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**Liquid biopsy in cholangiocarcinoma: Current status and future perspectives**

Rompianesi G *et al*. Liquid biopsy in cholangiocarcinoma

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**Abstract**

Cholangiocarcinoma (CCA) are a heterogeneous group of tumors in terms of aetiology, natural history, morphological subtypes, molecular alterations and management, but all sharing complex diagnosis, management, and poor prognosis. Several mutated genes and epigenetic changes have been detected in CCA, with the potential to identify diagnostic and prognostic biomarkers and therapeutic targets. Accessing tumoral components and genetic material is therefore crucial for the diagnosis, management and selection of targeted therapies; but sampling tumor tissue, when possible, is often risky and difficult to be repeated at different time points. Liquid biopsy (LB) represents a way to overcome these issues and comprises a diverse group of methodologies centering around detection of tumor biomarkers from fluid samples. Compared to the traditional tissue sampling methods LB is less invasive and can be serially repeated, allowing a real-time monitoring of the tumor genetic profile or the response to therapy. In this review, we analysis the current evidence on the possible roles of LB (circulating DNA, circulating RNA, exosomes, cytokines) in the diagnosis and management of patients affected by CCA.

**Key Words:** Liquid biopsy; Cholangiocarcinoma; Circulating biomarkers; Biliary tumors; Circulating DNA; Circulating RNA; Exosomes; Cytokines

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**Core Tip:** Liquid biopsy represents allows to access tumoral components and genetic material from fluid samples. In patients affected by cholangiocarcinoma could play a major role as minimally invasive screening and diagnostic biomarkers, prognostic tools and therapeutic monitoring targets but its role in the clinical practice is still marginal and further research is necessary.

**INTRODUCTION**

Cholangiocarcinoma (CCA) still represents a challenging entity despite the efforts of researchers and clinicians that led to constant advances in the characterization, diagnosis and treatment of this malignancy. It can arise from the bile duct epithelium at any level and is characterized as an adenocarcinoma in the vast majority of cases, although some other rare histological subtypes have been described[1,2]. The term CCA groups together tumors with substantial heterogeneity in terms of aetiology, natural history, morphological subtypes, molecular alterations and management. CCA is generally classified according to the anatomical location as: Intrahepatic CCA (iCCA) when arises from the second-order bile ducts or proximally, perihilar CCA (pCCA) when arises distal to the second-order bile ducts but above the cystic duct and distal CCA (dCCA) when affects the bile duct between the insertion of the cystic duct and the ampulla of Vater. The two most common forms are pCCA and dCCA, accounting for approximately 50%-60% and 30%-40% of the cases respectively[3,4], with iCCA representing only 10%-20% of the total[5]. CCA presents three distinct growth patterns according to the macroscopic appearance: Mass-forming (the most common variant, represented in two-thirds of cases), periductal infiltrating and intraductal growing[6-8]. A rare and independent entity that comprises features of both iCCA and hepatocellular carcinoma (HCC) is represented by the mixed HCC-CCA[9-11].

CCA is the second most common liver malignancy after HCC[12], and its incidence is highly variable across the World, ranging from 0.3/100000 population to as high as 85/100000 in parts of North East Thailand[6,12,13]. The mortality is similarly variable, with rates between 0.7-6/100000[14,15]. This represents a manifestation of the significant role of the genetic predisposition and the environment as risk factors for the CCA carcinogenesis, which is likely to be a multifactorial process leading to variations in the three subtypes[16]. Despite knowledge of aetiological factors, the majority of cases are attributable to sporadic events[2,17]. Multiple risk factors have been identified and have been shown to markedly increase the risk of CCA development in specific groups of patients or geographic areas. A predisposition to CCA has been observed in patients with biliary pathology [bile duct cysts[18,19], primary sclerosing cholangitis (PSC)[20], choledocholithiasis[21-23], pre-malignant biliary lesions[24-27]], liver diseases (cirrhosis[28], viral hepatitis[29-31]), inflammatory bowel disease[18,32], pancreatitis[18], parasitic infections (from *Clonorchis sinensis* and *Opisthorchis viverrini*)[33], metabolic disorders (diabetes[34] and non-alcoholic fatty liver disease[35]), alcohol consumption[36,37], tobacco use[18,37,38] and genetic polymorphism[16].

Despite the apparent heterogeneity in risk factors associated with CCA, all result in damage to the biliary epithelium secondary to combinations of cholestasis and chronic inflammation, indicating that these pathological processes are key to disease biology. Chronic inflammation leads to activation of cytokines and growth factors and is characterized by an influx of immune cells and fibroblasts. This generates an oncogenic microenvironment typified by unregulated epithelial proliferation, increased mutational burden and genomic instability which in turn drive the inflammatory cascade[39,40]. This process also promotes the development of an aberrant vascular network (the angiogenic switch[41]), which promotes uneven distribution of oxygenated blood and ultimately hypoxia; a key driver of metastatic dissemination. Whilst CCA may develop de-novo in a chronically inflamed bile duct, CCA also develops on the background of premalignant lesions including biliary epithelial neoplasia[25], intraductal papillary neoplasm of the bile duct[26,27,42], mucinous cystic neoplasm[43] and intraductal tubule papillary neoplasms[24,26]. Interestingly, only a proportion of these pre-malignant lesions will develop into malignancies and the role that chronic inflammation plays in driving their malignant progression remains unclear.

Mutation status has been extensively studied in CCA and intra- and extrahepatic forms can be defined as two distinct genetic entities characterized by substantial differences in their patterns of mutation[44-46]. Among the most commonly mutated genes are *IDH1/2*, *FGFR2* fusions, *BAP1*, *BRAF*, *ARID1A*, *KRAS*, *TP53* and *SMAD4* in iCCA; *KRAS*, *TP53*, *SMAD4*, *ERBB3*, *PRKACA–PRKACB* fusions and *ELF3* in pCCA and dCCA[15]. Some of these mutations are associated with prognosis, whilst others may indicate sensitivity to a particular pathway inhibitor or use as a predictive biomarker[46-48]. As well as mutational analysis, epigenetic changes have also been identified in CCA, including methylation-driven inactivation of several tumor suppressor genes, histone modification and aberrant expression of non-coding RNAs[49,50]. Dysregulated of non-coding RNAs has been linked to changes in gene expression that regulate cell survival, proliferation, and chemoresistance in CCA[15]. A better understanding of the epigenetic alterations in CCA therefore has the potential to identify new diagnostic and prognostic biomarkers and novel therapeutic targets[51].

pCCA or dCCA are often diagnosed following presentation with biliary obstruction, weight loss and non-specific abdominal pain, whereas iCCA less frequently cause jaundice and are detected at a later stage in their evolution[5,52], with 20%-25% of cases being an incidental finding[53]. In cases of suspected iCCA the diagnostic algorithm should include a contrast-enhanced computed tomography (CT) and/or a magnetic resonance imaging (MRI), in order to evaluate the relationship of the tumor with the adjacent structures, the extent of local invasion, calculate the future liver remnant volume, and can be associated to a chest CT for the detection of distant metastases[15,54,55]. One of the critical aspects is the difficulty in discriminating between iCCA and HCC, especially in case of small tumors < 2 cm of diameter[56,57]. This differentiation is of great importance considering that management, chemotherapy options and prognosis are different between these two forms. In case of pCCA or dCCA the majority of patients present with jaundice, and the initial investigation performed is an ultrasound in order to identify the site of biliary obstruction and exclude the presence of stones with a sensitivity of over 90%[58]. CT and MRI with magnetic resonance cholangiopancreatography are essential investigations to be performed in the diagnostic and staging process of pCCA and dCCA, where an important role is played by endoscopic procedures. Endoscopic ultrasonography (EUS) provides a high detection rate, especially in dCCA, and the possibility of sampling tumor tissue through fine-needle aspiration that comes at the price of risk of bleeding, infections and tumor seeding[59-61]. Endoscopic retrograde cholangiopancreatography can evaluate the biliary strictures and enables the placement of stents to relieve the biliary obstruction and the brushing sampling for cytological analysis. Percutaneous transhepatic cholangiography (PTC) is frequently performed in patients with CCA and can have both a diagnostic and a therapeutic role. PTC can achieve upstream biliary decompression of the future liver remnant in patients amenable of surgical resection or as a palliative procedure in case of unresectable malignancy, can provide accurate imaging of the biliary tree through cholangiography and allow endobiliary brush cytology[62]. Choledochoscopy is playing an increasing role in the diagnosis of extra-hepatic CCA, and with the implementation of the digital fiberoptic SpyGlass the sensitivity and specificity of this methodology are 90% and over[63,64]. Due to the difficult accessibility of CCA especially when located in the perihilar region and their highly desmoplastic and paucicellular nature, the biliary cytology sensitivity is as low as 20%-40%[65], but can be doubled when fluorescent *in situ* hybridization is used to analysis chromosomal instability[66,67]. This technique assumes a relevant role in discriminating malignant from benign strictures in patients affected by PSC, where the inflammatory changes can result in cellular alterations similar to neoplasia or by the multisystem fibroinflammatory disorder named immunoglobulin G4-related disease, where suspicious biliary strictures also can be present[68-70]. The difficulty in distinguishing a benign from a malignant stricture can result in a number of patients undergoing major surgical procedures burdened by a significant morbidity and mortality for a benign condition. Reports show that 3%-24% of patients undergoing surgery for suspected CCA are ultimately diagnosed with a benign condition[71-73]. The role of 18F-fluorodeoxyglucose positron emission tomography is marginal and controversial: Although can provide essential information in the staging process, especially regarding potential lymph node and distant metastases, and in the identification of tumor recurrence; its incorporation in the diagnostic algorithm is still not justified given the low sensitivity[74]. The serum level of carbohydrate antigen 19-9 (CA19-9) has been extensively investigated as a potential biomarker of CCA and its main limitations are the sensitivity too low to be reliable at early stages of the disease, the non-specificity for CCA and the levels significantly altered in case of cholangitis or cholestasis[75,76]. Serum levels < 100 U/L in non-PSC patients and < 129 U/L in PSC patients have shown a negative predictive value of 92% and 99% respectively[54]. Since surgery is the treatment of choice for patients affected by all types of CCA, establishing a correct diagnosis is essential but represents a challenging and complex process that can result in delays of the surgical or medical treatments and have a negative impact on the outcome. Over half of the patients diagnosed with CCA present at an advanced stage, when surgery is not a suitable option and the prognosis is very poor[40,77-80]. Tumor stage, although with different classifications for iCCA, pCCA and dCCA[81], correlates with patient expected survival, with 5-year relative survival rates between 2 and 24% for iCCA and between 2% and 15% for extrahepatic CCA[82].

In this setting, there is a strong need for novel tools that can assist clinicians in diagnosing, managing and monitoring CCA.

**LIQUID BIOPSY**

Thanks to very recent breakthroughs such as the sequencing of the whole genomes of dozens of tumors[83], it became possible to identify and analysis the genetic changes that contribute to cancerogenesis. Tumors have been historically classified by anatomical site and histopathology, but different types of tumors have shown to carry similar recurrent mutations and apparently similar cancers very different sets of mutations, making every tumor a unique entity and giving way to a novel individualized oncologic approach[84-86]. In this scenario, accessing tumoral components and genetic material could play a crucial role in all stages of cancer management, from the diagnosis to the monitoring of the genetic profile that may be subject to a dynamic evolution, and to define targeted therapies for a tailor-made approach. Sampling tumor tissue is often performed with invasive procedures carrying significant risks for the patients and can result in insufficient amount of material for the analysis or be impossible to be done in case of tumors situated in inaccessible locations. Tumors are continually evolving from a mutational and tumor microenvironmental view-point and display immense intra-tumoral heterogeneity. Therefore, if precision medicine is going to become a realistic option, serial sampling from multiple sites within the tumor will be required to continually adjust the personalised therapeutic algorithm. Moreover, being tumors such dynamic and evolving entities, a systematic monitoring of the molecular alterations through serially repeated tissue biopsies, even when possible, would be not easily feasible and anyway provide only information of the biopsied site. A possible way around these technical issues is the liquid biopsy (LB).

The term “LB” comprises a diverse group of methodologies, centering around detection of tumor biomarkers from fluid samples. Blood, plasma, urine or other fluids are accessed in a minimally invasive approach that can be used in a serial manner to interpreted disease biology. A range of technologies can be applied to sample analysis, with next generation sequencing (NGS) providing a high level of sensitivity and a lower limit of detection[87-89], such that even small amounts of tumor-derived genetic material derived can be detected. Greater sequencing depth is obtainable through NGS compared to traditional Sanger sequencing[90], enabling detection of a wide variety of genetic alterations inclusive of single-nucleotide polymorphisms, mutations, insertions and deletions with high accuracy (sensitivity and specificity in the range of 95%-100%), increasing the ability to investigate mutations in cancer and other diseases[91]. Compared to the traditional tissue sampling methods LBs are less invasive and can be serially repeated, allowing a real-time monitoring of the tumor genetic profile or the response to therapy.

***Types of LBs***

**Circulating tumor DNA:** Cell-free DNA (cfDNA) may enter the circulation through apoptosis or necrosis of cells[92,93] and is present as small fragments of 140-200 base pairs[94,95]. Higher yields of cfDNA have been observed in patients affected by inflammatory processes, trauma and cancer, although the pathophysiological mechanisms are not completely understood and the cfDNA concentrations may vary significantly[96]. The small fraction of cfDNA presenting tumor-specific alterations and originating from cancer cells during tumor progression and turnover, is named circulating tumor DNA (ctDNA)[93]. These fragments are generally characterized by the presence of somatic variants and can be difficult to isolate from cfDNA originating from normal cells[97]. The identification of cancer-specific mutations or methylation patterns that are indicative of the tumoral origin of the DNA can be obtained through the polymerase chain reaction (PCR) or NGS, the latter being more expensive but allowing a more comprehensive analysis of a larger set of mutations; an aspect valuable when considering the genetic heterogeneity of most tumors[98-100]. Detection is made difficult in tumors that display limited ctDNA shedding, stroma-rich cancers such as pancreatic adenocarcinoma or CCA[101-103] and from a technical perspective, most assays can only a small number of genes when compared to tissue-based panels[104]. This type of LB showed promising results as a potential screening and early diagnosis tool, as guidance for the selection of the most appropriate treatment strategy, for the detection of minimal residual disease and risk of relapse and prognosis[99,105,106].

**Exosomes, cytokines and proteins:** Exosomes are a subset of extracellular vesicles (EVs) originating from inward budding of the plasmatic membrane at the end of the endosomal pathway[107]. They can be found in all body fluids and are characterized by a small size (around 100 nm) and contain proteins, DNA and RNA[108], being involved in intercellular communication, cancer progression and metastasis[109,110]. Exosomes present both a set of protein regardless their tissue origin and other ones in common with their originating cell, giving them the possibility of being a potential diagnostic or prognostic tumor marker following isolation from body fluids[107,111-113]. An area of particular interest is the analysis of the genetic material that the exosomes carry contained within the phospholipid bilayer; a structure that effectively protects these materials from enzymatic degradation[114]. Upregulation of specific exosome micro-RNAs (miRNA) or the presence of mutations within exosome DNA has been associated with the presence of various cancers indicating that their assessment may have diagnostic value[108,115-117]. Cytokines are a broad and diverse group of proteins playing a key role in cellular communication but are also implicated in cancer development and progression[118,119]. Exosomes can deliver cytokines including interleukin (IL)-6, IL-8, IL-10, tumor necrosis factor-α and transforming growth factor-β, representing possible novel tumor biomarkers[120-122]. Moreover, it has been described how cancer cells-released EVs present a set of integral membrane proteins and membrane-anchored proteins that can be directly involved with tumor angiogenesis and anti-angiogenic therapies resistance[123].

**Circulating tumor RNA**: For over three decades extracellular RNA has been recognized as a tumor biomarker[124]. When in the bloodstream, is more unstable than cfDNA and with a half-life of only a few seconds when not incorporated within vesicles or bound to proteins[125]. Most circulating RNA does not originate from cell apoptosis or necrosis but from active secretion, enabling a real-time assessment of the cell population of origin[126]. The detection and analysis of circulating RNA is performed using techniques and technologies similar to the ones used for cfDNA, providing both quantitative and qualitative information about the circulating transcriptome[127,128]. All circulating RNA classes have been investigated as potential cancer biomarkers, including miRNA, long non-coding RNA and messenger RNA[129-133]. Having a key role in limiting the expression of tumor suppressors and increasing the activation of oncogenic pathways as well as mediating drug sensitivity and resistance mechanisms, circulating tumor RNA has a great potential of clinical significance in cancer early detection, treatment, monitoring and prognosis[134,135].

**Circulating tumor cells:** The ability of tumors to disseminate cells into the bloodstream was first described over 150 years ago[136]. They can originate from the primary or metastatic site and although could be a considerable amount, only a fraction of them will not be rapidly eliminated from the circulation where they can have entered even before the tumor is diagnosed[137-139]. Circulating tumor cells (CTC) can constitute an accessible source of tumor material which can be sampled repeatedly and not going through risky and invasive procedures such as biopsies. The CTC blood concentration is extremely low, in the range of 1-100 cells *per* mL, causing the counting process that usually requires the target cells to be enriched and then detected, isolated and released, to be challenging, time-consuming and expensive[140]. Despite the efforts in implementing new technologies that can ameliorate the CTC detection, the only Food and Drug Administration-approved technique is the CellSearchÒ. The discrimination of the CTC from circulating, mesenchymal-origin hematopoietic cells is obtained by targeting epithelial markers such as EPCAM and cytokeratins. However, these surface markers are heterogeneous and can be lost during the epithelial-mesenchymal transition that cancer cells undergo as they leave the primary site[99,141-144]. The genetic material that is possible to recover from each isolated CTC is small (2-7 picograms), but after amplification can be sufficient to allow genomic and proteomic profiling and molecular screening analysis[145]. In addition to their established role in basic cancer research, CTC can represent a biomarker in patients affected by various tumors[146-151]. DNA methylation profiling in CTC has been shown to be useful in tumor diagnosis, staging, therapeutic monitoring and prognostication[152-154].

**LB IN CCA**

Surgical resection is the only potential curative treatment for CCA and the possibility of obtaining an earlier diagnosis would be of paramount importance, considering that the majority of patients diagnosed with CCA present with an advanced stage precluding any surgical treatment. In this setting, LB would have the fascinating potential to increase the chances of obtaining a diagnosis at less advances stage, overcoming the difficulties and risks of tissue sampling and the diagnostic challenge when it comes to detecting iCCA over HCC. Additionally, it could offer the possibility to assess tumor heterogeneity, identify targeted therapeutic agents according to tumor biology and to evaluate treatment response and mechanisms of resistance to chemotherapy[155].

***cfDNA/ctDNA***

cfDNA and ctDNA are released into circulation from tumor cells undergoing metabolic secretion, apoptosis, or necrosis. The introduction of NGS assessments of cfDNA/ctDNA recently made possible the detection of genetic and epigenetic alterations associated with CCA, thus widening the potential diagnostic and therapeutic value of LB[156]. Mody *et al*[157] reported the largest profiling ctDNA series, based on 138 patients with biliary tract tumors. They found at least one genomic alteration in 89% of cases, including *TP53*, *BRAF*, *FGFR2* and *IDHA1* mutations, *ERBB2* amplifications, and *FGFR2* fusions. Nevertheless, the concordance between mutations observed in cfDNA/ctDNA with those seen in tumor tissue remains unknown. Zill *et al*[158] measured the concordance between tumor tissue biopsies and plasma-derived cfDNA in 26 pancreatobiliary malignancies (including 8 patients with CCA and 18 with pancreatic cancer). They reported a high concordance between mutations detected in tumor biopsies and cfDNA with the latter identifying the 90.3% of mutations also present in tissue biopsies. Similarly, Andersen and Jakobsen[159] screened 31 mutations in *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* in 11 CCA patients *vs* controls, finding a perfect agreement between mutations found in the tumor and in the plasma in all patients.

Importantly, some of the genetic changes seen in CCA are also demonstrated in benign or pre-malignant disease. For example, the identification of circulating BRCA-mutated DNA might imply a germline BRCA mutation without an underlying malignancy, a KRAS mutation as observed in patients with pancreatitis and circulating mutated TP53 might be related to clonal hematopoietic cells of indeterminate potential[160]. Another role for ctDNA/cfDNA is represented by the possibility of monitoring the response to chemotherapy and targeted therapy, thus tracking emergence of chemotherapy resistance, guiding use of second or third line agents[161,162]. In a German study by Ettrich *et al*[163] ctDNA and tumor tissue samples were collected from 24 patients with CCA before and during chemotherapy, the two samples were subjected to deep sequencing of 15 frequently mutated genes in CCA, including *TP53*, *ARID1A*, *KRAS*, *IDH1*, *BAP1*, *PBRM1*, *SMAD4*, *PIK3CA*, *FBXW7*, *CDKN2A*, *ERBB2*, *NRAS*, *IDH2*, *BRAF* and *BLC2*. The overall blood/tissue concordance was 74% and 92% overall and for intrahepatic tumors respectively, and 63% of chemotherapy-naïve patients displayed a change in their mutational profile during chemotherapy. This mutation drift in circulating mutation status was associated with progression-free survival. Another example is represented by the role of genomic alterations in *FGFR2* which have recently been related to targeted therapies tested in a number of clinical trials, including specific anti-FGFR2 antibodies such as BGJ39[164]. Goyal *et al*[165] analyzed the cfDNA in 4 patients enrolled in a Phase II trial assessing the role of BGJ39. Three out of the four included patients experienced an initial tumor regression followed by disease progression during the treatment, with cfDNA analysis demonstrating the presence of a V564F acquired mutation and multiple recurrent point mutations in the *FGFR2* kinase domain at progression. A high concordance was observed between tissue and plasma measurements, with post-progression tumor biopsy and autopsy confirming the different *FGFR2* mutations. Although based on a small group of patients, this study highlights the potential of cfDNA in monitoring CCA targeted therapy, where real-time detection of resistance mutations may provide useful information in guiding treatment selection.

Lastly, aside from using plasma as a source of ctDNA/cfDNA, assessment of bile has recently shown promising results as alternative DNA-containing body fluid in CCA[166,167]. A recent study by Shen *et al*[168] on 6 patients with CCA and 4 with gallbladder cancer, compared bile cfDNA and tumor DNA for mutational variants using a panel of 150 tumor-related genes. Their analysis demonstrated that bile cfDNA consisted of longer fragments, than those detected in plasma cfDNA. This was in agreement with the DNA lengths detected from the tumor tissue sample. Bile cfDNA and tumor DNA for single nucleotide variation, insertion and deletion revealed high sensitivity (94.7%) and specificity (99.9%). Therefore, the authors concluded that bile cfDNA could represent a promising source of tumor genetic material for LB in CCA patients, although having access to bile samples before or after surgery or in patients not undergoing an endobiliary procedures could be a difficult and demanding task.

***EVs and miRNAs***

EVs and miRNAs have received a special attention due to their stability and abundance in biofluids. Recent studies have demonstrated that bile EVs contain a significant amount of miRNAs[169]. These small, noncoding RNAs (18-25 nucleotides) play important roles in the regulation of a number of essential biological processes and are critical for the development of various cancer types[170]. A number of deregulated miRNAs have been recognized to function as oncogenic and/or tumor suppressors. One of the most extensively described miRNAs in CCA is miR-21, an oncogenic miRNA whose overexpression correlates with tumor stage and poor survival[171,172]. Correa-Gallego *et al*[173] compared miRNA-21 expression between 21 iCCA and 11 tumor-free liver samples. Significant overexpression of miR-21 and miR-221 was found in plasma from iCCA patients and circulating miR-21 demonstrated a high discriminatory ability between patients with iCCA and healthy controls [area under the curve (AUC): 0.94]. Similarly, Wang *et al*[174] analyzed the role of circulating miR-21 as a biomarker in patients with a variety of carcinomas in a recent meta-analysis. A total of 36 studies were included for the systemic review, with overall pooled results for sensitivity and specificity of 75.7% [95% confidence interval (CI): 67.1%-82.6%] and 79.3% (95%CI: 74.2%-83.5%), respectively. Interestingly, a higher circulating miR-21 expression was associated with poorer prognosis [odds ratio 2.37 (95%CI: 1.83-3.06, *P* < 0.001)], therefore the authors concluded that in addition to the role as a diagnostic biomarker, circulating miR-21 could have a prognostic value in patients with cancer. Nevertheless, similarly to ctDNA/cfDNA detection in plasma, the identification of miRNA should be interpreted with caution since this biomarker can also be identified in the serum of patients affected by HCC or other liver diseases[175]. Several other circulating miRNAs have shown potential as biomarkers with the ability to discriminate between CCA and other liver diseases[176-179]. Loosen *et al*[177] analyzed the concentrations of a 4 miRNAs panel (miR-122, miR-192, miR-29b and miR-155) in serum samples from 94 patients with CCA undergoing surgical resection and 40 healthy controls. The serum concentrations of all mi-RNAs were significantly elevated in patients with CCA compared to healthy controls or patients with PSC without malignant transformation. Furthermore, a strong postoperative decline of miR-122 serum levels was significantly associated with a more favorable prognosis and outcome. Similarly, Cheng *et al*[180] observed different expression levels of circulating miR-106a, sufficiently to discriminate patients affected by CCA from healthy controls and also from patients affected by benign bile duct diseases inclusive of biliary tract calculi and congenital biliary cysts. The presence and role of miRNA in bile samples has been recently investigated with some promising results[181,182]. Han *et al*[176] analyzed a panel of 1209 miRNAs in bile samples from 106 patients with obstructive biliary disease and found that miR-30d-5p and miR-92a-3p were significantly upregulated in patients with CCA. Similarly, Voigtländer *et al*[183] identified distinct circulating and bile miRNA profiles in patients affected by PSC with or without CCA, showing potential as a screening and diagnostic biomarker. CCA patients had a higher expression of miR-1281, miR-126, miR-26a, miR-30b and miR-122 in serum samples and different concentrations of miR-412, miR-640, miR-1537 and miR-3189 in bile samples compared to PSC patients. The diagnostic value of miRNAs for CCA has been reviewed and confirmed by two meta-analyses, that found an AUC of 0.88-0.9 and pooled sensitivities of 0.76-0.85 and specificities of 0.79-0.91[184,185]. Notably, in these analyses bile was the biological fluid that yielded the highest diagnostic capacity followed by serum, tissue and urine, with AUCs of 0.95, 0.913, 0.846 and 0.745 respectively.

Several studies have highlighted the potential of miRNAs as diagnostic and prognostic biomarkers for CCA, however there is still lack of strong evidence regarding the source of mi-RNA and which subgroup would provide the most reliable information to be translated into clinical practice. The available evidence is usually limited by super selected study populations with an artificially high prevalence of CCA, with the risk of overestimating the diagnostic accuracy. A possible option to overcome this issue could be to plan to prospectively collect and analysis samples as part of a trial.

***Proteins, cytokines and other serum metabolites***

Proteins, cytokines and other serum metabolites have recently been evaluated as potential diagnostic and prognostic biomarkers for CCA. Elevated circulating levels of cytokeratin-19 (CYFRA 21-1), MMP-7, osteopontin, periostin and IL-6 have been found in the serum of patients with CCA, differently from healthy individuals and patients with benign biliary diseases[186-193]. Huang *et al*[192] assessed the serum levels of CYFRA 21-1 in comparison with CA19-9 and carcinoembryonic antigen preoperatively, postoperatively and during follow-up in 134 patients with biliary tract cancers and 52 controls without tumors. CYFRA 21-1 had the best diagnostic performance with levels presenting a high concordance with the clinical and oncological status of the patients and showing a decline after surgical resection and an increase when the tumor recurred. Additionally, it represented an independent predictor of 1-year recurrence-free survival and overall survival on multivariate analysis. Osteopontin has also been investigated by Loosen *et al*[193] in 107 patients undergoing surgical resection for CCA. Here, elevated osteopontin was significantly elevated in CCA patients when compared to healthy controls and PSC patients, whilst pre- and postoperative serum concentrations correlated with patient survival. Banales *et al*[194] investigated whether serum metabolomes represent a useful source of biomarkers in the diagnosis of iCCA, HCC and PSC. They found several metabolites that demonstrated high diagnostic value for iCCA and developed two algorithms combining four and six metabolites in order to achieve the best diagnostic value in differentiating iCCA, HCC and PSC. Glycine, aspartic acid, SM (42:3), and SM (43:2) accurately differentiated iCCA from HCC with an AUC of 0.89, 75% sensitivity, and 90% specificity. The addition of PC (34:3) and histidine accurately permitted to differentiate iCCA from PSC with an impressive AUC of 0.99, 100% sensitivity, and 70% specificity. These results were subsequently validated in an independent cohort of patients.

Since cancer cells present a variety of marked metabolic alterations, measuring metabolites in distinct biological samples is now regarded as an encouraging way to identify accurate diagnostic and prognostic biomarkers. However, as yet, none have been adopted into routine clinical practice.

***CTC***

Although only a few studies have addressed the potential role of CTC as diagnostic and prognostic biomarkers in CCA, some recent research has postulated the association between the number of CTCs and patient survival in patients with biliary tract cancer[195]. After analysis the presence of CTC in 88 patients with CCA, Yang *et al*[196] found that a CTC count ≥ 5 was associated with tumor extent and more aggressive tumor characteristics, as well as independently predicting shorter survival [CTC ≥ 2 hazard ratio (HR) of 2.5; 95%CI: 1.1-5.4; *P* = 0.02 and CTC ≥ 5 HR of 4.1; 95%CI: 1.4-10.8; *P* = 0.01].

**CONCLUSION**

Despite the constant advances, CCA still represents an unsolved challenge for clinicians and researchers, burdened by difficult diagnosis, management, treatment and with a dismal patient survival. The only curative option is surgical resection, but it can be offered only to a fraction of patients and is burdened by high recurrence rates. Therefore, maximum effort should be applied to increase the current knowledge of this genetically highly heterogeneous group of tumors in order to obtain an earlier diagnosis, be able to identify patients that will benefit from a specific treatment and develop better targeted therapies. In this scenario, liquid biopsies could play a major role as minimally invasive screening and diagnostic biomarkers, prognostic tools and therapeutic monitoring targets (Table 1). The role of LB in the clinical practice of patients affected by CCA is still marginal and further research is necessary to appreciate its potential and move towards a multimodal, precision medicine approach.

**REFERENCES**

1 **Nakanuma Y**, Sato Y, Harada K, Sasaki M, Xu J, Ikeda H. Pathological classification of intrahepatic cholangiocarcinoma based on a new concept. *World J Hepatol* 2010; **2**: 419-427 [PMID: 21191517 DOI: 10.4254/wjh.v2.i12.419]

2 **Razumilava N,** Gores GJ. Cholangiocarcinoma. *Lancet* 2014; **383:** 2168-2179 [PMID: 24581682 DOI: 10.1016/S0140-6736(13)61903-0]

3 **DeOliveira ML,** Cunningham SC, Cameron JL, Kamangar F, Winter JM, Lillemoe KD, Choti MA, Yeo CJ, Schulick RD. Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. *Ann Surg* 2007; **245:** 755-762 [PMID: 17457168 DOI: 10.1097/01.sla.0000251366.62632.d3]

4 **Ghouri YA**, Mian I, Blechacz B. Cancer review: Cholangiocarcinoma. *J Carcinog* 2015; **14**: 1 [PMID: 25788866 DOI: 10.4103/1477-3163.151940]

5 **Nakeeb A**, Pitt HA, Sohn TA, Coleman J, Abrams RA, Piantadosi S, Hruban RH, Lillemoe KD, Yeo CJ, Cameron JL. Cholangiocarcinoma. A spectrum of intrahepatic, perihilar, and distal tumors. *Ann Surg* 1996; **224**: 463-73; discussion 473-5 [PMID: 8857851 DOI: 10.1097/00000658-199610000-00005]

6 **Banales JM,** Cardinale V, Carpino G, Marzioni M, Andersen JB, Invernizzi P, Lind GE, Folseraas T, Forbes SJ, Fouassier L, Geier A, Calvisi DF, Mertens JC, Trauner M, Benedetti A, Maroni L, Vaquero J, Macias RI, Raggi C, Perugorria MJ, Gaudio E, Boberg KM, Marin JJ, Alvaro D. Expert consensus document: Cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA). *Nat Rev Gastroenterol Hepatol* 2016; **13:** 261-280 [PMID: 27095655 DOI: 10.1038/nrgastro.2016.51]

7 **Nakanuma Y**, Xu J, Harada K, Sato Y, Sasaki M, Ikeda H, Kim J, Yu E. Pathological spectrum of intrahepatic cholangiocarcinoma arising in non-biliary chronic advanced liver diseases. *Pathol Int* 2011; **61**: 298-305 [PMID: 21501296 DOI: 10.1111/j.1440-1827.2011.02665.x]

8 **Vijgen S**, Terris B, Rubbia-Brandt L. Pathology of intrahepatic cholangiocarcinoma. *Hepatobiliary Surg Nutr* 2017; **6**: 22-34 [PMID: 28261592 DOI: 10.21037/hbsn.2016.11.04]

9 **Brunt E**, Aishima S, Clavien PA, Fowler K, Goodman Z, Gores G, Gouw A, Kagen A, Klimstra D, Komuta M, Kondo F, Miksad R, Nakano M, Nakanuma Y, Ng I, Paradis V, Nyun Park Y, Quaglia A, Roncalli M, Roskams T, Sakamoto M, Saxena R, Sempoux C, Sirlin C, Stueck A, Thung S, Tsui WMS, Wang XW, Wee A, Yano H, Yeh M, Zen Y, Zucman-Rossi J, Theise N. cHCC-CCA: Consensus terminology for primary liver carcinomas with both hepatocytic and cholangiocytic differentation. *Hepatology* 2018; **68**: 113-126 [PMID: 29360137 DOI: 10.1002/hep.29789]

10 **Roskams T**. Liver stem cells and their implication in hepatocellular and cholangiocarcinoma. *Oncogene* 2006; **25**: 3818-3822 [PMID: 16799623 DOI: 10.1038/sj.onc.1209558]

11 **Komuta M**, Govaere O, Vandecaveye V, Akiba J, Van Steenbergen W, Verslype C, Laleman W, Pirenne J, Aerts R, Yano H, Nevens F, Topal B, Roskams T. Histological diversity in cholangiocellular carcinoma reflects the different cholangiocyte phenotypes. *Hepatology* 2012; **55**: 1876-1888 [PMID: 22271564 DOI: 10.1002/hep.25595]

12 **Shaib Y**, El-Serag HB. The epidemiology of cholangiocarcinoma. *Semin Liver Dis* 2004; **24**: 115-125 [PMID: 15192785 DOI: 10.1055/s-2004-828889]

13 **Tyson GL**, El-Serag HB. Risk factors for cholangiocarcinoma. *Hepatology* 2011; **54**: 173-184 [PMID: 21488076 DOI: 10.1002/hep.24351]

14 **Bertuccio P,** Malvezzi M, Carioli G, Hashim D, Boffetta P, El-Serag HB, La Vecchia C, Negri E. Global trends in mortality from intrahepatic and extrahepatic cholangiocarcinoma. *J Hepatol* 2019; **71:** 104-114 [PMID: 30910538 DOI: 10.1016/j.jhep.2019.03.013]

15 **Banales JM**, Marin JJG, Lamarca A, Rodrigues PM, Khan SA, Roberts LR, Cardinale V, Carpino G, Andersen JB, Braconi C, Calvisi DF, Perugorria MJ, Fabris L, Boulter L, Macias RIR, Gaudio E, Alvaro D, Gradilone SA, Strazzabosco M, Marzioni M, Coulouarn C, Fouassier L, Raggi C, Invernizzi P, Mertens JC, Moncsek A, Rizvi S, Heimbach J, Koerkamp BG, Bruix J, Forner A, Bridgewater J, Valle JW, Gores GJ. Cholangiocarcinoma 2020: the next horizon in mechanisms and management. *Nat Rev Gastroenterol Hepatol* 2020; **17**: 557-588 [PMID: 32606456 DOI: 10.1038/s41575-020-0310-z]

16 **Khan SA**, Tavolari S, Brandi G. Cholangiocarcinoma: Epidemiology and risk factors. *Liver Int* 2019; **39 Suppl 1**: 19-31 [PMID: 30851228 DOI: 10.1111/liv.14095]

17 **Bagante F**, Gamblin TC, Pawlik TM. Cholangiocarcinoma risk factors and the potential role of aspirin. *Hepatology* 2016; **64**: 708-710 [PMID: 27112798 DOI: 10.1002/hep.28613]

18 **Petrick JL**, Yang B, Altekruse SF, Van Dyke AL, Koshiol J, Graubard BI, McGlynn KA. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma in the United States: A population-based study in SEER-Medicare. *PLoS One* 2017; **12**: e0186643 [PMID: 29049401 DOI: 10.1371/journal.pone.0186643]

19 **Söreide K**, Körner H, Havnen J, Söreide JA. Bile duct cysts in adults. *Br J Surg* 2004; **91**: 1538-1548 [PMID: 15549778 DOI: 10.1002/bjs.4815]

20 **Boonstra K,** Weersma RK, van Erpecum KJ, Rauws EA, Spanier BW, Poen AC, van Nieuwkerk KM, Drenth JP, Witteman BJ, Tuynman HA, Naber AH, Kingma PJ, van Buuren HR, van Hoek B, Vleggaar FP, van Geloven N, Beuers U, Ponsioen CY, Epi PSG. Population-based epidemiology, malignancy risk, and outcome of primary sclerosing cholangitis. *Hepatology* 2013; **58:** 2045-2055 [PMID: 23775876 DOI: 10.1002/hep.26565]

21 **Kim HJ**, Kim JS, Joo MK, Lee BJ, Kim JH, Yeon JE, Park JJ, Byun KS, Bak YT. Hepatolithiasis and intrahepatic cholangiocarcinoma: A review. *World J Gastroenterol* 2015; **21**: 13418-13431 [PMID: 26730152 DOI: 10.3748/wjg.v21.i48.13418]

22 **Lin CC**, Lin PY, Chen YL. Comparison of concomitant and subsequent cholangiocarcinomas associated with hepatolithiasis: Clinical implications. *World J Gastroenterol* 2013; **19**: 375-380 [PMID: 23372360 DOI: 10.3748/wjg.v19.i3.375]

23 **Schottenfeld D**, Beebe-Dimmer J. Chronic inflammation: a common and important factor in the pathogenesis of neoplasia. *CA Cancer J Clin* 2006; **56**: 69-83 [PMID: 16514135 DOI: 10.3322/canjclin.56.2.69]

24 **Katabi N**, Torres J, Klimstra DS. Intraductal tubular neoplasms of the bile ducts. *Am J Surg Pathol* 2012; **36**: 1647-1655 [PMID: 23073323 DOI: 10.1097/PAS.0b013e3182684d4f]

25 **Sato Y**, Harada K, Sasaki M, Nakanuma Y. Histological Characterization of Biliary Intraepithelial Neoplasia with respect to Pancreatic Intraepithelial Neoplasia. *Int J Hepatol* 2014; **2014**: 678260 [PMID: 24860672 DOI: 10.1155/2014/678260]

26 **Schlitter AM**, Born D, Bettstetter M, Specht K, Kim-Fuchs C, Riener MO, Jeliazkova P, Sipos B, Siveke JT, Terris B, Zen Y, Schuster T, Höfler H, Perren A, Klöppel G, Esposito I. Intraductal papillary neoplasms of the bile duct: stepwise progression to carcinoma involves common molecular pathways. *Mod Pathol* 2014; **27**: 73-86 [PMID: 23828315 DOI: 10.1038/modpathol.2013.112]

27 **Wan XS**, Xu YY, Qian JY, Yang XB, Wang AQ, He L, Zhao HT, Sang XT. Intraductal papillary neoplasm of the bile duct. *World J Gastroenterol* 2013; **19**: 8595-8604 [PMID: 24379576 DOI: 10.3748/wjg.v19.i46.8595]

28 **Chang JS**, Tsai CR, Chen LT. Medical risk factors associated with cholangiocarcinoma in Taiwan: a population-based case-control study. *PLoS One* 2013; **8**: e69981 [PMID: 23894567 DOI: 10.1371/journal.pone.0069981]

29 **Zhang H**, Zhu B, Zhang H, Liang J, Zeng W. HBV Infection Status and the Risk of Cholangiocarcinoma in Asia: A Meta-Analysis. *Biomed Res Int* 2016; **2016**: 3417976 [PMID: 27999794 DOI: 10.1155/2016/3417976]

30 **Li H**, Hu B, Zhou ZQ, Guan J, Zhang ZY, Zhou GW. Hepatitis C virus infection and the risk of intrahepatic cholangiocarcinoma and extrahepatic cholangiocarcinoma: evidence from a systematic review and meta-analysis of 16 case-control studies. *World J Surg Oncol* 2015; **13**: 161 [PMID: 25903488 DOI: 10.1186/s12957-015-0583-9]

31 **Ralphs S**, Khan SA. The role of the hepatitis viruses in cholangiocarcinoma. *J Viral Hepat* 2013; **20**: 297-305 [PMID: 23565610 DOI: 10.1111/jvh.12093]

32 **Huai JP**, Ding J, Ye XH, Chen YP. Inflammatory bowel disease and risk of cholangiocarcinoma: evidence from a meta-analysis of population-based studies. *Asian Pac J Cancer Prev* 2014; **15**: 3477-3482 [PMID: 24870743 DOI: 10.7314/apjcp.2014.15.8.3477]

33 **Shin HR**, Oh JK, Masuyer E, Curado MP, Bouvard V, Fang YY, Wiangnon S, Sripa B, Hong ST. Epidemiology of cholangiocarcinoma: an update focusing on risk factors. *Cancer Sci* 2010; **101**: 579-585 [PMID: 20085587 DOI: 10.1111/j.1349-7006.2009.01458.x]

34 **Jing W**, Jin G, Zhou X, Zhou Y, Zhang Y, Shao C, Liu R, Hu X. Diabetes mellitus and increased risk of cholangiocarcinoma: a meta-analysis. *Eur J Cancer Prev* 2012; **21**: 24-31 [PMID: 21857525 DOI: 10.1097/CEJ.0b013e3283481d89]

35 **Wongjarupong N**, Assavapongpaiboon B, Susantitaphong P, Cheungpasitporn W, Treeprasertsuk S, Rerknimitr R, Chaiteerakij R. Non-alcoholic fatty liver disease as a risk factor for cholangiocarcinoma: a systematic review and meta-analysis. *BMC Gastroenterol* 2017; **17**: 149 [PMID: 29216833 DOI: 10.1186/s12876-017-0696-4]

36 **Palmer WC**, Patel T. Are common factors involved in the pathogenesis of primary liver cancers? A meta-analysis of risk factors for intrahepatic cholangiocarcinoma. *J Hepatol* 2012; **57**: 69-76 [PMID: 22420979 DOI: 10.1016/j.jhep.2012.02.022]

37 **Petrick JL**, Thistle JE, Zeleniuch-Jacquotte A, Zhang X, Wactawski-Wende J, Van Dyke AL, Stampfer MJ, Sinha R, Sesso HD, Schairer C, Rosenberg L, Rohan TE, Robien K, Purdue MP, Poynter JN, Palmer JR, Newton CC, Linet MS, Liao LM, Lee IM, Koshiol J, Kitahara CM, Hofmann JN, Graubard BI, Giovannucci E, Gaziano MJ, Gapstur SM, Freedman ND, Chong DQ, Chan AT, Buring JE, Freeman LBE, Campbell PT, McGlynn KA. Body Mass Index, Diabetes and Intrahepatic Cholangiocarcinoma Risk: The Liver Cancer Pooling Project and Meta-analysis. *Am J Gastroenterol* 2018; **113**: 1494-1505 [PMID: 30177781 DOI: 10.1038/s41395-018-0207-4]

38 **Ye XH**, Huai JP, Ding J, Chen YP, Sun XC. Smoking, alcohol consumption, and the risk of extrahepatic cholangiocarcinoma: a meta-analysis. *World J Gastroenterol* 2013; **19**: 8780-8788 [PMID: 24379600 DOI: 10.3748/wjg.v19.i46.8780]

39 **Andersen JB**. Molecular pathogenesis of intrahepatic cholangiocarcinoma. *J Hepatobiliary Pancreat Sci* 2015; **22**: 101-113 [PMID: 25174625 DOI: 10.1002/jhbp.155]

40 **Squadroni M**, Tondulli L, Gatta G, Mosconi S, Beretta G, Labianca R. Cholangiocarcinoma. *Crit Rev Oncol Hematol* 2017; **116**: 11-31 [PMID: 28693792 DOI: 10.1016/j.critrevonc.2016.11.012]

41 **Bergers G**, Benjamin LE. Tumorigenesis and the angiogenic switch. *Nat Rev Cancer* 2003; **3**: 401-410 [PMID: 12778130 DOI: 10.1038/nrc1093]

42 **Gordon-Weeks AN**, Jones K, Harriss E, Smith A, Silva M. Systematic Review and Meta-analysis of Current Experience in Treating IPNB: Clinical and Pathological Correlates. *Ann Surg* 2016; **263**: 656-663 [PMID: 26501712 DOI: 10.1097/SLA.0000000000001426]

43 **Nakajima T**, Sugano I, Matsuzaki O, Nagao K, Kondo Y, Miyazaki M, Wada K. Biliary cystadenocarcinoma of the liver. A clinicopathologic and histochemical evaluation of nine cases. *Cancer* 1992; **69**: 2426-2432 [PMID: 1314687 DOI: 10.1002/1097-0142(19920515)69:10<2426::aid-cncr2820691007>3.0.co;2-3]

44 **Churi CR**, Shroff R, Wang Y, Rashid A, Kang HC, Weatherly J, Zuo M, Zinner R, Hong D, Meric-Bernstam F, Janku F, Crane CH, Mishra L, Vauthey JN, Wolff RA, Mills G, Javle M. Mutation profiling in cholangiocarcinoma: prognostic and therapeutic implications. *PLoS One* 2014; **9**: e115383 [PMID: 25536104 DOI: 10.1371/journal.pone.0115383]

45 **Javle M**, Bekaii-Saab T, Jain A, Wang Y, Kelley RK, Wang K, Kang HC, Catenacci D, Ali S, Krishnan S, Ahn D, Bocobo AG, Zuo M, Kaseb A, Miller V, Stephens PJ, Meric-Bernstam F, Shroff R, Ross J. Biliary cancer: Utility of next-generation sequencing for clinical management. *Cancer* 2016; **122**: 3838-3847 [PMID: 27622582 DOI: 10.1002/cncr.30254]

46 **Ruzzenente A**, Fassan M, Conci S, Simbolo M, Lawlor RT, Pedrazzani C, Capelli P, D'Onofrio M, Iacono C, Scarpa A, Guglielmi A. Cholangiocarcinoma Heterogeneity Revealed by Multigene Mutational Profiling: Clinical and Prognostic Relevance in Surgically Resected Patients. *Ann Surg Oncol* 2016; **23**: 1699-1707 [PMID: 26717940 DOI: 10.1245/s10434-015-5046-6]

47 **Lowery MA**, Ptashkin R, Jordan E, Berger MF, Zehir A, Capanu M, Kemeny NE, O'Reilly EM, El-Dika I, Jarnagin WR, Harding JJ, D'Angelica MI, Cercek A, Hechtman JF, Solit DB, Schultz N, Hyman DM, Klimstra DS, Saltz LB, Abou-Alfa GK. Comprehensive Molecular Profiling of Intrahepatic and Extrahepatic Cholangiocarcinomas: Potential Targets for Intervention. *Clin Cancer Res* 2018; **24**: 4154-4161 [PMID: 29848569 DOI: 10.1158/1078-0432.CCR-18-0078]

48 **Jain A**, Kwong LN, Javle M. Genomic Profiling of Biliary Tract Cancers and Implications for Clinical Practice. *Curr Treat Options Oncol* 2016; **17**: 58 [PMID: 27658789 DOI: 10.1007/s11864-016-0432-2]

49 **Tischoff I**, Wittekind C, Tannapfel A. Role of epigenetic alterations in cholangiocarcinoma. *J Hepatobiliary Pancreat Surg* 2006; **13**: 274-279 [PMID: 16858537 DOI: 10.1007/s00534-005-1055-3]

50 **Chiang NJ**, Shan YS, Hung WC, Chen LT. Epigenetic regulation in the carcinogenesis of cholangiocarcinoma. *Int J Biochem Cell Biol* 2015; **67**: 110-114 [PMID: 26100596 DOI: 10.1016/j.biocel.2015.06.012]

51 **O'Rourke CJ**, Lafuente-Barquero J, Andersen JB. Epigenome Remodeling in Cholangiocarcinoma. *Trends Cancer* 2019; **5**: 335-350 [PMID: 31208696 DOI: 10.1016/j.trecan.2019.05.002]

52 **Blechacz B**, Komuta M, Roskams T, Gores GJ. Clinical diagnosis and staging of cholangiocarcinoma. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 512-522 [PMID: 21808282 DOI: 10.1038/nrgastro.2011.131]

53 **Alvaro D,** Bragazzi MC, Benedetti A, Fabris L, Fava G, Invernizzi P, Marzioni M, Nuzzo G, Strazzabosco M, Stroffolini T, committee AC. Cholangiocarcinoma in Italy: A national survey on clinical characteristics, diagnostic modalities and treatment. Results from the "Cholangiocarcinoma" committee of the Italian Association for the Study of Liver disease. *Dig Liver Dis* 2011; **43:** 60-65 [PMID: 20580332 DOI: 10.1016/j.dld.2010.05.002]

54 **Blechacz B**. Cholangiocarcinoma: Current Knowledge and New Developments. *Gut Liver* 2017; **11**: 13-26 [PMID: 27928095 DOI: 10.5009/gnl15568]

55 **You MW**, Yun SJ. Differentiating between hepatocellular carcinoma and intrahepatic cholangiocarcinoma using contrast-enhanced MRI features: a systematic review and meta-analysis. *Clin Radiol* 2019; **74**: 406.e9-406.e18 [PMID: 30704667 DOI: 10.1016/j.crad.2018.12.016]

56 **Iavarone M**, Piscaglia F, Vavassori S, Galassi M, Sangiovanni A, Venerandi L, Forzenigo LV, Golfieri R, Bolondi L, Colombo M. Contrast enhanced CT-scan to diagnose intrahepatic cholangiocarcinoma in patients with cirrhosis. *J Hepatol* 2013; **58**: 1188-1193 [PMID: 23485522 DOI: 10.1016/j.jhep.2013.02.013]

57 **Kim SH**, Lee CH, Kim BH, Kim WB, Yeom SK, Kim KA, Park CM. Typical and atypical imaging findings of intrahepatic cholangiocarcinoma using gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid-enhanced magnetic resonance imaging. *J Comput Assist Tomogr* 2012; **36**: 704-709 [PMID: 23192208 DOI: 10.1097/RCT.0b013e3182706562]

58 **Sharma MP**, Ahuja V. Aetiological spectrum of obstructive jaundice and diagnostic ability of ultrasonography: a clinician's perspective. *Trop Gastroenterol* 1999; **20**: 167-169 [PMID: 10769604]

59 **Rizvi S**, Khan SA, Hallemeier CL, Kelley RK, Gores GJ. Cholangiocarcinoma - evolving concepts and therapeutic strategies. *Nat Rev Clin Oncol* 2018; **15**: 95-111 [PMID: 28994423 DOI: 10.1038/nrclinonc.2017.157]

60 **Chhieng DC**. Fine needle aspiration biopsy of liver - an update. *World J Surg Oncol* 2004; **2**: 5 [PMID: 15025788 DOI: 10.1186/1477-7819-2-5]

61 **Weilert F**, Bhat YM, Binmoeller KF, Kane S, Jaffee IM, Shaw RE, Cameron R, Hashimoto Y, Shah JN. EUS-FNA is superior to ERCP-based tissue sampling in suspected malignant biliary obstruction: results of a prospective, single-blind, comparative study. *Gastrointest Endosc* 2014; **80**: 97-104 [PMID: 24559784 DOI: 10.1016/j.gie.2013.12.031]

62 **Xing GS**, Geng JC, Han XW, Dai JH, Wu CY. Endobiliary brush cytology during percutaneous transhepatic cholangiodrainage in patients with obstructive jaundice. *Hepatobiliary Pancreat Dis Int* 2005; **4**: 98-103 [PMID: 15730930]

63 **Dimas ID,** Fragaki M, Vardas E, Paspatis GA. Digital cholangioscopy (Spyglass) in the diagnosis of cholangiocarcinoma. *Ann Gastroenterol* 2017; **30:** 253 [PMID: 28243051 DOI: 10.20524/aog.2016.0110]

64 **Nimura Y**. Staging cholangiocarcinoma by cholangioscopy. *HPB (Oxford)* 2008; **10**: 113-115 [PMID: 18773067 DOI: 10.1080/13651820801992658]

65 **Trikudanathan G**, Navaneethan U, Njei B, Vargo JJ, Parsi MA. Diagnostic yield of bile duct brushings for cholangiocarcinoma in primary sclerosing cholangitis: a systematic review and meta-analysis. *Gastrointest Endosc* 2014; **79**: 783-789 [PMID: 24140129 DOI: 10.1016/j.gie.2013.09.015]

66 **Navaneethan U**, Njei B, Venkatesh PG, Vargo JJ, Parsi MA. Fluorescence in situ hybridization for diagnosis of cholangiocarcinoma in primary sclerosing cholangitis: a systematic review and meta-analysis. *Gastrointest Endosc* 2014; **79**: 943-950.e3 [PMID: 24360654 DOI: 10.1016/j.gie.2013.11.001]

67 **Barr Fritcher EG,** Voss JS, Brankley SM, Campion MB, Jenkins SM, Keeney ME, Henry MR, Kerr SM, Chaiteerakij R, Pestova EV, Clayton AC, Zhang J, Roberts LR, Gores GJ, Halling KC, Kipp BR. An Optimized Set of Fluorescence In Situ Hybridization Probes for Detection of Pancreatobiliary Tract Cancer in Cytology Brush Samples. *Gastroenterology* 2015; **149:** 1813-1824 e1 [PMID: 26327129 DOI: 10.1053/j.gastro.2015.08.046]

68 **Ghazale A**, Chari ST, Zhang L, Smyrk TC, Takahashi N, Levy MJ, Topazian MD, Clain JE, Pearson RK, Petersen BT, Vege SS, Lindor K, Farnell MB. Immunoglobulin G4-associated cholangitis: clinical profile and response to therapy. *Gastroenterology* 2008; **134**: 706-715 [PMID: 18222442 DOI: 10.1053/j.gastro.2007.12.009]

69 **Tanaka A**, Tazuma S, Okazaki K, Nakazawa T, Inui K, Chiba T, Takikawa H. Clinical Features, Response to Treatment, and Outcomes of IgG4-Related Sclerosing Cholangitis. *Clin Gastroenterol Hepatol* 2017; **15**: 920-926.e3 [PMID: 28111336 DOI: 10.1016/j.cgh.2016.12.038]

70 **Kendall T**, Verheij J, Gaudio E, Evert M, Guido M, Goeppert B, Carpino G. Anatomical, histomorphological and molecular classification of cholangiocarcinoma. *Liver Int* 2019; **39 Suppl 1**: 7-18 [PMID: 30882996 DOI: 10.1111/liv.14093]

71 **Clayton RA**, Clarke DL, Currie EJ, Madhavan KK, Parks RW, Garden OJ. Incidence of benign pathology in patients undergoing hepatic resection for suspected malignancy. *Surgeon* 2003; **1**: 32-38 [PMID: 15568422 DOI: 10.1016/s1479-666x(03)80006-9]

72 **Otsuka S**, Ebata T, Yokoyama Y, Igami T, Mizuno T, Yamaguchi J, Onoe S, Watanabe N, Shimoyama Y, Nagino M. Benign hilar bile duct strictures resected as perihilar cholangiocarcinoma. *Br J Surg* 2019; **106**: 1504-1511 [PMID: 31386198 DOI: 10.1002/bjs.11257]

73 **Geramizadeh B**, Moughali M, Shahim-Aein A, Memari S, Ghetmiri Z, Taghavi A, Bagheri Lankarani K. False negative and false positive rates in common bile duct brushing cytology, a single center experience. *Gastroenterol Hepatol Bed Bench* 2018; **11**: 296-300 [PMID: 30425807]

74 **Lamarca A**, Barriuso J, Chander A, McNamara MG, Hubner RA, ÓReilly D, Manoharan P, Valle JW. 18F-fluorodeoxyglucose positron emission tomography (18FDG-PET) for patients with biliary tract cancer: Systematic review and meta-analysis. *J Hepatol* 2019; **71**: 115-129 [PMID: 30797051 DOI: 10.1016/j.jhep.2019.01.038]

75 **Aljiffry M**, Walsh MJ, Molinari M. Advances in diagnosis, treatment and palliation of cholangiocarcinoma: 1990-2009. *World J Gastroenterol* 2009; **15**: 4240-4262 [PMID: 19750567 DOI: 10.3748/wjg.15.4240]

76 **Sinakos E,** Saenger AK, Keach J, Kim WR, Lindor KD. Many patients with primary sclerosing cholangitis and increased serum levels of carbohydrate antigen 19-9 do not have cholangiocarcinoma. *Clin Gastroenterol Hepatol* 2011; **9:** 434-9.e1 [PMID: 21334457 DOI: 10.1016/j.cgh.2011.02.007]

77 **Waseem D**, Tushar P. Intrahepatic, perihilar and distal cholangiocarcinoma: Management and outcomes. *Ann Hepatol* 2017; **16**: 133-139 [PMID: 28051802 DOI: 10.5604/16652681.1226927]

78 **Brandi G,** Venturi M, Pantaleo MA, Ercolani G; GICO. Cholangiocarcinoma: Current opinion on clinical practice diagnostic and therapeutic algorithms: A review of the literature and a long-standing experience of a referral center. *Dig Liver Dis* 2016; **48:** 231-241 [PMID: 26769568 DOI: 10.1016/j.dld.2015.11.017]

79 **Forner A**, Vidili G, Rengo M, Bujanda L, Ponz-Sarvisé M, Lamarca A. Clinical presentation, diagnosis and staging of cholangiocarcinoma. *Liver Int* 2019; **39 Suppl 1**: 98-107 [PMID: 30831002 DOI: 10.1111/liv.14086]

80 **Jarnagin WR,** Fong Y, DeMatteo RP, Gonen M, Burke EC, Bodniewicz BS J, Youssef BA M, Klimstra D, Blumgart LH. Staging, resectability, and outcome in 225 patients with hilar cholangiocarcinoma. *Ann Surg* 2001; **234:** 507-17; discussion 517-9 [PMID: 11573044 DOI: 10.1097/00000658-200110000-00010]

81 **Amin MB**, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, Meyer L, Gress DM, Byrd DR, Winchester DP. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA Cancer J Clin* 2017; **67**: 93-99 [PMID: 28094848 DOI: 10.3322/caac.21388]

82 **American Cancer Society.** Cancer Facts & Figures 2021. [cited 10 January 2021] Available from: https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2021.html

83 **ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium.** Pan-cancer analysis of whole genomes. *Nature* 2020; **578:** 82-93 [PMID: 32025007 DOI: 10.1038/s41586-020-1969-6]

84 **Ashley EA**. Towards precision medicine. *Nat Rev Genet* 2016; **17**: 507-522 [PMID: 27528417 DOI: 10.1038/nrg.2016.86]

85 **Hyman DM**, Taylor BS, Baselga J. Implementing Genome-Driven Oncology. *Cell* 2017; **168**: 584-599 [PMID: 28187282 DOI: 10.1016/j.cell.2016.12.015]

86 **McGranahan N**, Swanton C. Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future. *Cell* 2017; **168**: 613-628 [PMID: 28187284 DOI: 10.1016/j.cell.2017.01.018]

87 **Rivas MA,** Beaudoin M, Gardet A, Stevens C, Sharma Y, Zhang CK, Boucher G, Ripke S, Ellinghaus D, Burtt N, Fennell T, Kirby A, Latiano A, Goyette P, Green T, Halfvarson J, Haritunians T, Korn JM, Kuruvilla F, Lagacé C, Neale B, Lo KS, Schumm P, Törkvist L; National Institute of Diabetes and Digestive Kidney Diseases Inflammatory Bowel Disease Genetics Consortium (NIDDK IBDGC); United Kingdom Inflammatory Bowel Disease Genetics Consortium; International Inflammatory Bowel Disease Genetics Consortium, Dubinsky MC, Brant SR, Silverberg MS, Duerr RH, Altshuler D, Gabriel S, Lettre G, Franke A, D'Amato M, McGovern DP, Cho JH, Rioux JD, Xavier RJ, Daly MJ. Deep resequencing of GWAS loci identifies independent rare variants associated with inflammatory bowel disease. *Nat Genet* 2011; **43:** 1066-1073 [PMID: 21983784 DOI: 10.1038/ng.952]

88 **Shendure J**, Ji H. Next-generation DNA sequencing. *Nat Biotechnol* 2008; **26**: 1135-1145 [PMID: 18846087 DOI: 10.1038/nbt1486]

89 **Schuster SC**. Next-generation sequencing transforms today's biology. *Nat Methods* 2008; **5**: 16-18 [PMID: 18165802 DOI: 10.1038/nmeth1156]

90 **Sanger F**, Coulson AR. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *J Mol Biol* 1975; **94**: 441-448 [PMID: 1100841 DOI: 10.1016/0022-2836(75)90213-2]

91 **Robin JD**, Ludlow AT, LaRanger R, Wright WE, Shay JW. Comparison of DNA Quantification Methods for Next Generation Sequencing. *Sci Rep* 2016; **6**: 24067 [PMID: 27048884 DOI: 10.1038/srep24067]

92 **Stroun M**, Lyautey J, Lederrey C, Olson-Sand A, Anker P. About the possible origin and mechanism of circulating DNA apoptosis and active DNA release. *Clin Chim Acta* 2001; **313**: 139-142 [PMID: 11694251 DOI: 10.1016/s0009-8981(01)00665-9]

93 **Crowley E**, Di Nicolantonio F, Loupakis F, Bardelli A. Liquid biopsy: monitoring cancer-genetics in the blood. *Nat Rev Clin Oncol* 2013; **10**: 472-484 [PMID: 23836314 DOI: 10.1038/nrclinonc.2013.110]

94 **Mouliere F**, Robert B, Arnau Peyrotte E, Del Rio M, Ychou M, Molina F, Gongora C, Thierry AR. High fragmentation characterizes tumour-derived circulating DNA. *PLoS One* 2011; **6**: e23418 [PMID: 21909401 DOI: 10.1371/journal.pone.0023418]

95 **Batth IS**, Mitra A, Manier S, Ghobrial IM, Menter D, Kopetz S, Li S. Circulating tumor markers: harmonizing the yin and yang of CTCs and ctDNA for precision medicine. *Ann Oncol* 2017; **28**: 468-477 [PMID: 27998963 DOI: 10.1093/annonc/mdw619]

96 **Schwarzenbach H,** Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. *Nat Rev Cancer* 2011; **11:** 426-437 [PMID: 21562580 DOI: 10.1038/nrc3066]

97 **Bronkhorst AJ**, Ungerer V, Holdenrieder S. The emerging role of cell-free DNA as a molecular marker for cancer management. *Biomol Detect Quantif* 2019; **17**: 100087 [PMID: 30923679 DOI: 10.1016/j.bdq.2019.100087]

98 **Zhang W,** Xia W, Lv Z, Ni C, Xin Y, Yang L. Liquid Biopsy for Cancer: Circulating Tumor Cells, Circulating Free DNA or Exosomes? *Cell Physiol Biochem* 2017; **41:** 755-768 [PMID: 28214887 DOI: 10.1159/000458736]

99 **Palmirotta R**, Lovero D, Cafforio P, Felici C, Mannavola F, Pellè E, Quaresmini D, Tucci M, Silvestris F. Liquid biopsy of cancer: a multimodal diagnostic tool in clinical oncology. *Ther Adv Med Oncol* 2018; **10**: 1758835918794630 [PMID: 30181785 DOI: 10.1177/1758835918794630]

100 **Xu RH**, Wei W, Krawczyk M, Wang W, Luo H, Flagg K, Yi S, Shi W, Quan Q, Li K, Zheng L, Zhang H, Caughey BA, Zhao Q, Hou J, Zhang R, Xu Y, Cai H, Li G, Hou R, Zhong Z, Lin D, Fu X, Zhu J, Duan Y, Yu M, Ying B, Zhang W, Wang J, Zhang E, Zhang C, Li O, Guo R, Carter H, Zhu JK, Hao X, Zhang K. Circulating tumour DNA methylation markers for diagnosis and prognosis of hepatocellular carcinoma. *Nat Mater* 2017; **16**: 1155-1161 [PMID: 29035356 DOI: 10.1038/nmat4997]

101 **Waghray M**, Yalamanchili M, di Magliano MP, Simeone DM. Deciphering the role of stroma in pancreatic cancer. *Curr Opin Gastroenterol* 2013; **29**: 537-543 [PMID: 23892539 DOI: 10.1097/MOG.0b013e328363affe]

102 **Brivio S**, Cadamuro M, Strazzabosco M, Fabris L. Tumor reactive stroma in cholangiocarcinoma: The fuel behind cancer aggressiveness. *World J Hepatol* 2017; **9**: 455-468 [PMID: 28396716 DOI: 10.4254/wjh.v9.i9.455]

103 **Gentilini A**, Pastore M, Marra F, Raggi C. The Role of Stroma in Cholangiocarcinoma: The Intriguing Interplay between Fibroblastic Component, Immune Cell Subsets and Tumor Epithelium. *Int J Mol Sci* 2018; **19** [PMID: 30249019 DOI: 10.3390/ijms19102885]

104 **Rizzo A**, Ricci AD, Tavolari S, Brandi G. Circulating Tumor DNA in Biliary Tract Cancer: Current Evidence and Future Perspectives. *Cancer Genomics Proteomics* 2020; **17**: 441-452 [PMID: 32859625 DOI: 10.21873/cgp.20203]

105 **Chen M**, Zhao H. Next-generation sequencing in liquid biopsy: cancer screening and early detection. *Hum Genomics* 2019; **13**: 34 [PMID: 31370908 DOI: 10.1186/s40246-019-0220-8]

106 **Smania MA**. Liquid biopsy for cancer screening, diagnosis, and treatment. *J Am Assoc Nurse Pract* 2020; **32**: 5-7 [PMID: 31913212 DOI: 10.1097/JXX.0000000000000359]

107 **Théry C**, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol* 2002; **2**: 569-579 [PMID: 12154376 DOI: 10.1038/nri855]

108 **Thakur BK**, Zhang H, Becker A, Matei I, Huang Y, Costa-Silva B, Zheng Y, Hoshino A, Brazier H, Xiang J, Williams C, Rodriguez-Barrueco R, Silva JM, Zhang W, Hearn S, Elemento O, Paknejad N, Manova-Todorova K, Welte K, Bromberg J, Peinado H, Lyden D. Double-stranded DNA in exosomes: a novel biomarker in cancer detection. *Cell Res* 2014; **24**: 766-769 [PMID: 24710597 DOI: 10.1038/cr.2014.44]

109 **Harding CV**, Heuser JE, Stahl PD. Exosomes: looking back three decades and into the future. *J Cell Biol* 2013; **200**: 367-371 [PMID: 23420870 DOI: 10.1083/jcb.201212113]

110 **Weidle UH**, Birzele F, Kollmorgen G, Rüger R. The Multiple Roles of Exosomes in Metastasis. *Cancer Genomics Proteomics* 2017; **14**: 1-15 [PMID: 28031234 DOI: 10.21873/cgp.20015]

111 **Logozzi M**, De Milito A, Lugini L, Borghi M, Calabrò L, Spada M, Perdicchio M, Marino ML, Federici C, Iessi E, Brambilla D, Venturi G, Lozupone F, Santinami M, Huber V, Maio M, Rivoltini L, Fais S. High levels of exosomes expressing CD63 and caveolin-1 in plasma of melanoma patients. *PLoS One* 2009; **4**: e5219 [PMID: 19381331 DOI: 10.1371/journal.pone.0005219]

112 **Nilsson J**, Skog J, Nordstrand A, Baranov V, Mincheva-Nilsson L, Breakefield XO, Widmark A. Prostate cancer-derived urine exosomes: a novel approach to biomarkers for prostate cancer. *Br J Cancer* 2009; **100**: 1603-1607 [PMID: 19401683 DOI: 10.1038/sj.bjc.6605058]

113 **Costa-Silva B**, Aiello NM, Ocean AJ, Singh S, Zhang H, Thakur BK, Becker A, Hoshino A, Mark MT, Molina H, Xiang J, Zhang T, Theilen TM, García-Santos G, Williams C, Ararso Y, Huang Y, Rodrigues G, Shen TL, Labori KJ, Lothe IM, Kure EH, Hernandez J, Doussot A, Ebbesen SH, Grandgenett PM, Hollingsworth MA, Jain M, Mallya K, Batra SK, Jarnagin WR, Schwartz RE, Matei I, Peinado H, Stanger BZ, Bromberg J, Lyden D. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. *Nat Cell Biol* 2015; **17**: 816-826 [PMID: 25985394 DOI: 10.1038/ncb3169]

114 **Huang X**, Yuan T, Tschannen M, Sun Z, Jacob H, Du M, Liang M, Dittmar RL, Liu Y, Liang M, Kohli M, Thibodeau SN, Boardman L, Wang L. Characterization of human plasma-derived exosomal RNAs by deep sequencing. *BMC Genomics* 2013; **14**: 319 [PMID: 23663360 DOI: 10.1186/1471-2164-14-319]

115 **Madhavan B**, Yue S, Galli U, Rana S, Gross W, Müller M, Giese NA, Kalthoff H, Becker T, Büchler MW, Zöller M. Combined evaluation of a panel of protein and miRNA serum-exosome biomarkers for pancreatic cancer diagnosis increases sensitivity and specificity. *Int J Cancer* 2015; **136**: 2616-2627 [PMID: 25388097 DOI: 10.1002/ijc.29324]

116 **Kahlert C**, Melo SA, Protopopov A, Tang J, Seth S, Koch M, Zhang J, Weitz J, Chin L, Futreal A, Kalluri R. Identification of double-stranded genomic DNA spanning all chromosomes with mutated KRAS and p53 DNA in the serum exosomes of patients with pancreatic cancer. *J Biol Chem* 2014; **289**: 3869-3875 [PMID: 24398677 DOI: 10.1074/jbc.C113.532267]

117 **Dai J**, Su Y, Zhong S, Cong L, Liu B, Yang J, Tao Y, He Z, Chen C, Jiang Y. Exosomes: key players in cancer and potential therapeutic strategy. *Signal Transduct Target Ther* 2020; **5**: 145 [PMID: 32759948 DOI: 10.1038/s41392-020-00261-0]

118 **Yao M**, Brummer G, Acevedo D, Cheng N. Cytokine Regulation of Metastasis and Tumorigenicity. *Adv Cancer Res* 2016; **132**: 265-367 [PMID: 27613135 DOI: 10.1016/bs.acr.2016.05.005]

119 **Tan C**, Hu W, He Y, Zhang Y, Zhang G, Xu Y, Tang J. Cytokine-mediated therapeutic resistance in breast cancer. *Cytokine* 2018; **108**: 151-159 [PMID: 29609137 DOI: 10.1016/j.cyto.2018.03.020]

120 **Wang Y**, Yi J, Chen X, Zhang Y, Xu M, Yang Z. The regulation of cancer cell migration by lung cancer cell-derived exosomes through TGF-β and IL-10. *Oncol Lett* 2016; **11**: 1527-1530 [PMID: 26893774 DOI: 10.3892/ol.2015.4044]

121 **Martinez VG**, O'Neill S, Salimu J, Breslin S, Clayton A, Crown J, O'Driscoll L. Resistance to HER2-targeted anti-cancer drugs is associated with immune evasion in cancer cells and their derived extracellular vesicles. *Oncoimmunology* 2017; **6**: e1362530 [PMID: 29209569 DOI: 10.1080/2162402X.2017.1362530]

122 **Jung HH**, Kim JY, Lim JE, Im YH. Cytokine profiling in serum-derived exosomes isolated by different methods. *Sci Rep* 2020; **10**: 14069 [PMID: 32826923 DOI: 10.1038/s41598-020-70584-z]

123 **Ko SY**, Naora H. Extracellular Vesicle Membrane-Associated Proteins: Emerging Roles in Tumor Angiogenesis and Anti-Angiogenesis Therapy Resistance. *Int J Mol Sci* 2020; **21** [PMID: 32751440 DOI: 10.3390/ijms21155418]

124 **Wieczorek AJ**, Sitaramam V, Machleidt W, Rhyner K, Perruchoud AP, Block LH. Diagnostic and prognostic value of RNA-proteolipid in sera of patients with malignant disorders following therapy: first clinical evaluation of a novel tumor marker. *Cancer Res* 1987; **47**: 6407-6412 [PMID: 2445471]

125 **De Rubis G**, Rajeev Krishnan S, Bebawy M. Liquid Biopsies in Cancer Diagnosis, Monitoring, and Prognosis. *Trends Pharmacol Sci* 2019; **40**: 172-186 [PMID: 30736982 DOI: 10.1016/j.tips.2019.01.006]

126 **Javidi MA**, Ahmadi AH, Bakhshinejad B, Nouraee N, Babashah S, Sadeghizadeh M. Cell-free microRNAs as cancer biomarkers: the odyssey of miRNAs through body fluids. *Med Oncol* 2014; **31**: 295 [PMID: 25362261 DOI: 10.1007/s12032-014-0295-y]

127 **Sole C**, Arnaiz E, Manterola L, Otaegui D, Lawrie CH. The circulating transcriptome as a source of cancer liquid biopsy biomarkers. *Semin Cancer Biol* 2019; **58**: 100-108 [PMID: 30684535 DOI: 10.1016/j.semcancer.2019.01.003]

128 **Zaporozhchenko IA**, Ponomaryova AA, Rykova EY, Laktionov PP. The potential of circulating cell-free RNA as a cancer biomarker: challenges and opportunities. *Expert Rev Mol Diagn* 2018; **18**: 133-145 [PMID: 29307231 DOI: 10.1080/14737159.2018.1425143]

129 **Xu YF**, Hannafon BN, Zhao YD, Postier RG, Ding WQ. Plasma exosome miR-196a and miR-1246 are potential indicators of localized pancreatic cancer. *Oncotarget* 2017; **8**: 77028-77040 [PMID: 29100367 DOI: 10.18632/oncotarget.20332]

130 **Jin X**, Chen Y, Chen H, Fei S, Chen D, Cai X, Liu L, Lin B, Su H, Zhao L, Su M, Pan H, Shen L, Xie D, Xie C. Evaluation of Tumor-Derived Exosomal miRNA as Potential Diagnostic Biomarkers for Early-Stage Non-Small Cell Lung Cancer Using Next-Generation Sequencing. *Clin Cancer Res* 2017; **23**: 5311-5319 [PMID: 28606918 DOI: 10.1158/1078-0432.CCR-17-0577]

131 **Santangelo A**, Imbrucè P, Gardenghi B, Belli L, Agushi R, Tamanini A, Munari S, Bossi AM, Scambi I, Benati D, Mariotti R, Di Gennaro G, Sbarbati A, Eccher A, Ricciardi GK, Ciceri EM, Sala F, Pinna G, Lippi G, Cabrini G, Dechecchi MC. A microRNA signature from serum exosomes of patients with glioma as complementary diagnostic biomarker. *J Neurooncol* 2018; **136**: 51-62 [PMID: 29076001 DOI: 10.1007/s11060-017-2639-x]

132 **Tan SK**, Pastori C, Penas C, Komotar RJ, Ivan ME, Wahlestedt C, Ayad NG. Serum long noncoding RNA HOTAIR as a novel diagnostic and prognostic biomarker in glioblastoma multiforme. *Mol Cancer* 2018; **17**: 74 [PMID: 29558959 DOI: 10.1186/s12943-018-0822-0]

133 **Zhang K**, Shi H, Xi H, Wu X, Cui J, Gao Y, Liang W, Hu C, Liu Y, Li J, Wang N, Wei B, Chen L. Genome-Wide lncRNA Microarray Profiling Identifies Novel Circulating lncRNAs for Detection of Gastric Cancer. *Theranostics* 2017; **7**: 213-227 [PMID: 28042329 DOI: 10.7150/thno.16044]

134 **Jiang N**, Pan J, Fang S, Zhou C, Han Y, Chen J, Meng X, Jin X, Gong Z. Liquid biopsy: Circulating exosomal long noncoding RNAs in cancer. *Clin Chim Acta* 2019; **495**: 331-337 [PMID: 31054913 DOI: 10.1016/j.cca.2019.04.082]

135 **Ratti M**, Lampis A, Ghidini M, Salati M, Mirchev MB, Valeri N, Hahne JC. MicroRNAs (miRNAs) and Long Non-Coding RNAs (lncRNAs) as New Tools for Cancer Therapy: First Steps from Bench to Bedside. *Target Oncol* 2020; **15**: 261-278 [PMID: 32451752 DOI: 10.1007/s11523-020-00717-x]

136 **Ashworth TR**. A case of cancer in which cells similar to those in the tumors were seen in the blood after death. *Med J Aust* 1869; **14**: 146-147

137 **Young R**, Pailler E, Billiot F, Drusch F, Barthelemy A, Oulhen M, Besse B, Soria JC, Farace F, Vielh P. Circulating tumor cells in lung cancer. *Acta Cytol* 2012; **56**: 655-660 [PMID: 23207444 DOI: 10.1159/000345182]

138 **Steinert G**, Schölch S, Niemietz T, Iwata N, García SA, Behrens B, Voigt A, Kloor M, Benner A, Bork U, Rahbari NN, Büchler MW, Stoecklein NH, Weitz J, Koch M. Immune escape and survival mechanisms in circulating tumor cells of colorectal cancer. *Cancer Res* 2014; **74**: 1694-1704 [PMID: 24599131 DOI: 10.1158/0008-5472.CAN-13-1885]

139 **Meng S**, Tripathy D, Frenkel EP, Shete S, Naftalis EZ, Huth JF, Beitsch PD, Leitch M, Hoover S, Euhus D, Haley B, Morrison L, Fleming TP, Herlyn D, Terstappen LW, Fehm T, Tucker TF, Lane N, Wang J, Uhr JW. Circulating tumor cells in patients with breast cancer dormancy. *Clin Cancer Res* 2004; **10**: 8152-8162 [PMID: 15623589 DOI: 10.1158/1078-0432.CCR-04-1110]

140 **Esmaeilsabzali H**, Beischlag TV, Cox ME, Parameswaran AM, Park EJ. Detection and isolation of circulating tumor cells: principles and methods. *Biotechnol Adv* 2013; **31**: 1063-1084 [PMID: 23999357 DOI: 10.1016/j.biotechadv.2013.08.016]

141 **Alix-Panabières C**, Pantel K. Technologies for detection of circulating tumor cells: facts and vision. *Lab Chip* 2014; **14**: 57-62 [PMID: 24145967 DOI: 10.1039/c3lc50644d]

142 **Ferreira MM,** Ramani VC, Jeffrey SS. Circulating tumor cell technologies. *Mol Oncol* 2016; **10:** 374-394 [PMID: 26897752 DOI: 10.1016/j.molonc.2016.01.007]

143 **Yadavalli S**, Jayaram S, Manda SS, Madugundu AK, Nayakanti DS, Tan TZ, Bhat R, Rangarajan A, Chatterjee A, Gowda H, Thiery JP, Kumar P. Data-Driven Discovery of Extravasation Pathway in Circulating Tumor Cells. *Sci Rep* 2017; **7**: 43710 [PMID: 28262832 DOI: 10.1038/srep43710]

144 **Bankó P,** Lee SY, Nagygyörgy V, Zrínyi M, Chae CH, Cho DH, Telekes A. Technologies for circulating tumor cell separation from whole blood. *J Hematol Oncol* 2019; **12:** 48 [PMID: 31088479 DOI: 10.1186/s13045-019-0735-4]

145 **Palmirotta R**, Lovero D, Silvestris E, Felici C, Quaresmini D, Cafforio P, Silvestris F. Next-generation Sequencing (NGS) Analysis on Single Circulating Tumor Cells (CTCs) with No Need of Whole-genome Amplification (WGA). *Cancer Genomics Proteomics* 2017; **14**: 173-179 [PMID: 28446532 DOI: 10.21873/cgp.20029]

146 **Cristofanilli M**, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, Reuben JM, Doyle GV, Allard WJ, Terstappen LW, Hayes DF. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med* 2004; **351**: 781-791 [PMID: 15317891 DOI: 10.1056/NEJMoa040766]

147 **Krebs MG**, Hou JM, Ward TH, Blackhall FH, Dive C. Circulating tumour cells: their utility in cancer management and predicting outcomes. *Ther Adv Med Oncol* 2010; **2**: 351-365 [PMID: 21789147 DOI: 10.1177/1758834010378414]

148 **Mavroudis D**. Circulating cancer cells. *Ann Oncol* 2010; **21 Suppl 7**: vii95-vi100 [PMID: 20943650 DOI: 10.1093/annonc/mdq378]

149 **Paterlini-Brechot P**, Benali NL. Circulating tumor cells (CTC) detection: clinical impact and future directions. *Cancer Lett* 2007; **253**: 180-204 [PMID: 17314005 DOI: 10.1016/j.canlet.2006.12.014]

150 **Pantel K**, Alix-Panabières C. The clinical significance of circulating tumor cells. *Nat Clin Pract Oncol* 2007; **4**: 62-63 [PMID: 17259923 DOI: 10.1038/ncponc0737]

151 **Devriese LA**, Voest EE, Beijnen JH, Schellens JH. Circulating tumor cells as pharmacodynamic biomarker in early clinical oncological trials. *Cancer Treat Rev* 2011; **37**: 579-589 [PMID: 21592671 DOI: 10.1016/j.ctrv.2011.04.006]

152 **Pixberg CF**, Schulz WA, Stoecklein NH, Neves RP. Characterization of DNA Methylation in Circulating Tumor Cells. *Genes (Basel)* 2015; **6**: 1053-1075 [PMID: 26506390 DOI: 10.3390/genes6041053]

153 **Chimonidou M**, Strati A, Tzitzira A, Sotiropoulou G, Malamos N, Georgoulias V, Lianidou ES. DNA methylation of tumor suppressor and metastasis suppressor genes in circulating tumor cells. *Clin Chem* 2011; **57**: 1169-1177 [PMID: 21700955 DOI: 10.1373/clinchem.2011.165902]

154 **Friedlander TW**, Ngo VT, Dong H, Premasekharan G, Weinberg V, Doty S, Zhao Q, Gilbert EG, Ryan CJ, Chen WT, Paris PL. Detection and characterization of invasive circulating tumor cells derived from men with metastatic castration-resistant prostate cancer. *Int J Cancer* 2014; **134**: 2284-2293 [PMID: 24166007 DOI: 10.1002/ijc.28561]

155 **Bettegowda C**, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, Bartlett BR, Wang H, Luber B, Alani RM, Antonarakis ES, Azad NS, Bardelli A, Brem H, Cameron JL, Lee CC, Fecher LA, Gallia GL, Gibbs P, Le D, Giuntoli RL, Goggins M, Hogarty MD, Holdhoff M, Hong SM, Jiao Y, Juhl HH, Kim JJ, Siravegna G, Laheru DA, Lauricella C, Lim M, Lipson EJ, Marie SK, Netto GJ, Oliner KS, Olivi A, Olsson L, Riggins GJ, Sartore-Bianchi A, Schmidt K, Shih lM, Oba-Shinjo SM, Siena S, Theodorescu D, Tie J, Harkins TT, Veronese S, Wang TL, Weingart JD, Wolfgang CL, Wood LD, Xing D, Hruban RH, Wu J, Allen PJ, Schmidt CM, Choti MA, Velculescu VE, Kinzler KW, Vogelstein B, Papadopoulos N, Diaz LA Jr. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med* 2014; **6**: 224ra24 [PMID: 24553385 DOI: 10.1126/scitranslmed.3007094]

156 **Newman AM,** Bratman SV, To J, Wynne JF, Eclov NC, Modlin LA, Liu CL, Neal JW, Wakelee HA, Merritt RE, Shrager JB, Loo BW, Jr., Alizadeh AA, Diehn M. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. *Nat Med* 2014; **20:** 548-554 [PMID: 24705333 DOI: 10.1038/nm.3519]

157 **Mody K**, Kasi PM, Yang JD, Surapaneni PK, Ritter A, Roberts A, Nagy R, Borad MJ. Feasibility of circulating tumor DNA testing in hepatocellular carcinoma. *J Gastrointest Oncol* 2019; **10**: 745-750 [PMID: 31392055 DOI: 10.21037/jgo.2019.02.10]

158 **Zill OA**, Greene C, Sebisanovic D, Siew LM, Leng J, Vu M, Hendifar AE, Wang Z, Atreya CE, Kelley RK, Van Loon K, Ko AH, Tempero MA, Bivona TG, Munster PN, Talasaz A, Collisson EA. Cell-Free DNA Next-Generation Sequencing in Pancreatobiliary Carcinomas. *Cancer Discov* 2015; **5**: 1040-1048 [PMID: 26109333 DOI: 10.1158/2159-8290.CD-15-0274]

159 **Andersen RF**, Jakobsen A. Screening for circulating RAS/RAF mutations by multiplex digital PCR. *Clin Chim Acta* 2016; **458**: 138-143 [PMID: 27181912 DOI: 10.1016/j.cca.2016.05.007]

160 **Jaiswal S**, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, Lindsley RC, Mermel CH, Burtt N, Chavez A, Higgins JM, Moltchanov V, Kuo FC, Kluk MJ, Henderson B, Kinnunen L, Koistinen HA, Ladenvall C, Getz G, Correa A, Banahan BF, Gabriel S, Kathiresan S, Stringham HM, McCarthy MI, Boehnke M, Tuomilehto J, Haiman C, Groop L, Atzmon G, Wilson JG, Neuberg D, Altshuler D, Ebert BL. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med* 2014; **371**: 2488-2498 [PMID: 25426837 DOI: 10.1056/NEJMoa1408617]

161 **Lucci A,** Hall CS, Lodhi AK, Bhattacharyya A, Anderson AE, Xiao L, Bedrosian I, Kuerer HM, Krishnamurthy S. Circulating tumour cells in non-metastatic breast cancer: a prospective study. *Lancet Oncol* 2012; **13:** 688-695 [PMID: 22677156 DOI: 10.1016/S1470-2045(12)70209-7]

162 **Ansari J**, Yun JW, Kompelli AR, Moufarrej YE, Alexander JS, Herrera GA, Shackelford RE. The liquid biopsy in lung cancer. *Genes Cancer* 2016; **7**: 355-367 [PMID: 28191282 DOI: 10.18632/genesandcancer.127]

163 **Ettrich TJ**, Schwerdel D, Dolnik A, Beuter F, Blätte TJ, Schmidt SA, Stanescu-Siegmund N, Steinacker J, Marienfeld R, Kleger A, Bullinger L, Seufferlein T, Berger AW. Genotyping of circulating tumor DNA in cholangiocarcinoma reveals diagnostic and prognostic information. *Sci Rep* 2019; **9**: 13261 [PMID: 31519967 DOI: 10.1038/s41598-019-49860-0]

164 **Lamarca A**, Barriuso J, McNamara MG, Valle JW. Molecular targeted therapies: Ready for "prime time" in biliary tract cancer. *J Hepatol* 2020; **73**: 170-185 [PMID: 32171892 DOI: 10.1016/j.jhep.2020.03.007]

165 **Goyal L,** Saha SK, Liu LY, Siravegna G, Leshchiner I, Ahronian LG, Lennerz JK, Vu P, Deshpande V, Kambadakone A, Mussolin B, Reyes S, Henderson L, Sun JE, Van Seventer EE, Gurski JM, Jr., Baltschukat S, Schacher-Engstler B, Barys L, Stamm C, Furet P, Ryan DP, Stone JR, Iafrate AJ, Getz G, Porta DG, Tiedt R, Bardelli A, Juric D, Corcoran RB, Bardeesy N, Zhu AX. Polyclonal Secondary FGFR2 Mutations Drive Acquired Resistance to FGFR Inhibition in Patients with FGFR2 Fusion-Positive Cholangiocarcinoma. *Cancer Discov* 2017; **7:** 252-263 [PMID: 28034880 DOI: 10.1158/2159-8290.CD-16-1000]

166 **Adrait A,** Dumonceau JM, Delhaye M, Annessi-Ramseyer I, Frossard JL, Couté Y, Farina A. Liquid Biopsy of Bile based on Targeted Mass Spectrometry for the Diagnosis of Malignant Biliary Strictures. *Clin Transl Sci* 2021; 14: 148-152 [PMID: 33048472 DOI: 10.1111/cts.12890]

167 **Kinugasa H**, Nouso K, Ako S, Dohi C, Matsushita H, Matsumoto K, Kato H, Okada H. Liquid biopsy of bile for the molecular diagnosis of gallbladder cancer. *Cancer Biol Ther* 2018; **19**: 934-938 [PMID: 29580156 DOI: 10.1080/15384047.2018.1456604]

168 **Shen N**, Zhang D, Yin L, Qiu Y, Liu J, Yu W, Fu X, Zhu B, Xu X, Duan A, Chen Z, Wang X, Cao X, Zhao T, Zhou Z, Yu L, Qin H, Fang Z, Li JY, Liu Y, Xiong L, Yuan B, Li F, Zhang Y. Bile cell‑free DNA as a novel and powerful liquid biopsy for detecting somatic variants in biliary tract cancer. *Oncol Rep* 2019; **42**: 549-560 [PMID: 31173267 DOI: 10.3892/or.2019.7177]

169 **Li L**, Masica D, Ishida M, Tomuleasa C, Umegaki S, Kalloo AN, Georgiades C, Singh VK, Khashab M, Amateau S, Li Z, Okolo P, Lennon AM, Saxena P, Geschwind JF, Schlachter T, Hong K, Pawlik TM, Canto M, Law J, Sharaiha R, Weiss CR, Thuluvath P, Goggins M, Shin EJ, Peng H, Kumbhari V, Hutfless S, Zhou L, Mezey E, Meltzer SJ, Karchin R, Selaru FM. Human bile contains microRNA-laden extracellular vesicles that can be used for cholangiocarcinoma diagnosis. *Hepatology* 2014; **60**: 896-907 [PMID: 24497320 DOI: 10.1002/hep.27050]

170 **Yang G**, Zhang L, Li R, Wang L. The role of microRNAs in gallbladder cancer. *Mol Clin Oncol* 2016; **5**: 7-13 [PMID: 27330755 DOI: 10.3892/mco.2016.905]

171 **Kishimoto T**, Eguchi H, Nagano H, Kobayashi S, Akita H, Hama N, Wada H, Kawamoto K, Tomokuni A, Tomimaru Y, Umeshita K, Doki Y, Mori M. Plasma miR-21 is a novel diagnostic biomarker for biliary tract cancer. *Cancer Sci* 2013; **104**: 1626-1631 [PMID: 24118467 DOI: 10.1111/cas.12300]

172 **Liu CH**, Huang Q, Jin ZY, Xie F, Zhu CL, Liu Z, Wang C. Circulating microRNA-21 as a prognostic, biological marker in cholangiocarcinoma. *J Cancer Res Ther* 2018; **14**: 220-225 [PMID: 29516989 DOI: 10.4103/0973-1482.193125]

173 **Correa-Gallego C**, Maddalo D, Doussot A, Kemeny N, Kingham TP, Allen PJ, D'Angelica MI, DeMatteo RP, Betel D, Klimstra D, Jarnagin WR, Ventura A. Circulating Plasma Levels of MicroRNA-21 and MicroRNA-221 Are Potential Diagnostic Markers for Primary Intrahepatic Cholangiocarcinoma. *PLoS One* 2016; **11**: e0163699 [PMID: 27685844 DOI: 10.1371/journal.pone.0163699]

174 **Wang Y**, Gao X, Wei F, Zhang X, Yu J, Zhao H, Sun Q, Yan F, Yan C, Li H, Ren X. Diagnostic and prognostic value of circulating miR-21 for cancer: a systematic review and meta-analysis. *Gene* 2014; **533**: 389-397 [PMID: 24076132 DOI: 10.1016/j.gene.2013.09.038]

175 **Afonso MB**, Rodrigues PM, Simão AL, Castro RE. Circulating microRNAs as Potential Biomarkers in Non-Alcoholic Fatty Liver Disease and Hepatocellular Carcinoma. *J Clin Med* 2016; **5** [PMID: 26950158 DOI: 10.3390/jcm5030030]

176 **Han HS**, Kim MJ, Han JH, Yun J, Kim HK, Yang Y, Kim KB, Park SM. Bile-derived circulating extracellular miR-30d-5p and miR-92a-3p as potential biomarkers for cholangiocarcinoma. *Hepatobiliary Pancreat Dis Int* 2020; **19**: 41-50 [PMID: 31784323 DOI: 10.1016/j.hbpd.2019.10.009]

177 **Loosen SH**, Lurje G, Wiltberger G, Vucur M, Koch A, Kather JN, Paffenholz P, Tacke F, Ulmer FT, Trautwein C, Luedde T, Neumann UP, Roderburg C. Serum levels of miR-29, miR-122, miR-155 and miR-192 are elevated in patients with cholangiocarcinoma. *PLoS One* 2019; **14**: e0210944 [PMID: 30653586 DOI: 10.1371/journal.pone.0210944]

178 **Meijer LL**, Puik JR, Le Large TYS, Heger M, Dijk F, Funel N, Wurdinger T, Garajová I, van Grieken NCT, van de Wiel MA, Giovannetti E, Kazemier G. Unravelling the Diagnostic Dilemma: A MicroRNA Panel of Circulating MiR-16 and MiR-877 as A Diagnostic Classifier for Distal Bile Duct Tumors. *Cancers (Basel)* 2019; **11** [PMID: 31443224 DOI: 10.3390/cancers11081181]

179 **Salem PES**, Ghazala RA, El Gendi AM, Emara DM, Ahmed NM. The association between circulating MicroRNA-150 level and cholangiocarcinoma. *J Clin Lab Anal* 2020; **34**: e23397 [PMID: 33161598 DOI: 10.1002/jcla.23397]

180 **Cheng Q**, Feng F, Zhu L, Zheng Y, Luo X, Liu C, Yi B, Jiang X. Circulating miR-106a is a Novel Prognostic and Lymph Node Metastasis Indicator for Cholangiocarcinoma. *Sci Rep* 2015; **5**: 16103 [PMID: 26534789 DOI: 10.1038/srep16103]

181 **Shigehara K**, Yokomuro S, Ishibashi O, Mizuguchi Y, Arima Y, Kawahigashi Y, Kanda T, Akagi I, Tajiri T, Yoshida H, Takizawa T, Uchida E. Real-time PCR-based analysis of the human bile microRNAome identifies miR-9 as a potential diagnostic biomarker for biliary tract cancer. *PLoS One* 2011; **6**: e23584 [PMID: 21858175 DOI: 10.1371/journal.pone.0023584]

182 **Yan IK**, Berdah VX, Patel T. Isolation of Extracellular RNA from Bile. *Methods Mol Biol* 2018; **1740**: 59-67 [PMID: 29388136 DOI: 10.1007/978-1-4939-7652-2\_6]

183 **Voigtländer T,** Gupta SK, Thum S, Fendrich J, Manns MP, Lankisch TO, Thum T. MicroRNAs in Serum and Bile of Patients with Primary Sclerosing Cholangitis and/or Cholangiocarcinoma. *PLoS One* 2015; **10:** e0139305 [PMID: 26431155 DOI: 10.1371/journal.pone.0139305]

184 **Liang Z**, Liu X, Zhang Q, Wang C, Zhao Y. Diagnostic value of microRNAs as biomarkers for cholangiocarcinoma. *Dig Liver Dis* 2016; **48**: 1227-1232 [PMID: 27476468 DOI: 10.1016/j.dld.2016.07.006]

185 **Zhou J**, Liu Z, Yang S, Li X. Identification of microRNAs as biomarkers for cholangiocarcinoma detection: A diagnostic meta-analysis. *Clin Res Hepatol Gastroenterol* 2017; **41**: 156-162 [PMID: 27939910 DOI: 10.1016/j.clinre.2016.10.007]

186 **Leelawat K**, Narong S, Udomchaiprasertkul W, Wannaprasert J, Treepongkaruna SA, Subwongcharoen S, Ratanashu-ek T. Prognostic relevance of circulating CK19 mRNA in advanced malignant biliary tract diseases. *World J Gastroenterol* 2012; **18**: 175-181 [PMID: 22253524 DOI: 10.3748/wjg.v18.i2.175]

187 **Zhou KQ**, Liu WF, Yang LX, Sun YF, Hu J, Chen FY, Zhou C, Zhang XY, Peng YF, Yu L, Zhou J, Fan J, Wang Z. Circulating osteopontin per tumor volume as a prognostic biomarker for resectable intrahepatic cholangiocarcinoma. *Hepatobiliary Surg Nutr* 2019; **8**: 582-596 [PMID: 31929985 DOI: 10.21037/hbsn.2019.03.14]

188 **Julich-Haertel H**, Urban SK, Krawczyk M, Willms A, Jankowski K, Patkowski W, Kruk B, Krasnodębski M, Ligocka J, Schwab R, Richardsen I, Schaaf S, Klein A, Gehlert S, Sänger H, Casper M, Banales JM, Schuppan D, Milkiewicz P, Lammert F, Krawczyk M, Lukacs-Kornek V, Kornek M. Cancer-associated circulating large extracellular vesicles in cholangiocarcinoma and hepatocellular carcinoma. *J Hepatol* 2017; **67**: 282-292 [PMID: 28267620 DOI: 10.1016/j.jhep.2017.02.024]

189 **Xu H**, Inagaki Y, Tang W, Guo Q, Wang F, Seyama Y, Midorikawa Y, Gai R, Kokudo N, Sugawara Y, Nakata M, Makuuchi M. Elevation of serum KL-6 mucin levels in patients with cholangiocarcinoma. *Hepatogastroenterology* 2008; **55**: 2000-2004 [PMID: 19260467]

190 **Leelawat K**, Sakchinabut S, Narong S, Wannaprasert J. Detection of serum MMP-7 and MMP-9 in cholangiocarcinoma patients: evaluation of diagnostic accuracy. *BMC Gastroenterol* 2009; **9**: 30 [PMID: 19405942 DOI: 10.1186/1471-230X-9-30]

191 **Kobayashi S**, Werneburg NW, Bronk SF, Kaufmann SH, Gores GJ. Interleukin-6 contributes to Mcl-1 up-regulation and TRAIL resistance via an Akt-signaling pathway in cholangiocarcinoma cells. *Gastroenterology* 2005; **128**: 2054-2065 [PMID: 15940637 DOI: 10.1053/j.gastro.2005.03.010]

192 **Huang L**, Chen W, Liang P, Hu W, Zhang K, Shen S, Chen J, Zhang Z, Chen B, Han Y, Meng F, DeMorrow S, Yin X, Lai J, Liang L. Serum CYFRA 21-1 in Biliary Tract Cancers: A Reliable Biomarker for Gallbladder Carcinoma and Intrahepatic Cholangiocarcinoma. *Dig Dis Sci* 2015; **60**: 1273-1283 [PMID: 25487191 DOI: 10.1007/s10620-014-3472-0]

193 **Loosen SH**, Roderburg C, Kauertz KL, Pombeiro I, Leyh C, Benz F, Vucur M, Longerich T, Koch A, Braunschweig T, Ulmer TF, Heidenhain C, Tacke F, Binnebösel M, Schmeding M, Trautwein C, Neumann UP, Luedde T. Elevated levels of circulating osteopontin are associated with a poor survival after resection of cholangiocarcinoma. *J Hepatol* 2017; **67**: 749-757 [PMID: 28668580 DOI: 10.1016/j.jhep.2017.06.020]

194 **Banales JM**, Iñarrairaegui M, Arbelaiz A, Milkiewicz P, Muntané J, Muñoz-Bellvis L, La Casta A, Gonzalez LM, Arretxe E, Alonso C, Martínez-Arranz I, Lapitz A, Santos-Laso A, Avila MA, Martínez-Chantar ML, Bujanda L, Marin JJG, Sangro B, Macias RIR. Serum Metabolites as Diagnostic Biomarkers for Cholangiocarcinoma, Hepatocellular Carcinoma, and Primary Sclerosing Cholangitis. *Hepatology* 2019; **70**: 547-562 [PMID: 30325540 DOI: 10.1002/hep.30319]

195 **Al Ustwani O**, Iancu D, Yacoub R, Iyer R. Detection of circulating tumor cells in cancers of biliary origin. *J Gastrointest Oncol* 2012; **3**: 97-104 [PMID: 22811877 DOI: 10.3978/j.issn.2078-6891.2011.047]

196 **Yang JD**, Campion MB, Liu MC, Chaiteerakij R, Giama NH, Ahmed Mohammed H, Zhang X, Hu C, Campion VL, Jen J, Venkatesh SK, Halling KC, Kipp BR, Roberts LR. Circulating tumor cells are associated with poor overall survival in patients with cholangiocarcinoma. *Hepatology* 2016; **63**: 148-158 [PMID: 26096702 DOI: 10.1002/hep.27944]

197 **Okamura R**, Kurzrock R, Mallory RJ, Fanta PT, Burgoyne AM, Clary BM, Kato S, Sicklick JK. Comprehensive genomic landscape and precision therapeutic approach in biliary tract cancers. *Int J Cancer* 2021; **148**: 702-712 [PMID: 32700810 DOI: 10.1002/ijc.33230]

198 **Kasi PM**. Favorable Outcomes in FGFR Fusion-Positive Cholangiocarcinomas and Evolution on Treatment Noted on Circulating Tumor DNA Liquid Biopsies. *Case Rep Oncol* 2020; **13**: 941-947 [PMID: 32999653 DOI: 10.1159/000509075]

199 **Tan Y**, Pan T, Ye Y, Ge G, Chen L, Wen D, Zou S. Serum microRNAs as potential biomarkers of primary biliary cirrhosis. *PLoS One* 2014; **9**: e111424 [PMID: 25347847 DOI: 10.1371/journal.pone.0111424]

200 **Puik JR**, Meijer LL, Le Large TY, Prado MM, Frampton AE, Kazemier G, Giovannetti E. miRNA profiling for diagnosis, prognosis and stratification of cancer treatment in cholangiocarcinoma. *Pharmacogenomics* 2017; **18**: 1343-1358 [PMID: 28832247 DOI: 10.2217/pgs-2017-0010]

201 **Wang S**, Yin J, Li T, Yuan L, Wang D, He J, Du X, Lu J. Upregulated circulating miR-150 is associated with the risk of intrahepatic cholangiocarcinoma. *Oncol Rep* 2015; **33**: 819-825 [PMID: 25482320 DOI: 10.3892/or.2014.3641]

202 **Silakit R**, Loilome W, Yongvanit P, Chusorn P, Techasen A, Boonmars T, Khuntikeo N, Chamadol N, Pairojkul C, Namwat N. Circulating miR-192 in liver fluke-associated cholangiocarcinoma patients: a prospective prognostic indicator. *J Hepatobiliary Pancreat Sci* 2014; **21**: 864-872 [PMID: 25131257 DOI: 10.1002/jhbp.145]

203 **Shen L**, Chen G, Xia Q, Shao S, Fang H. Exosomal miR-200 family as serum biomarkers for early detection and prognostic prediction of cholangiocarcinoma. *Int J Clin Exp Pathol* 2019; **12**: 3870-3876 [PMID: 31933776]

204 **Xue XY**, Liu YX, Wang C, Gu XJ, Xue ZQ, Zang XL, Ma XD, Deng H, Liu R, Pan L, Liu SH. Identification of exosomal miRNAs as diagnostic biomarkers for cholangiocarcinoma and gallbladder carcinoma. *Signal Transduct Target Ther* 2020; **5**: 77 [PMID: 32527999 DOI: 10.1038/s41392-020-0162-6]

205 **Lapitz A**, Arbelaiz A, O'Rourke CJ, Lavin JL, Casta A, Ibarra C, Jimeno JP, Santos-Laso A, Izquierdo-Sanchez L, Krawczyk M, Perugorria MJ, Jimenez-Aguero R, Sanchez-Campos A, Riaño I, Gónzalez E, Lammert F, Marzioni M, Macias RIR, Marin JJG, Karlsen TH, Bujanda L, Falcón-Pérez JM, Andersen JB, Aransay AM, Rodrigues PM, Banales JM. Patients with Cholangiocarcinoma Present Specific RNA Profiles in Serum and Urine Extracellular Vesicles Mirroring the Tumor Expression: Novel Liquid Biopsy Biomarkers for Disease Diagnosis. *Cells* 2020; **9** [PMID: 32183400 DOI: 10.3390/cells9030721]

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**Table 1 Liquid biopsy biomarkers**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Alteration** | **Source** | **Potential application** | **Ref.** |
| **DNA** | | | | |
| ARID1A, BAP1, BLC2, BRAF, CDKN2A, FBXW7, FGFR2, IDHA1, IDH2, KRAS, NRAS, PBRM1, PIK3CA, SMAD4, TP53 | Mutations | Serum | Diagnostic value | [157-159,163,197] |
| ERBB2 | Amplifications | Diagnostic value | [157] |
| FGFR2 | Fusions | Diagnostic and prognostic value | [157,198] |
| **microRNA** | | | | |
| miR-9, miR-21, miR-29b, miR-106, miR-122, miR-150, miR-155, miR-192, miR-200 family | ↑ Expression | Serum | Diagnostic and prognostic value | [171-173,177,179-181,183,199-203] |
| miR-26a, miR-26a-5p, miR-30b, miR-126, miR-141-3p, miR-551B, miR-604, miR-1281, miR-96-5p, miR-151a-5p, miR-191-5p, miR-4732-3p | Diagnostic value | [183,199,204,205] |
| miR-30d-5p, miR-92a-3p, miR-412, miR-640, miR-1537, miR-3189, miR-191, miR-486-3p, miR-1274b, miR-16 and miR-484 | Bile | Diagnostic value | [169,176,183] |
| **Proteins and cytokines** | | | | |
| Cytokeratin-19, MMP-1, MMP-7, MMP-9, MMP-10, Periostin, IL-6, CYFRA 21-1, Osteopontin | ↑ Expression | Serum | Diagnostic and prognostic value | [186,187,190-193] |
| **Serum metabolites** | | | | |
| Glycine, aspartic acid, SM (42:3), and SM (43:2) | ↑ Expression | Serum | Diagnostic and prognostic value | [194] |