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The New Era of Immunotherapy in Bile Duct Cancer Management

*Cosmas Rinaldi Adithya Lesmana
and Baiq Kirana D. Mandasari*

Abstract

Bile duct carcinoma or well known as cholangiocarcinoma (CCA) is the second most common of primary liver malignancy after hepatocellular carcinoma (HCC). Although cholangiocarcinoma is a rare cancer, it has an aggressive feature with very poor prognosis. The epidemiological profile of cholangiocarcinoma varies widely across the world, which is reflecting the exposure of different risk factors, such as chronic inflammatory disease of the biliary tract, specific infectious disease, and congenital malformation. Diagnosis of CCA is quite challenging. CCA is generally asymptomatic in the early stages. Therefore, the management of this malignancy is often delayed due to late diagnosed, where the metastasis has already present or even when it is causing bile duct obstruction. Treatment for CCA is often difficult and should be managed in the tertiary referral hospital with a multidisciplinary team approach. Surgical treatment with complete resection could be benefit only for patient with early stage of the disease. Other treatment modalities as adjuvant therapy are also have been developed to improve survival of the patient, such as chemotherapy, radiotherapy, molecular targeted therapy, targeting angiogenesis and EGFR, and immunotherapy. Recently, immunotherapy has also been developed as a new cancer treatment option and showed a promising result. Whether immunotherapy can be useful for treatment biliary malignancy is still controversial. Hence, a lot of studies is still required to confirm the preliminary findings.

Keywords: bile duct carcinoma, bile duct carcinoma management, immunotherapy, gastroenterology

1. Introduction

The bile duct carcinoma or known as cholangiocarcinoma (CCA) by the definition is a malignancy that originate from cholangiocytes lining the biliary tree. It is included in liver malignancy and become the second most common primary liver malignancy after hepatocellular carcinoma. [1, 2] Incidence of this malignancy is 10–20% cases of all hepatic cancer. [2, 3] Although cholangiocarcinoma is a rare cancer, it has an aggressive feature with very poor prognosis. The data showed that the incidence of cholangiocarcinoma among gastrointestinal cancer approximately reaches 3% but has nearly 20% of death from all hepatobiliary cancer. [3, 4] In addition, cholangiocarcinoma is a clinically silent disease at early stage. Therefore, the diseases are usually diagnosed at advanced stage with poor prognosis.

CCA may occur anywhere in the biliary tract, however, based on where the tumor arises in the biliary tree, it is classified into intrahepatic (iCCA) and extrahepatic bile duct cholangiocarcinoma (eCCA). Extrahepatic bile duct cholangiocarcinoma is divided into two types, perihilar (pCCA) and distal (dCCA) cholangiocarcinoma. iCCAs arise above the second - order of the bile ducts. In contrast, the point anatomical which is distinction pCCA and dCCA is the insertion of the cystic duct. The majority of cholangiocarcinoma are in the perihilar (50–60% cases) and distal region (20–30% cases), and only 10% of CCA are located in intrahepatic. [5]

The tumor is considered rare in most countries with incidence rate from 2001 to 2015 was 1.26 cases per 100,000 persons and has a mortality rate 1–6 per 100,000. [1, 6] Nevertheless, this malignancy is still an endemic disease with high prevalence and incidence in some countries or regions such as Thailand and South Korea. The epidemiological profile of cholangiocarcinoma varies widely across the world, which is reflecting the exposure of different risk factor, such as chronic inflammatory disease of the biliary tract, specific infectious disease, and congenital malformation. In western countries, primary sclerosing cholangitis (PSC) causing biliary obliterative fibrosis, is the major etiology of CCA. [7] Specific in endemic area, Northeast Thailand, with incidence rate 118.5 per 100,000, which is 100 times higher than the global rate. [8] Number of mortality cases from liver and bile duct cancer is the leading cause of death in Thai males and places the third place in female with total number 28,000 deaths per year. [9] Northeast region of Thailand showed the highest number of liver mortality, comprising 70% of cases. [9] In this area, incidence of CCA is strongly related to liver fluke infestation that is endemic in Mekong River. Liver fluke infection is caused by water-borne parasites known as *Opisthorchis viverrini*, *Clonorchis sinensis*, and *Opisthorchis felinus*. These parasites are transmitted to human by the consumption of raw, pickled, or undercooked infected fish associated with local tradition and poor income. [10–12]

The life cycle of this parasite is quite complex, involving two intermediate host (snail to fish) and including several changes of morphological feature. Fish contaminated with metacercariae is ingested by the human. [2] Infected human excretes the egg produced by the mature adult worms in their feces. [2, 13] Feces then contaminated the fresh water and then ingested by snail and the larva develop and hatch in the digestive tract of the snail. [2, 13] After that, thousands of cercariae were excreted into the water and penetrate the skin fish, encyst, and forming metacercariae. In the body of human, this parasite excyst in the duodenum and ascend to the bile duct via the ampulla of Vater then migrate further into the smaller and proximal bile duct, then become mature worm and able to sexually produce. [2, 13] Adult worm could survive up to 25 years in the biliary tree and causing mild symptoms such as malaise, abdominal discomfort, and diarrhea. Long term complication of this infection associated with hepatomegaly, chronic infection, cholecystitis, gallstone, and periportal fibrosis. [2, 13] Long term of chronic inflammation found to be a major etiological precursor of hepatobiliary malignancy, predominantly of CCA. Once a person is infected and suffered from chronic infection and inflammation, the risk for having CCA is increasing and could present within 30–40 years after infection. [11] Until now, the prognosis of CCA is remain poor and death tend to occur within 3–6 months after diagnosis. [11] There are several hypothesized on the mechanism or pathway how the chronic infection could develop become malignancy: 1) mechanical damage caused by the fluke sucker, 2) fluke toxic secretory product, and 3) immunopathological host response. [11] These pathways then caused proliferative response and formation of precursor lesion such as epithelial and adenomatous hyperplasia, and goblet cell metaplasia. [11]

Beside parasite infection, primary sclerosing cholangitis (PSC) is another common etiology of cholangiocarcinoma, especially in the western population. PSC is a progressive cholestatic biliary characterized by the chronic inflammation that leads

to destruction of the intra and extrahepatic bile duct. [14] The incidence rate of PSC ranges from 0 to 1.3 per 100.000 people. [15] At early stage, PSC is asymptomatic and is usually already diagnosed at advanced stage whereas jaundice and pruritus are the major complaint due to cholestasis. It has been also strongly associated with inflammatory bowel disease (IBD). On the other hand, PSC is often found with portal hypertension, cirrhosis, and in hepatobiliary and colorectal malignancies. [16, 17]

The other risk factor for developing CCA is biliary stones which is formed in the biliary tree, substantially in intrahepatic bile duct or known as hepatolithiasis. Biliary stones are typically concomitant with biliary stasis, cholangitis, strictures, and bacterial infection, leading to long term inflammation and biliary injury, and at the end, increasing the risk of malignant cholangiocytes growth. [18] Abnormal morphological also increase the risk for malignant transformation. Choledochal cysts is a rare congenital malformation characterized by dilatation of the biliary tree, can be single or multiple, and can be developed in the intra or extra hepatic bile ducts. [17, 19] Moreover, the coincidence of abnormal pancreatobiliary duct junctions increases the possibility of cholangiocarcinogenesis. This due to pancreatic enzyme reflux, cholestasis, and elevated bile acid concentrations. [19]

Exposure to chemical carcinogens such as Thorotrast, halogenated hydrocarbon solvent, and 1,2-dichloropropane were found to be associated with CCA incidence. [20, 21] Carcinogens-induced liver insult has been showed to promote hepatocyte remodeling, genomic instability, DNA methylation, and disrupt the liver architecture. Moreover, some studies reported few genetic mutations related to hepatobiliary malignancy. [22] Hepatic disease associated with CCA include alcoholic liver disease, cirrhosis, and cholangitis are included become risk factor. [17]

Diagnosis of CCA is quite challenging. CCA is generally asymptomatic in the early stage. Therefore, management of this malignancy is often delayed due to late diagnosed, where it already metastasis or compress the bile duct. The clinical features of CCA are heterogenous, with general malaise, cachexia, abdominal pain, night sweats, fatigue, weight loss, asthenia, and/or jaundice which is more frequent symptom in pCCA and dCCA due to biliary tract obstruction. [23, 24] Diagnosis of CCA is usually confirmed by combining nonspecific biomarkers in serum, biopsy specimens, and imaging technique. To date, there is no specific serum marker available for diagnosing CCA. Liver function parameters such as serum bilirubin, alkaline phosphatase, and aminotransferase enzyme usually elevate when biliary obstruction is presence. [24, 25] However, it is not specific signs for biliary malignancy. Serum tumor marker such as carbohydrate antigen (CA) 19-9, CA-125, and carcinoembryonic antigen (CEA) are the most widely used markers for suspected CCA. [25] But this diagnostic tool should not be used alone due to their poor diagnostic performance and inherent limitations.

Imaging techniques which are required to help diagnosis CCA are trans-abdominal ultrasonography (US), contrast-enhanced ultrasonography (CEUS), CT scan, and MRI. Becoming diagnostic tools, imaging techniques play a key role in the management of CCA in term of diagnosis, staging, follow-up, and assessment of favorable treatment response. The accuracy of diagnosis is depending on the anatomical location and growth pattern of CCA. Magnetic resonance cholangiopancreatography (MRCP) has the higher diagnostic accuracy for sizing strictures and localizing. [24, 25]

But unfortunately, there are no specific CCA radiology pattern exists. Therefore, histopathology or cytological analysis is also necessary for confirming the diagnosis. Definitive diagnosis is usually made by undergoing endoscopic retrograde cholangiopancreatography (ERCP) procedure for fluid cytology, brush cytology, fluorescence in situ hybridization (FISH), and cholangioscope or chromoendoscopy-guided biopsy. [26–28] Those multiple diagnostic modalities are required to 1) establish

strictures anatomical location; 2) distinguish between benign and malignant strictures; 3) differentiate CCA from gallbladder cancer; 4) stage and grade the tumor; and 5) plan treatment approach. Based on WHO classification of biliary tract cancer it is showing an adenocarcinoma or mucinous carcinoma, with tubular and/or papillary structures and a variable fibrous stroma. [24, 25]

Determine staging of CCA is important for choosing the treatment, its resectability, and the outcome of the treatment. TNM classification system of American Joint Committee on Cancer (AJCC) and Union for International Cancer Control (UICC) has been used at present to determine the staging of CCA. TNM staging system is based on imaging tests which is evaluating the number of primary nodules, vascular invasion, direct extension in neighboring tissue, and bile duct involvement. [29] pCCA can be further divided according to the Bismuth-Corlette classification, depending on the size of the tumor, disease extension in the main bile duct, hepatic artery and/or portal involvement, lymph node involvement, distant metastasis, and remnant liver volume after resection. [30] iCCA could be classified based on 3 growth pattern which has different prognosis of each pattern: mass-forming (MF-iCCA), periductal infiltration (PI-iCCA), and intraductal growth (IG-iCCA). [31]

Treatment for managing cholangiocarcinoma is quite difficult too and should be managed in the tertiary hospital with a multidisciplinary team experienced in endoscopic, percutaneous, and surgical approaches. Management of this malignancy also depends on the staging of the tumor. Surgical treatment with complete resection could give benefit only for patient with early stage of the disease [32].

Resection could be performed in approximately 30% of patient with CCA. This is the only option that provides a real possibility for long-term survival in patient diagnosed with CCA. The indication and extension of surgery are determined based on clinical features of the patient, functional liver reserve, and the location and extension of the tumor, which include the association with vascular structure and negative metastatic disease. [33, 34]

Criteria for patients who are considered as absolute unresectability are the presence of nonresectable extrahepatic, hepatic metastases, bilateral extension of the tumor with involvement of the secondary biliary tract, complete occlusion of the main portal vein, thrombosis in portal vein contralateral to the tumor. [23] The most common postoperative complications are hemorrhage, infection, liver failure, cardiorespiratory failure, and adrenal failure. Mortality and morbidity for postoperative patient are still remaining high, 8,2% and 50%, respectively. [35] In several condition, drainage should be applied. But in the recent years, increasing number of patients with unresectable intrahepatic and extrahepatic CCA are being included to be candidate for liver transplant. Other treatment modalities as adjuvant therapy are also developed to improve the survival of the patient, such as chemotherapy, radiotherapy, molecular targeted therapy, targeting angiogenesis and EGFR, and immunotherapy.

2. Role of immunotherapy in cancer management

2.1 History and definition

Cancer immunotherapy is significantly progressing and rapidly advancing. In the recent years, immunotherapy is considered to be the fifth pillar of cancer therapy and management modality besides surgery, cytotoxic chemotherapy, radiation, and targeted therapy. The mechanism of immunotherapy in cancer management is to determine a manipulation of the immune system by using immune

agents such as vaccine, cytokine, cell therapies and humoral, transfection agent. Cancer immunotherapy has to stimulate the host anti-tumor response by increasing the effector cell number and production of soluble mediators, decrease the host's suppressor mechanism by inducing tumor killing environment, and could modulate immune checkpoint. [36, 37]

In 1891, William Coley, who is known today as the Father of Immunotherapy, injected heat inactivated bacteria or known as Coley toxins to the sarcoma patient who was inoperable. [38] This first experiment resulted in long term regression of the sarcoma after an erysipelas infection after injecting the toxin. [38] By late 1970s, immunotherapy for managing cancer was discovered. The first experiment was done in bladder cancer case which is managed by using BCG (Bacillus Calmette-Guerin). Then, it is continued with IFN therapy in malignant melanoma. [39] Brief background review of immune system is classically considered to be comprised of the innate and adaptive arms. Immune system which are included in innate immune system are dendritic cells, natural killer cells (NK), macrophages, neutrophils, eosinophils, basophils, and mast cells. As we known, this group of immune system does not need prior stimulation by antigen, and it plays role as first line of defense against foreign antigens. In the contrary, adaptive immune system consists of B lymphocytes, CD4 helper T lymphocytes, and CD 8 cytotoxic T lymphocytes (CTLs). This group of immune system requires formal presentation by antigen presenting cells (APCs) for its activation. [40, 41]

Several kinds of malignant cells are able to evade the tumor immunosurveillance system by manipulating their own characteristic as well as the cells in their microenvironment to become successful tumors. The concept that the immune system is capable for detecting and killing nascent non-self-malignant cells was developed. Elimination, equilibrium, and escape are three main phases of immunoeediting process. [42] The elimination phase is the initial damage process and destruction of the tumor cell by innate immune system, then tumor antigens are presented to the dendritic cells, followed by presentation to the T cell and then create tumor-specific CD4 and CD8 T-cells. Second phase occurs when tumor cells survive after the initial destruction but are not able to progress and being maintained in an equilibrium state. The last phase is escape phase. [42] In this phase, tumor cells are growing rapidly, followed by metastasize of tumor cell due to loss control of the immune system and the tumor cells do not presented antigens on its surface or even losing their MHC class1 expression. Tumor cell could protect their self from T cell by expressing immune checkpoint (IC) molecules on their surface. [42]

The ability of this malignant cells to evade immune destruction by modulating its own cellular characteristic and creating its own "tumor microenvironment" by recruiting apparently normal immune cells to help shield it from attack of immune system. In addition, tumor cell can influence the systemic environment by altering hematopoiesis and tissue parenchyma of organs at distant sites. Cancer immunotherapies play role in manipulating these tumor microenvironments. But the loss of MHC class 1 expression manipulating is remaining challenge. [43–45]

First, older, and non-specific immunotherapies are the kind of immune stimulator cytokines such as interleukin-2 (IL-2) and interferon (IFN). [46] Beside that, synthetic analogue of bacterial cell wall called L-MTP could activate monocytes and macrophages is one of the immunostimulatory cytokines. Vaccine trials using multiple neoantigens specific to and individual patient's tumor have shown promising results in two small early trials with the aim to expose patients to those tumor antigens which can provoke an antitumor immune response via the generation of tumor specific antibodies and T cells. [46] BCG was the first vaccine used as cancer immunotherapy for treating bladder carcinoma. [47]

Oncolytic viruses are the combination of biologic therapy and immunotherapy. Viruses which are used for this method has genetically modified to lack virulence against normal cell but has a selective feature to invade and lyse cancer cells. Viral-induced tumor cell destruction undergoing further attack by an immune system. [48]

Adoptive cell therapy (ACT) is one type of immunotherapy which involves in the isolation and in-vitro expansion of tumor-specific T-cells, which is given through infusion in the cancer patient. ACT using NK cells could be used to treat solid tumor metastasis and hematological cancers. [49] Several forms of ACT using different techniques are culturing tumor infiltrating lymphocytes directly from the tumor, isolating and expanding one particular T-cell or the clone, using T cell which have been engineered in vitro so that it could recognize and attack the tumor cells or known as chimeric antigen receptor T-cell (CAR T-cell) therapy. ACT has produced remarkable result in clinical trials with melanoma and hematologic malignancies. But some studies reported death have occurred in the trial phase due to cytokine release syndrome or cytokine storm. [50]

Another immunotherapy, Immune checkpoint, work by targeting molecules that serve as checks in the regulation of immune responses and block inhibitory molecules or activate stimulatory molecules and enhance pre-existing anti-cancer immune response. [51]

2.2 The clinical importance of immunotherapy

Cancer immunotherapy works to stimulate the host's anti-tumor response. The mechanism included are increasing the effector cell number and production of soluble mediators, decreasing the host's suppressor mechanism by inducing tumor killing environment, and modulating immune checkpoints. The usefulness of cancer immunotherapy was introduced in the beginning to manage bladder cancer. The overall 5-year survival after transmitting immunotherapy is 77%. [39] Patients with moderate and high-grade bladder cancer who received intravesical immunotherapy with BCG have shown good result. Immune checkpoint inhibitors showed a promising clinical research in managing anti-cancer immune responses. Several studies using Nivolumab, Ipilimumab, and Pertuzumab are still on progress in metastatic bladder cancer. Some cytokines which are messenger molecules, play a role to control the growth and activity of immune system cells. [52] Treatment using cytokines as immunotherapy can enhance the activity of the immune system against tumors. The link of IL-2 to the antibody, ALT-801, and cytokines can target IL-2 to cancer cells. [53] Oncolytic virus therapy could also be used to treat bladder cancer using adenovirus which expresses the immune stimulating cytokine GM-CSF. [54]

Immunotherapy is developed to manage some immunogenic cancer cases besides bladder cancer. The using of immunotherapy for managing breast cancer have been improved and approved in the recent years. Although the best treatment of breast cancer is surgery, but combination therapy followed by chemotherapy, radiation therapy, or immunotherapy could increase clinical outcome for patient. A promising immunotherapy using immune checkpoint inhibitors that work by targeting molecules that serve as checks in the regulation of immune response and block inhibitory molecules or activate stimulatory molecules. [39] The other form of immunotherapy which can be used for breast cancer is monoclonal antibodies and adoptive T cell transfer. By definition, adoptive T cell transfer is a process of removing T cell from the patient, then it would be modified genetically or treated with chemical to enhance its activity and re-introduced into the patient. Specifically, in breast cancer, T cell genetically is modified to target the carcinoembryonic antigen (CEA). [55]

Another immunogenic cancer is cervical cancer caused by infection of human papillomavirus (HPV). Cervical cancer is the third most frequent cancer among women in the world. [56] The prevalence of this cancer is decreasing due to development widespread of screening tools Pap test and vaccine to prevent HPV infection. In the recent years, monoclonal antibodies, checkpoint inhibitor, and adoptive T cell transfer have become additional therapy for managing progressivity of cancer cell. [39]

Immunotherapies are also developed as a new modality treatment to treat brain cancer, colorectal cancer, esophageal cancer, and biliary tract cancer. Probably, in time, immunotherapy could lead to personalized medicine that will increase overall survival and progression free survival for many treatments. [39]

2.3 The role of immunotherapy in managing bile duct malignancy

Biliary tract malignancy is an invasive carcinoma which can be originated from gallbladder or bile duct. It has been known that the immune system in human body has a significant role in the surveillance and eradication of cancer cells. Tumor that lack the mismatch repair system harbor more mutation than tumor without this deficiency. Thus, the neoantigen generated and be recognize as immunogenic antigen. The characteristic of mismatch repair deficient tumors is microsatellite instability (MSI). There are approximately 3% of CCA are mismatch repair-deficient/MSI-high. [56] This feature makes the tumor cells are susceptible to programmed cell death protein 1 (PD-1) inhibitors. Zhu et al. studied about efficacy and safety of gemcitabine, oxaliplatin, and bevacizumab in advanced biliary-tract cancers and the correlation of changes in 18-fluorodeoxyglucose PET with clinical outcome in a phase 2 study showed that combination of chemotherapy and immunotherapy have anti-tumor effect with tolerable safety and promising efficacy for managing advanced biliary tract malignancy. This combination treatment was generally well tolerated with less adverse event and manageable toxicity. [57]

Another clinical data about immune-directed therapy in CCA is still scanty. Vaccine for preventing CCA has been developed and tested but no data has showed successful result. [58] CAR T cell immunotherapy in recent years has been developed. Guo et al. in their study about expanded and parallel clinical trial of EGFR-specific chimeric antigen receptor-engineered autologous T (CART) cell immunotherapy. The aim of this study is to assess the safety and activity of CART-EGFR cell therapy in EGFR-positive advanced unresectable, relapsed/metastatic biliary tract cancer. Total sample of this study is 19 patients and showed that CART-EGFR cell infusion was tolerated, 1 achieved complete response and 10 achieved stable disease. We can conclude that CART-EGFR cell immunotherapy was a safe and active strategy for EGFR-positive advanced biliary tract cancer. [59] Wei et al. showed that in some patients, immune checkpoint blockade using monoclonal antibodies has shown remarkable and durable response rate in a many kind of malignancy cell. [60] Le et al. in their study concluded that mismatch-repair status predicted clinical benefit of immune checkpoint blockade with pembrolizumab and achieving objective responses in up to 40% of patients. [61] Study by Ott et al. in KEYNOTE-028 basket trial of pembrolizumab included patients with advanced biliary tract cancer resulted the objective response rate was 17% with median progression-free survival of 1.8 months. [62] However, further studies are required either combination immunotherapeutic approaches targeting both the innate and adaptive immune system or combined strategies involving chemotherapy or radiation.

3. Conclusions

Bile duct cancer is still one of the challenging malignancies in the gastroenterology field due to the difficulty in early detection and most of patients come in the late stage of the disease. Chemotherapy is still the main option of management despite surgery and biliary drainage. Immunotherapy is a promising treatment option in the future; however, further studies would be needed to give strong evidence before it can be used in common clinical practice.

Author details

Cosmas Rinaldi Adithya Lesmana^{1,2*} and Baiq Kirana D. Mandasari¹

1 Division of Hepatobiliary, Department of Internal Medicine, Dr. Cipto Mangunkusumo National General Hospital, Medical Faculty Universitas Indonesia, Jakarta, Indonesia

2 Digestive Disease and GI Oncology Centre, Medistra Hospital, Jakarta, Indonesia

*Address all correspondence to: medicaldr2001id@yahoo.com

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