

The impact of gender and immune system determinants on long-term survival in biliary tract cancer

Massimiliano Salati^{1,2}, Francesco Caputo¹, Krisida Cerma¹, Luigi Marcheselli¹, Chiara Braconi^{1,2}, Stefano Cascinu¹

¹Department of Oncology, University Hospital of Modena and Reggio Emilia, Modena, Italy; ²Division of Cancer Therapeutics, The Institute of Cancer Research and Gastrointestinal Unit, The Royal Marsden Hospital, London and Sutton, UK

Summary. Advanced biliary tract cancers are poor prognosis tumors with limited therapeutic options. The conventional treatment consists of combination chemotherapy resulting in overall survival of less than 12 months. Recently, a subgroup of patients characterized by an exceedingly favorable prognosis has been reported in the literature. In our study, we assessed the prevalence and provided a clinicopathological characterization of this subset of patients in the context of daily practice. In particular, we were able to identify female sex and neutrophil-to-lymphocyte ratio as independent predictors of long-term survival. Based on these premises, we deem it necessary to prompt translational research projects aimed at molecularly characterized long-term survivors, particularly focusing on sex- and immune-related determinants.

Key words. Gender differences, immune system determinants, biliary tract cancer.

Impatto del genere e dei determinanti del sistema immunitario sulla sopravvivenza a lungo termine nel carcinoma biliare

Riassunto. I tumori delle vie biliari in stadio avanzato sono neoplasie a prognosi sfavorevole con opzioni terapeutiche limitate. Il trattamento convenzionale consiste nella chemioterapia di combinazione con conseguente sopravvivenza globale inferiore a 12 mesi. Recentemente, un sottogruppo di pazienti caratterizzati da una prognosi estremamente favorevole è stato riportato in letteratura. Nel nostro studio, abbiamo valutato la prevalenza e fornito una caratterizzazione clinicopatologica di questo sottogruppo di pazienti nel contesto della pratica clinica quotidiana. In particolare, siamo stati in grado di identificare il sesso femminile ed il rapporto tra neutrofili e linfociti come predittori indipendenti di sopravvivenza a lungo termine. Sulla base di queste premesse, consideriamo mandatorio promuovere progetti di ricerca traslazionale volti a caratterizzare a livello molecolare i pazienti lungo-sopravvivenza, concentrandosi in particolare su determinanti di genere e immunitari.

Parole chiave. Differenze di genere, determinanti del sistema immunitario, tumori delle vie biliari.

Introduction

Biliary tract cancers (BTCs) are a heterogeneous group of tumors encompassing cholangiocarcinoma (CCA), which is further subdivided into intrahepatic (iCCA), perihilar (pCCA), distal (dCCA) and gallbladder carcinoma (GBC). The incidence of BTCs varies greatly worldwide with the highest rates recorded in Northeast Thailand (>80 cases every 100,000) and much lower rates in Western countries (0.3-3.5 cases every 100,000)¹. However, their incidence is increasing globally, in particular, that of iCCA. Primary sclerosing cholangitis is the most established predisposing factor in Western countries, while hepatobiliary fluke infestation and hepatolithiasis are well-documented risk factors in Eastern countries².

Surgery is the only potentially curative treatment option, though feasible in only 10-20% of cases and associated with recurrence rates as high as 60-80%³. The vast majority of patients presents with advanced disease at diagnosis when palliative chemotherapy is an obliged choice resulting in median overall survival (mOS) hardly exceeding 12 months⁴.

As a consequence, 5-year OS is a disappointing 15-30% and <5% in early-stages and unresectable advanced disease, respectively. In spite of this commonly poor prognosis, a subgroup of outliers has been described among advanced BTCs that shows a surprisingly long-term survival when treated with conventional chemotherapy⁵. The aim of our study was to investigate this subset of patients in a real-world setting, looking at clinicopathological determinants linked to a higher likelihood of achieving long-lasting tumor control.

Materials and methods

Electronic medical records of patients with BTCs treated with first-line chemotherapy at The Modena Cancer Centre between 2010 and 2017 were retrospectively reviewed. Major inclusion criteria included: cyto-histologically proven unresectable recurrent, locally advanced and metastatic BTC (i.e., iCCA, pCCA, dCCA, GBC), treatment with first-line chemotherapy, and availability of clinical, pathological and biochemical data. Patients

with mixed hepatocellular-cholangiocellular carcinoma as well as ampullary carcinoma were excluded and radiotherapy treatment was not allowed.

Long-term survivors were defined as those patients surviving more than 24 months since the diagnosis of advanced disease.

Overall survival was calculated using Kaplan-Meier estimators from the date of the first cycle of front-line chemotherapy to the date of death for any cause or last follow-up visit. Statistical comparisons between curves were performed with the log-rank test.

Univariate and multiple logistic regression analyses were performed to investigate factors associated with the likelihood of surviving longer than 24 months.

Results

A total of 153 patients fulfilling the abovementioned criteria were identified at our center. Median OS in the entire advanced BTC population was 9.5 months (95% CI 5-11) (Figure 1A). Among these patients, 22 (13%) of them survived ≥ 2 years. The median age of long-term survivors was 67 years (range 29-80) and 82% were female. ECOG PS was 0 in 18 cases (82%) and 1 in 4 cases (18%). 13 (60%) had an intrahepatic tumor, 4 (18%) had a perihilar tumor, 4 (18%) had a gallbladder tumor and 1 (4%) had a distal tumor. Disease status was metastatic in 18 cases (82%) and locally advanced in 4 cases (18%). As first-line treatment, 10 patients received cisplatin/gemcitabine (45%), 7 (32%) monochemotherapy (i.e., gemcitabine) and 5 (23%) other chemotherapy doublets (i.e., mFOLFOX6 - 5-fluorouracil, leucovorin and oxaliplatin). A total of 18 patients (82%) received a second-line, 14 (64%) a third-line and 5 (22%) a fourth-line treatment. Median progression-free survival (mPFS) and median overall survival (mOS) were 12 and 36 months, respectively (Figure 1B). In univariate logistic regression, ECOG PS 0, female sex and locally advanced disease, neutrophil/lymphocyte ratio (NLR) ≤ 3.0 , and CEA < 9.5 were associated with a higher likelihood of surviving ≥ 2 years. Female sex and NLR ≤ 3.0 retained statistical significance in multiple logistic regression (Table 1). Women with an NLR ≤ 3 display the highest chance of surviving ≥ 2 years (45%).

Discussion

In this study, we provided the first real-world clinico-pathologic description of long-term survivors in the overall population of advanced BTC patients. More interestingly, we found that both NLR ≤ 3 and female sex were positive prognostic factors in this setting. In particular, the latter turned out to be the one showing the

strongest statistical correlation with long-term survival ($p = 0.021$) in our cohort.

Although the prognosis of advanced BTC is generally very poor, a subgroup of outliers experiencing prolonged disease control and long-term survival has recently been described. Bridgewater et al reported that 11% (45) of patients treated in the ABC-02 trial survived longer than 24 months showing a mOS of 31.4 months⁵, which is considerably better than commonly reported. Of note, a higher number of female patients was found among long-term survivors (26 vs 19). In fact, sex, to-

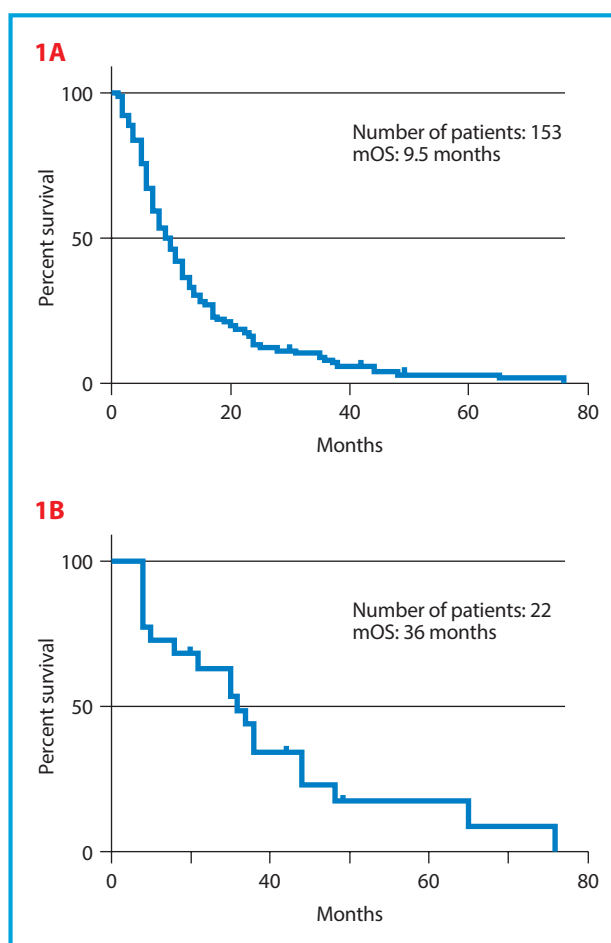


Figure 1. Overall survival in the whole advanced BTC population (1A) and in the long-term survivor subgroup (1B).

Table 1. Multiple logistic regression for OS >24 months		
Factor	OR (95CI)	P value
Female gender	4.11 (1.23-13.7)	0.021
Metastatic	0.27 (0.06-1.26)	0.097
ECOG PS >0	0.33 (0.10-1.08)	0.068
NLR ≤ 3	2.73 (1.17-6.36)	0.030
CEA >9.5	0.16 (0.02-1.33)	0.090

gether with hemoglobin, disease status, bilirubin, neutrophils, white blood count and ECOG PS, was identified as an independent risk factor for OS ($p = 0.037$)⁶. In addition, in one of the largest second-line study ever performed in BTC, it has been shown that female sex was among clinical features linked to a greater chance of receiving salvage treatments (31% versus 21%, $p = 0.03$)⁷. Notably, though the benefit of second-line treatment is still controversial in BTC, it is reasonable speculating that at least a further line of therapy can contribute to prolonging OS in a number of patients.

A different degree in chemotherapy tolerability according to patient's sex could explain dissimilar outcomes between males and females. Indeed, various reports suggest that renal insult from potential nephrotoxic agents (i.e., gentamycin, amphotericin B, tobramycin and phenobarbital) is more likely to occur in men than in women⁸⁻¹¹. Among them is cisplatin which is one of the reference cytotoxic drugs employed in the treatment of BTC. In an animal preclinical setting, it has been demonstrated that renal function markers, such as serum creatinine, blood urea nitrogen, magnesium and kidney malondialdehyde, were all significantly higher in male rats than female ones when treated with cisplatin¹². The pathophysiology of this observation has been suggested to lie in a differential effect of cisplatin on angiotensin system receptors within the kidneys¹³. In male rats, cisplatin is thought to reduce renal blood inflow leading to a less effective drug excretion. Albeit preclinical, this evidence is intriguing and deserves further assessment also in humans. In fact, better renal elimination of cisplatin in women could result in less adverse events and discontinuations of treatment and/or withdrawals with an increase in dose intensity and a favorable impact of treatment on survival outcomes.

Some emerging data are also suggesting that sex hormones and their metabolic pathways might play a role in sex-related biological diversities seen in BTC. Kaewlert et al focused on CYP19A1 (also called aromatase, a member of the cytochrome P450 superfamily), a key enzyme in estrogen biosynthesis, catalyzing the aromatization of aromatizable androgens (androstenedione and testosterone) into estrogens¹⁴. The authors showed that male CCA patients who present with higher tissue expression of CYP19A1 have a higher level of circulating estrogens and a worse prognosis. Elevated serum levels of estrogens are known to cause aberrant estrogen-ER interactions that in turn can trigger expressions of estrogen-responsive genes via activating nuclear and/or membrane receptor (IGF1-R) signalling cascade, among which are VEGF-A, VEGF-C, and the receptors VEGFR-1, -2 and -3¹⁵. This can ultimately result in CCA progression and more aggressive clinical behavior.

With regard to the prognostic value of NLR, our results are consistent with those of larger retrospective

experiences. McNamara et al showed that an $NLR \geq 3.0$ was an independent prognostic factor for OS in their cohort of 864 patients with both early and advanced stage BTC¹⁶. Median OS was 21.6 months versus 12.0 months for patients with $NLR < 3.0$ versus $NLR \geq 3.0$, respectively. The ratio between neutrophils and lymphocytes mirrors immune system polarization and elevated NLR, resulting from a rise in absolute neutrophil count and/or decrease in lymphocyte count, suggests insufficient anti-tumor immunological reaction and thus worse clinical outcomes.

To this end, sex is a biological variable that has been shown to affect immune response diversity between males and females against both self and foreign antigens and cancer. Several factors have been implicated such as genes encoded on the X chromosome (e.g., cytokine receptors, transcriptional factors, microRNAs and long non-coding RNA) and hormonal mediators (e.g., estradiol, progesterone, androgens) that are differentially expressed between the sexes¹⁷. Notably, it is well-known that males have an almost two-fold greater risk of mortality from all malignancies than females, with the greatest differences seen for larynx, esophagus, bladder and lung cancers¹⁸. Thus, sex-based differences in immune function seem to contribute to the prognosis of cancer patients and deserve further investigation.

Although our results warrant prospective confirmation, they offer insights into how sex and immune system determinants could contribute to the outcome of advanced BTC receiving standard treatment. Remarkably, the finding that female patients seem to survive longer than male patients highlights the need for a translational sex-oriented approach in order to unveil biological reasons for diversity across sexes.

Key messages

- Biliary tract cancers are rare tumours with limited therapeutic options.
- Most patients present with advanced-stage disease only amenable to palliative chemotherapy with survival < 12 months.
- Growing evidence suggests that a small subset of selected patients can survive longer than 2 years from the diagnosis of advanced disease.
- Female sex and neutrophil-to-lymphocyte ratio ≤ 3 have been found associated to the chance of long-term survival in our study.
- Future translational studies are warranted to better characterize this uncommon population of outliers.

References

1. Bridgewater J, Galle PR, Khan SA, Llovet JM, Park JW, Patel T, et al. Guidelines for the diagnosis and management of intrahepatic cholangiocarcinoma. *J Hepatol.* 2014;60:1268-89.
2. Saha SK, Zhu AX, Fuchs CS, Brooks GA. Forty-year trends in cholangiocarcinoma incidence in the US: intrahepatic disease on the rise. *Oncologist.* 2016;21:594-9.
3. Razumilava N, Gores GJ. Cholangiocarcinoma. *Lancet.* 2014;383:2168-79.
4. Valle J, Wasan H, Palmer DH, Cunningham D, Anthony A, Maraveyas A, et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N Engl J Med.* 2010;362:1273-81.
5. Bridgewater J, Lopes A, Palmer D, Cunningham D, Anthony A, Maraveyas A, et al. Quality of life, long-term survivors and long-term outcome from the ABC-02 study. *Br J Cancer.* 2010;114:965-71.
6. Bridgewater J, Lopes A, Wasan H, Malka D, Jensen L, Okusaka T, et al. Prognostic factors for progression-free and overall survival in advanced biliary tract cancer. *Ann Oncol.* 2016;27:134-40.
7. Walter T, Horgan AM, McNamara M, McKeever L, Min T, Hedley D, et al. Feasibility and benefits of second-line chemotherapy in advanced biliary tract cancer: a large retrospective study. *Eur J Cancer.* 2013;49:329-35.
8. Bennett WM, Parker RA, Elliott WC, Gilbert DN, Houghton DC. Sex-related differences in the susceptibility of rats to gentamicin nephrotoxicity. *J Infect Dis.* 1982;145:370-3.
9. Laniado-Laborin R, Cabrales-Vargas MN. Amphotericin B: side effects and toxicity. *Rev Iberoam Micol.* 2009;26:223-7.
10. Goodrich JA, Hottendorf GH. Tobramycin gender-related nephrotoxicity in Fischer but not Sprague-Dawley rats. *Toxicol Lett.* 1995;75(1-3):127-31.
11. Hong SK, Anestis DK, Valentovic MA, Ball JG, Brown PI, Rankin GO. Gender differences in the potentiation of N-(3,5-dichlorophenyl) succinimide metabolite nephrotoxicity by phenobarbital. *J Toxicol Environ Health A.* 2001;64:241-56.
12. Nematbakhsh M, Ebrahimian S, Tooyserkani M, Eshraghi-Jazi F, Talebi A, Ashrafi F. Gender difference in cisplatin-induced nephrotoxicity in a rat model: greater intensity of damage in male than female. *Nephrourol Mon.* 2013;5:818-21.
13. Safari T, Nematbakhsh M, Hilliard LM, Evans RG, Denton KM. Sex differences in the renal vascular response to angiotensin II involves the Mas receptor. *Acta Physiol (Oxf).* 2012;206:150-6.
14. Kaewlert W, Sakonsinsiri C, Namwat N, Sawanyawisuth K, Ungarreevittaya P, Khuntikeo N, et al. The importance of CYP19A1 in estrogen receptor-positive cholangiocarcinoma. *Horm Cancer.* 2018;9:408-19.
15. Mancino A, Mancino MG, Glaser SS, Alpini G, Bolognese A, Izzo L, et al. Estrogens stimulate the proliferation of human cholangiocarcinoma by inducing the expression and secretion of vascular endothelial growth factor. *Dig Liver Dis.* 2009;41:156-63.
16. McNamara M, Templeton A, Maganti M, Walter T, Horgan AM, McKeever L, et al. Neutrophil/lymphocyte ratio as a prognostic factor in biliary tract cancer. *Eur J Cancer* 2014; 50 (9): 1581-9.
17. Ortona E. Immunology and immune-mediated diseases. *Ital J Gender-Specific Med.* 2017;3:165-6.
18. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol.* 2016;16: 626-38.

Conflict of interest statement: the Authors declare no conflicts of interest.

Correspondence to:

Massimiliano Salati

Department of Oncology

University Hospital of Modena and Reggio Emilia
Modena, Italy

Division of Cancer Therapeutics

The Institute of Cancer Research and Gastrointestinal Unit

The Royal Marsden Hospital

London and Sutton, UK

email maxsalati@live.it