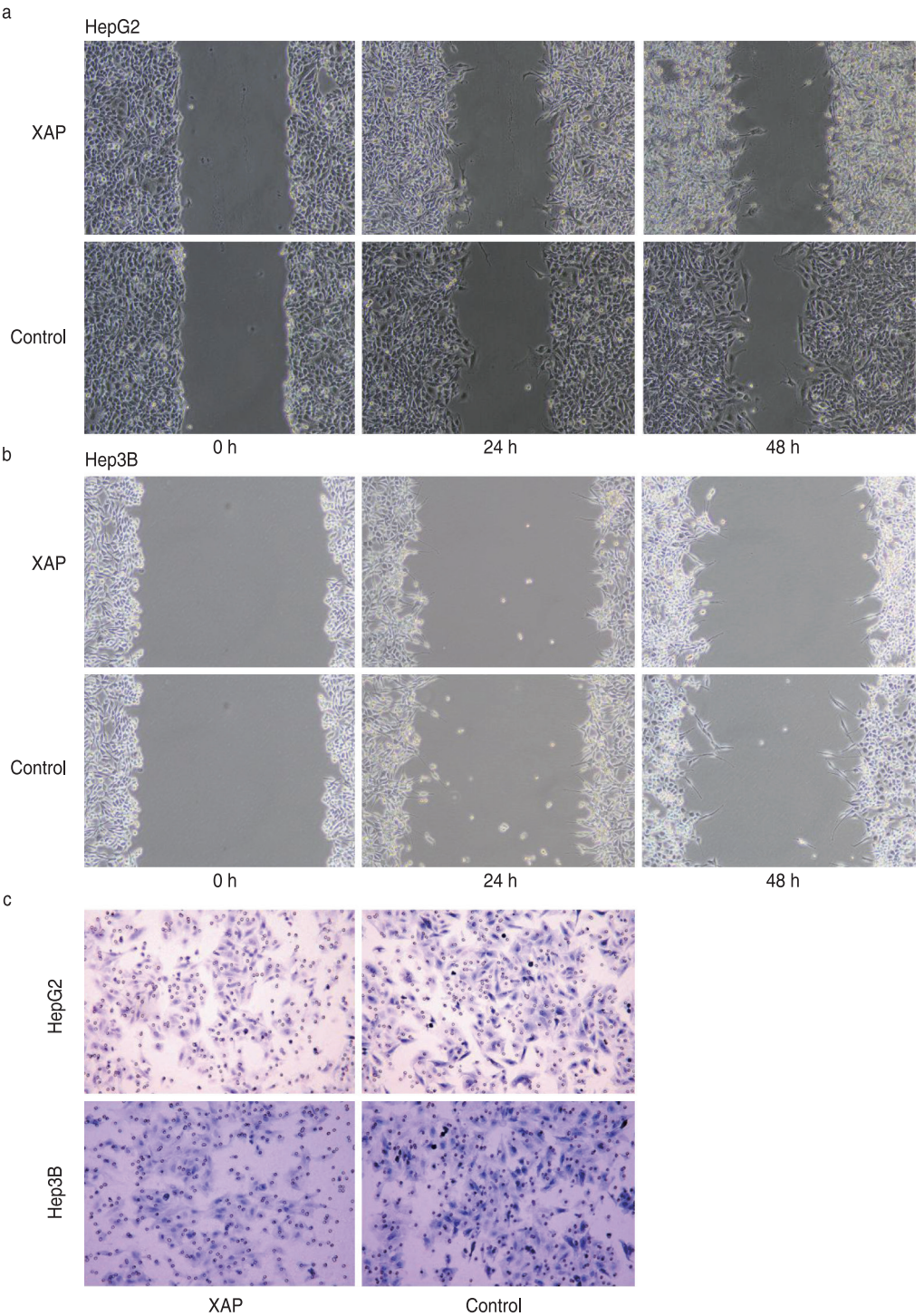

Fig. 1 Xiaoaiping (XAP) can inhibit the invasion and metastasis of hepatocellular carcinoma (HCC). (a, b) The migration distance of the cell scratch between XAP group and the control group are measured at 0 h, 24 h, and 48 h; (c) Comparison of metastatic ability between XAP and control group cells in Hep3B and HepG2 using a Transwell metastasis assay. The corresponding results indicate that the number of cells in the Hep3B/XAP and HepG2/XAP groups were significantly lower than in the corresponding controls ($P < 0.05$)

with XAP injection was significantly lower than that of the control group, suggesting that XAP may exert its clinical anti-tumor effects by influencing the distant metastasis of HCC; these findings, therefore, address our previous questions. These findings are consistent with a previously reported conclusion that XAP has an inhibitory effect on the invasion and metastasis of A549 cells through regulation of the CCR5–CCL5 axis, Rho C,

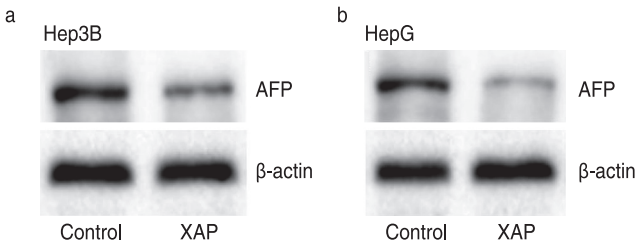

Fig. 2 Xiaoaiping (XAP) inhibits the protein expression of alpha-fetoprotein (AFP). The expression of AFP in the XAP group was significantly lower than that in control group

with AFP expression vectors, when compared with control cells transfected with an empty vector ($P < 0.05$), indicating that AFP can promote HCC cell invasion *in vitro* [17]. Additionally, Zhu has reported that AFP induces the expression of CXCR4 by activating the AKT/mTOR signaling pathway, thus enhancing the ability of liver cancer invasion and metastasis [20]. These findings are consistent with our experimental results, which demonstrate that the expression level of AFP influences the invasion and metastasis of Hep3B cells. Further, we confirmed that overexpression of AFP promoted invasion

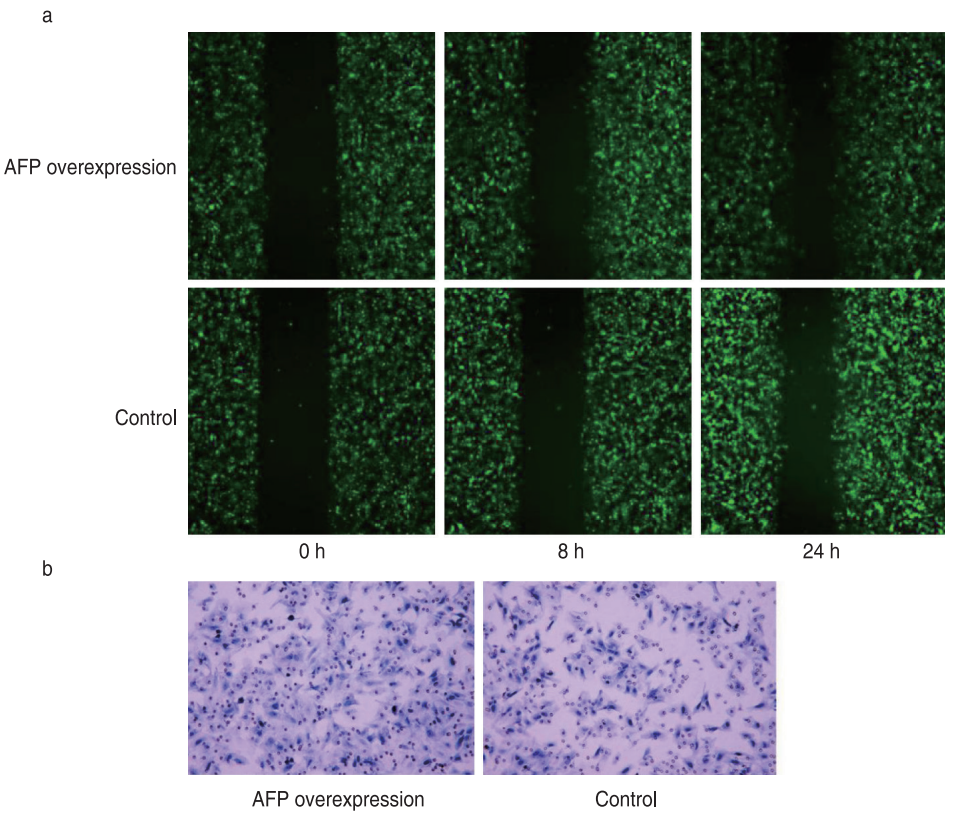

Fig. 3 Effect of alpha-fetoprotein (AFP) overexpression on invasion and metastasis of hepatocellular carcinoma. (a) Overexpression of AFP enhances the ability of cell invasion and metastasis of Hep3B, illustrated by a cell scratch test. The migration distance in the AFP overexpression group is significantly shorter than that in the control group ($P < 0.05$); (b) Migration of Hep3B cells after enhancing AFP expression using a Transwell chamber assay. When AFP is up-regulated, the number of cells significantly increases ($P < 0.05$)

and phosphorylated FAK [21].

To date, prior studies have shown that AFP plays a critical role in the effects of anti-tumor drugs in HCC cells [22]; further, AFP expression has been positively correlated with HCC invasion and metastasis [23, 24]. Previous studies have shown that the serum AFP level is closely related to the invasion and metastasis of HCC. Specifically, in our previous study, we confirmed that when the serum AFP level reached above 400 μg/L, HCC patients possessed a higher risk of tumor invasion and postoperative metastasis [8]. According to Wang, the migratory capacity of HCC cells can be significantly enhanced following transfection and metastasis of Hep3B cells, whereas the migration ability was inhibited by AFP silencing using a Transwell assay and scratch test.

The results from the present study explore the mechanisms of XAP treatment and suggest that XAP inhibits the expression of AFP protein and can weaken the invasion and metastasis of HCC cells. Since MMPs are the only known matrix degradation enzymes of interstitial collagen that can degrade all components of the extracellular matrix (ECM) released from polysaccharides, we examined the influence of XAP on

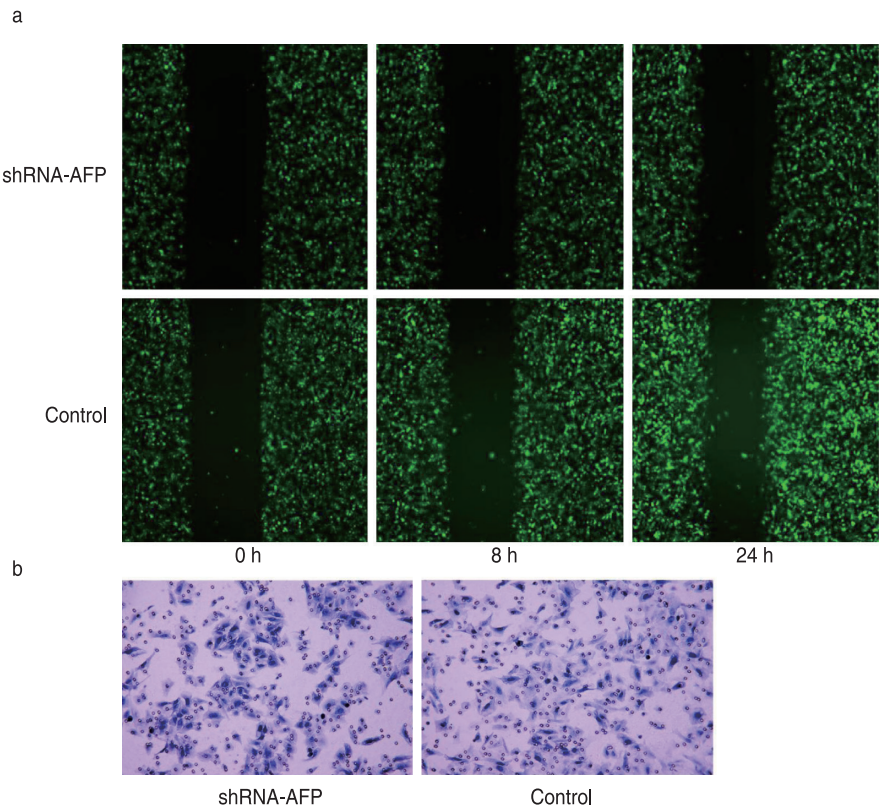

Fig. 4 The results showed that the metastatic ability of Hep3B cells was reduced after AFP expression was silenced. (a) The cell scratch test showed that alpha-fetoprotein (AFP) depletion resulted in reduced cell migration and invasion; (b) The cell migration in Hep3B cells after silencing AFP by using the transwell chamber experiment among the two groups

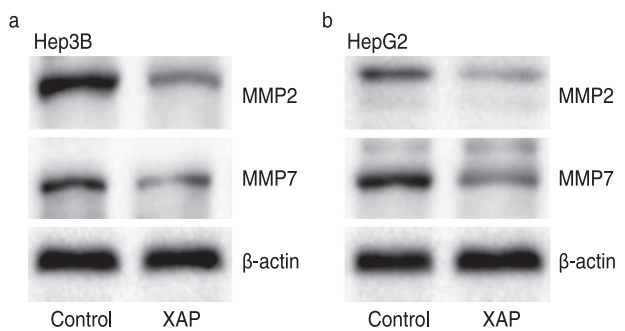

Fig. 5 Expression of MMP-2 and MMP-7 was significantly reduced in two cell lines treated with XAP. (a) Compared with the control group, XAP could down-regulate the expression of MMP-2 and MMP-7 expressions in Hep3B cells; (b) Compared with the control group, XAP could down-regulate the expression of MMP-2 and MMP-7 expressions in HepG2 cells

the expression of certain members of the MMP family by using western blotting. Consequently, it was observed that the expression of MMP-2 and MMP-7 proteins was downregulated in XAP-treated cells. It is well established that MMPs can participate in the regulation of the tumor microenvironment, predominantly through both proteolytic and non-proteolytic processes; consequently, these enzymes play an important role in ECM circulation, cell growth, inflammation, angiogenesis, and tumor cell migration [25]. Since the discovery of the first MMP in 1964, MMP-1, the number of MMP family members has increased to 26, with high homology among their corresponding genes [26]. Blood analysis of patients with liver disease and liver cancer demonstrated that the MMP-2 expression was significantly higher than normal in these groups [27]. In addition, upregulation of MMP-2 expression and activity has been shown to play a key role in a variety of human cancers with metastatic ability [28]. MMP-2 is the main enzyme in the MMP family; specifically, MMP-2 degrades type IV collagen and participates in the degradation of the ECM and basement membrane, thereby playing an important role in tumor invasion and metastasis [29, 30]. MMP-7 is a secreted protein involved in the destruction of the ECM in many cancers and can promote the metastasis of liver cancer [31, 32]. Lynch reported that abnormal expression of MMP-7 may initiate of cellular processes and promotion of cellular migration by converting the cell adhesion protein E-cadherin into a soluble form in tumor cells; this can lead to the freeing of cancer cells from large tumor tissues, improving cell migration [33]. This understanding highlights the understanding that the expression levels of MMP-2 and MMP-7 are important indices for evaluating tumor

invasion and metastasis. Therefore, this is a reasonable explanation for the reduced expression levels of MMP-2 and MMP-7 observed after XAP treatment in our study, which were accompanied by weakened invasion and metastatic ability of liver cancer cells.

Conclusion

XAP injection inhibits the invasion and metastatic ability of HCC by influencing the expression of AFP; further, the corresponding inhibition of AFP is achieved by XAP-mediated downregulation of MMP-2 and MMP-7 expression.

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Conflicts of interest
The authors declare that they have no conflicts of interest.

Author contributions
Not applicable.

Data availability statement
All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Ethical approval
Not applicable.

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ORIGINAL ARTICLE

Effect of postoperative adjuvant chemotherapy on the prognosis of patients with ypT0-3N0 rectal cancer undergoing neoadjuvant chemoradiotherapy*

Jueyi Huang, Yongqian Cai, Biao Wang (✉)

Department of General Surgery, Dazhou Central Hospital, Dazhou 635000, China

Abstract

Objective The aim of this study was to investigate the effect of adjuvant chemotherapy (AC) on the prognosis of patients with ypT0-3N0 rectal cancer undergoing neoadjuvant chemoradiotherapy.

Methods The study participants were 110 patients with locally advanced rectal cancer. Thirty-four patients did not receive postoperative AC treatment, and the other 76 patients received postoperative AC treatment. The differences in the 5-year overall survival (OS) and disease-free survival (DFS) between the two groups were compared.

Results Age was an important determinant of the patients' decision to undergo postoperative treatment. Patients who did not receive AC treatment were significantly older than those who received AC treatment ($P < 0.05$). The tumor location (distance above anal margin) in the AC group was significantly larger than that in the non-AC group ($P < 0.05$). Moreover, there was no significant difference in the 5-year DFS and OS between the two groups. Postoperative AC did not significantly improve the prognosis of patients with rectal cancer. Age, tumor differentiation, and the number of resected lymph nodes were independent factors affecting the OS of patients ($P < 0.05$). Older patients, patients with lower degree of tumor differentiation, and patients with <12 resected lymph nodes showed worse prognosis ($P < 0.05$).

Conclusion Patients with rectal cancer whose ypT0-3N0 stage is reduced after neoadjuvant chemoradiotherapy, especially those without adverse prognostic factors, do not need AC after surgery.

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Key words: rectal cancer; postoperative adjuvant chemotherapy; neoadjuvant chemoradiotherapy; total mesorectal excision

Neoadjuvant chemoradiotherapy (NCRT) combined with radical resection is the main treatment strategy for locally advanced rectal cancer [1]. Compared with postoperative radiotherapy and chemotherapy, NCRT can effectively reduce the local recurrence rate and reduce the toxicity and side effects. NCRT can also promote tumor shrinkage and increase the success rate of sphincter preserving surgery, which helps patients undergo better functional rehabilitation [2]. The 2020 National Comprehensive Cancer Network (NCCN) ®) guidelines point out that for patients with locally advanced rectal cancer undergoing NCRT and radical surgery, a 6-month perioperative adjuvant chemotherapy (AC) should be generally implemented regardless of postoperative pathological stage [3]. However, previous studies have failed to observe the survival benefit of AC in some patients who have reached the descending stage, such as patients with ypT1-3 or ypN0 [4]. Therefore, the purpose of this retrospective study was to evaluate the need for AC in the subgroup of patients with ypT0-3N0 rectal cancer and to identify potential prognostic factors affecting recurrence and mortality in these patients.

Materials and methods

Research object

The study participants were 110 patients with locally advanced rectal cancer treated in the general surgery department of Dazhou Central Hospital from January 2010 to December 2016. The inclusion criteria were (1) age 18–70 years; (2) first definite diagnosis; (3) having received NCRT and radical surgery; (4) middle and low

✉ Correspondence to: Biao Wang. Email: weixbb2021@163.com
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rectal cancer (< 12 cm from the anal verge); (5) pathological stage was T0–T3, with no lymph node (LN) metastasis or distant metastasis (ypT0-3N0M0). The exclusion criteria were (1) patients who died at the hospital; (2) those with incomplete clinical data or follow-up data; and (3) those who underwent intestinal surgery for other diseases. This retrospective study was approved by the ethics review committee of Dazhou Central Hospital, which waived the need for obtaining patients' informed consent. The demographic characteristics of patients and intraoperative and postoperative parameters, such as age, gender, tumor location, operation type, pathological stage, and LNs, were collected through the electronic medical record system and tumor differentiation.

Treatment protocol

All patients underwent NCRT according to the standard treatment protocol. The treatment included two courses of preoperative chemotherapy: (1) fluorouracil (5-FU) 2000 mg/m^2, calcium folinate (LV) 200 mg/m^2, intravenous drip once a day for 8 weeks; and (2) oral capecitabine (Shanghai Roche Pharmaceutical Co., Ltd., China, gyzz h20073024, specification 0.5 g), 800 mg/m^2, twice a day for 8 weeks. The irradiation dose was 180 cGy/D, divided into 25 doses. The patients received a total dose of 4500–5040 cGy within 5 weeks. The whole pelvis was treated with radiotherapy using the three-dimensional conformal radiotherapy planning system. The upper edge of the tumor bed field was the L5/S1 junction, and the lower edge was the lower edge of the sciatic tubercle. The lateral edge of radiotherapy was located 1.5 cm outside the pelvis and the posterior edge wrapped the whole sacrum. Radical resection of rectal cancer was performed 6–8 weeks after the completion of NCRT. The types of surgery included low anterior resection, abdominal resection, or sphincter preserving surgery (colostomy). Total mesorectal resection (TME) and LN resection were performed according to the following principles: (1) high/low ligation of inferior mesenteric artery (IMA), LN was resected along the vascular route; and (2) the mesorectal capsule containing the rectum and adjacent lymphovascular tissue was completely resected. The AC regimen included (1) intravenous drip of 5-FU 2000 mg/m^2 and LV 200 mg/m^2 for 24 h, once every 2 weeks for 16 weeks; and (2) capecitabine 800 mg/m^2, twice a day for 16 weeks.

Follow-up

All patients underwent regular follow-up, including physical examination, colonoscopy, and blood tests such as whole blood cell count and serum carcinoembryonic antigen levels. Patients also underwent imaging examinations such as abdominal ultrasound and chest X-ray. When recurrence was suspected, computed tomography or magnetic resonance imaging was performed.

Statistical analyses

SPSS 20.0 and GraphPad prism software (version 5.0) were used for data processing. The discrete data are expressed as the number of cases, and the continuous data are expressed as mean ± standard deviation. Disease-free survival (DFS) was defined as the time between the date of initial operation and the date of recurrence. Overall survival (OS) was defined as the date of the first operation and the time of the last visit or death. Survival was calculated using Kaplan–Meier curve. The significance of the difference among subgroups was calculated by log-rank test. Stepwise multivariate Cox regression analysis was used to find out the independent prognostic factors related to survival. Results having $P < 0.05$ were considered statistically significant.

Results

General information of the whole group of participants

Pathological examination confirmed that only two cases had local recurrence, with a recurrence rate of 1.8%. While 76 patients received 5-FU-based AC treatment, 38 patients failed to receive AC treatment due to other complications, older age, or patients' rejection of AC treatment.

Comparison of clinical characteristics between the two groups

Among the 110 patients, 34 (30.9%) underwent TME alone and 76 (69.1%) underwent TME combined with 5-FU-based AC. Univariate analysis showed that age was an important determinant affecting the patients' choice of postoperative treatment. Patients who did not receive AC treatment were significantly older than those who received AC treatment ($P < 0.05$). The tumor location, defined according to its distance from the anal margin, was significantly larger in the AC group than in the non-AC group ($P < 0.05$). Other clinical parameters, including sex, pathological T stage, degree of differentiation, type of operation, and LN resection, showed no significant difference between the two groups ($P > 0.05$; Table 1).

Effect of AC on patient survival

Subgroup analysis was performed based on the age of patients. There was no significant difference in the 3-year DFS ($\chi^2 = 0.068$, $P = 0.793$) and 3-year OS ($\chi^2 = 0.063$, $P = 0.801$) between the TME + AC group and TME group of patients aged ≤ 60 years (Fig. 1). Moreover, there was no significant difference in the 3-year DFS ($\chi^2 = 3.147$, $P = 0.076$) and 3-year OS ($\chi^2 = 1.783$, $P = 0.181$) between the

Table 1 Comparison of the clinical characteristics between the two groups of patients

Index	TME + AC (n = 76)	TME (n = 34)	t/χ^2	P
Age	61.8 ± 12.5	69.8 ± 10.3	–3.266	0.001
Sex			0.052	0.820
Female	52	24		
Male	24	10		
Distance from the anal margin (cm)			7.616	0.022
< 4	20	10		
4–7.9	31	21		
8–12	25	3		
Degree of differentiation			0.292	0.589
Poorly differentiated	4	1		
Well-differentiated	72	33		
Modus operandi			0.146	0.702
Low pre-excision	65	30		
Abdominal perineum was combined with radical resection	11	4		
T stage			0.148	0.929
0	6	2		
1	30	14		
2	40	18		
Number of lymph node excisions			0.028	0.866
< 12	46	20		
≥ 12	30	14		

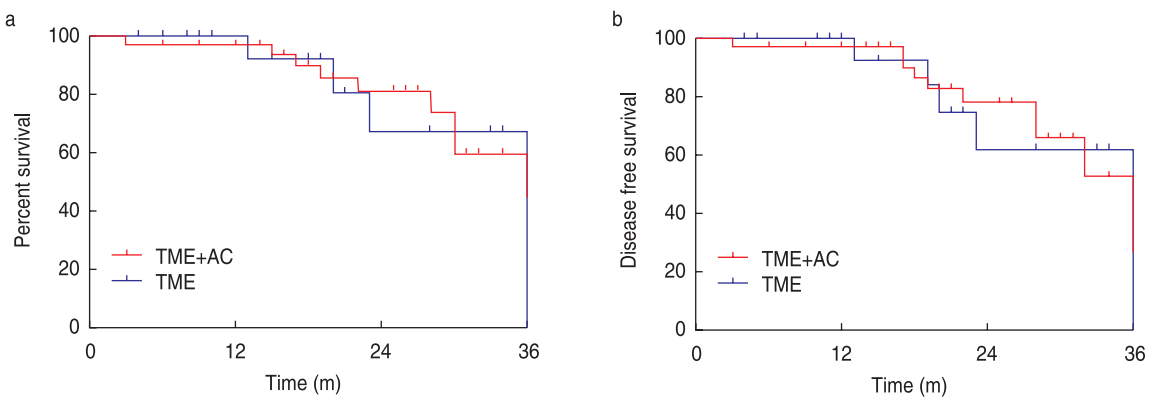

Fig. 1 The 3-year OS and DFS of patients aged ≥ 60 years in the TME + AC group and TME group. (a) The 3-year OS of patients aged ≥ 60 years in the TME + AC group and TME group; (b) The 3-year DFS of patients aged ≥ 60 years in the TME + AC group and TME group

TME + AC group and TME group of patients aged > 60 years (Fig. 2).

Multivariate analysis of the overall survival of patients with rectal cancer

Cox multivariate analysis showed that age, tumor differentiation, and the number of resected LNs were independent factors affecting the OS of patients (P < 0.05). Older patients, patients with lower degree of tumor differentiation, and patients with < 12 resected LNs showed worse prognosis (P < 0.05; Table 2).

Discussion

The survival benefit of AC in patients with lower stage of ypT0-3N0 rectal cancer after NCRT remains controversial. Our study shows that postoperative AC has no survival benefit for patients with lower stage of ypT0-3N0 rectal cancer, which suggests that careful consideration be taken when administering AC in these patients, especially for patients with rectal cancer without adverse prognostic factors. The EORTC 22921 randomized controlled study also failed to support the use of 5-FU AC in patients with 5-year OS [5]. In addition,

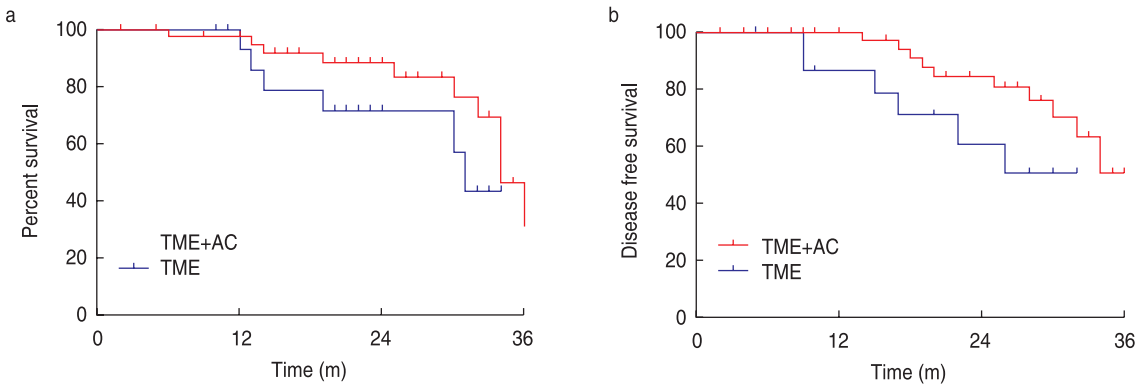

Fig. 2 The 3-year OS and DFS of the patients aged < 60 years in the TME + AC group and TME group. (a) The 3-year OS of the patients aged < 60 years in the TME + AC group and TME group; (b) The 3-year DFS of the patients aged < 60 years in the TME + AC group and TME group

Table 2 Multivariate analysis affecting overall survival in patients with rectal cancer

Index	B	SE	Wald	P	RR	95%CI
Age (≥ 60/< 60)	0.852	0.352	7.174	0.010	2.587	1.264–4.564
Sex (female/male)	0.239	0.213	1.154	0.298	1.336	0.787–1.874
Tumor differentiation (low/high)	1.284	0.529	4.726	0.032	3.554	1.036–11.935
AC (yes/no)	−1.512	0.137	2.689	0.089	0.328	0.115–0.551
T stage (T2/T0-1)	0.102	0.354	0.250	0.587	1.103	0.625–2.120
No. of lymph node (≥ 12/< 12)	−0.744	0.230	2.541	0.000	0.642	0.379–1.109

three other randomized prospective trials (PROCTOR-SCRIPT, CHRONICLE, and I-CNR-RT) showed that AC had no significant benefit on promoting survival in such patients. The PROCTOR-SCRIPT and CHRONICLE studies, which used 5-FU/LV or capecitabine as the AC protocol, showed that AC did not provide benefits in terms of DFS, OS, or recurrence rate [6]. The I-CNR-RT study used a smaller dose of 5-FU/LV as AC (5-FU 350 mg/m^2 and folic acid 20 mg/m^2) and reported that AC could not improve DFS, OS, or the rate of distant metastasis [7].

However, some retrospective cohort studies found that postoperative AC had significant survival benefits for some patients with locally advanced rectal cancer [8]. Garlipp *et al.* [9] conducted propensity score matching analysis on 1040 patients with rectal cancer who received 5-FU/capecitabine/oxaliplatin AC pretreatment and revealed improvement in the DFS of these patients. Tiselieus *et al.* [10] retrospectively investigated 436 patients with stage III rectal cancer who received NCRT, surgical treatment, and 5-FU/LV as AC and reported that AC could improve the prognosis of patients. Overall, conflicting conclusions from previous studies have posed a dilemma regarding the use of AC in patients with rectal cancer undergoing NCRT and surgery. Therefore, it is necessary to evaluate the need for AC in descending rectal cancer and identify specific populations that could benefit from this treatment.

The response of patients with locally advanced rectal cancer to NCRT is difficult to predict. Therefore, postoperative pathological stage rather than preoperative clinical stage may be a reliable predictor and can be used as the basis for determining the necessity of AC [11]. The results of this study and those of another study by Govindarajan *et al.* show that the reduction of ypT0-3N0 in patients before NCRT does not benefit from AC [12]. Yu *et al.* [13] conducted a retrospective cohort study of 203 patients with ypT0-3N0 and showed that the addition of AC had no effect on the 5-year DFS, which is consistent with our results.

In rectal cancer, the number of resected LNs is regarded as an indicator of radical surgery and accurate staging. According to the NCCN guidelines, only patients with at least 12 LNs can fully meet the staging criteria [14]. However, some studies questioned this guideline because it was observed that patients who received NCRT seemed to have fewer resected LNs than did those who did not receive NCRT. Furthermore, it was found that resection of < 12 LNs was associated with good DFS and OS. The reduction in the number of resected LNs can be considered as an individual's response to radiotherapy and chemotherapy, rather than a sign of insufficient surgical clearance. LNs on the mesorectum are vulnerable to irradiation. Therefore, radiotherapy can lead to lymphocyte apoptosis or interstitial atrophy [15]. In addition, from an anatomical perspective, the total number and size of LNs in rectal specimens are lesser than

those in colon specimens. Therefore, anatomical features and radiation effects are attributed to the reduced number of LNs harvested in patients receiving NCRT [16]. In our study, poor differentiation of tumor was identified as a prognostic factor corresponding to the poor 5-year DFS.

Microscopically, poorly differentiated tumors have a high tendency for invasion, which is a high-risk feature of stage II rectal cancer. In the study of Park *et al.*, poorly differentiated tumor was recognized as an independent adverse prognostic factor affecting DFS.

In conclusion, this study found that patients with ypt0-3n0 rectal cancer may not need AC, especially those without adverse prognostic factors. One of the limitations of this study is that age is still an important determinant and poses a potential choice bias in the decision regarding postoperative treatment. In our study, the age distribution between AC and non-AC patients was significantly unbalanced in patients receiving NCRT and surgery. In addition, AC was administered to younger patients with fewer complications and better physical capability. Another limitation is the retrospective nature of data collection, which lowers the level of evidence. For example, the tumor location (distance above the anal margin) is different between patients treated with AC and patients not treated with AC. Finally, this study was a single institution retrospective cohort study, which may also lead to potential selection bias. Larger prospective, randomized studies may provide more convincing evidence in the future. Nevertheless, we believe that the current results provide important information for clinical judgment on the effectiveness of AC in the subgroup of patients with ypT0-T3N0 rectal cancer.

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Author contributions
All authors contributed to data acquisition, data interpretation, and reviewed and approved the final version of this manuscript.

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Editorial office of Oncology and Translational Medicine
Tongji Hospital
Tongji Medical College
Huazhong University of Science and Technology
Jie Fang Da Dao 1095
430030 Wuhan, China
Tel.: 86-27-83662395
Email: otm.office@tjh.tjmu.edu.cn;
dmedizin@sina.com

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