REVIEW

DOI: http://dx.doi.org/10.15403/jgld-4634

Bile Composition - which Components Can Have Clinical Significance? A Review of the Literature

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Received: 28.09.2022
Accepted: 21.01.2023

ABSTRACT

Over the years, scientific research concerning the qualitative analysis of bile and its use in diagnostics and treatment, have been very limited. Due to unsatisfactory results of detection, *inter alia*, cholangiocarcinoma or gallbladder carcinoma, and the necessity to discover more efficient techniques of diagnostics, bile has become an interesting direction to study. Nowadays, thanks to the latest research, analysis of concentration i.e. specific bile salts, proteins, nucleic or fatty acids in bile or imbalance of biliary microbiome, could play a crucial role in cancer detection or prognosis of progression such diseases as primary sclerosing cholangitis/choledocholithiasis. This review article provides an overview of individual biliary solutes, which may play a role in diagnostics improvement.

Key words: bile – bile composition – biliary tract – cholangiocarcinoma – acids – gallbladder.

Abbreviations: CA: cholic acid; CCA: cholangiocarcinoma; CDCA: chenodeoxycholic acid; cfDNA: cell free DNA; CSI: lithogenicity index; DCA: deoxycholic acid; ERCP: endoscopic retrograde cholangiopancreatography; FFA: free fatty acids; GCA: glycocholic acid; GDCA: glycochenodeoxycholic acid; LCA: litocholic acid; LPC: lysophosphatidylcholine; microRNA: miRNA; PBC: primary biliary cholangitis; PC: pancreatic cancer; PSC: primary sclerosing cholangitis; PtC: phosphatidylcholine; PUFA: polyunsaturated fatty acids; SOD: sphincter of Oddi dysfunction; TCA: taurocholic acid; TDCA: taurochenodeoxycholic acid; UDCA: ursodeoxycholic acid.

INTRODUCTION

The bile is a highly complex solution, liquid in its nature, which contains less than 5% solid organic and inorganic solutes. It is produced in hepatocytes and modified distally by absorptive and secretory transport systems in the bile duct epithelium. The biliary tree in the liver helps route the bile into the gallbladder, where it is accumulated. Every food consumption triggers hormonal stimulus (release of cholecystokinin), that induces contraction of a gallbladder and excretion of the bile into the intestinal lumen. The bile has several important functions: it aids in the digestion of fat via fat emulsification, in the absorption of fat and fat-soluble vitamins and excretion of bilirubin. Moreover, it provides an alkaline fluid in the duodenum to neutralize the acidic pH of the chyme that comes from the stomach and provides bactericidal activity against microorganisms present in the ingested food. The possibility of making use of this secretion to diagnose or manage various diseases has great potential, but until the XXI century, was not exploited. Knowledge of the mechanisms of bile formation has progressed rapidly in recent years and has provided the basis for further diagnosis and treatment. Nevertheless, there is still a great scope of unexamined dependencies and connections. The main limitation of making a bile examination more common is still a great difficulty to gain this fluid. The fundamental technique for bile collection, endoscopic retrograde cholangiopancreatography (ERCP), is a highly invasive procedure, which carries the risk of several complications, mainly a development of pancreatitis (due to irritation of the pancreatic duct by the contrast material or catheter), which can occur in 5-10% of all procedures, post-endoscopic intestinal wall perforation or bleeding [1]. On the other hand, ERCP plays a crucial role in the diagnosis and management of biliary strictures, which are mostly secondary to malignant diseases. Other techniques including analysis of bile after cholecystectomy or analysis of bile components in stool are either very difficult, or their accessibility is limited in a clinical practice. The composition of bile is quite stable in humans - most of its volume constitutes water, in which there are dissolved a number of endogenous solid constituents including mainly bile salts, bilirubin, phospholipids, cholesterol, amino acids, steroids, enzymes, porphyrins, vitamins, and heavy metals, as well as exogenous drugs, xenobiotics and environmental toxins [2, 3] (Fig. 1).

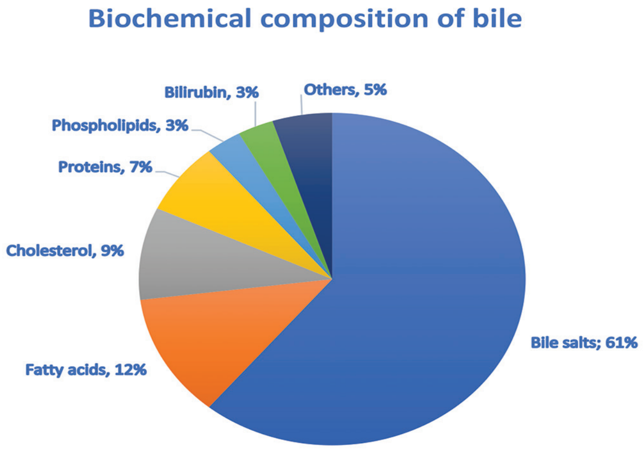

Fig. 1. Biochemical composition of bile. The chart shows a percentage distribution of biliary solutes. Adapted from [3].

BILE SALTS

The most abundant organic solutes in the bile are bile salts, which are 24-carbon water-soluble products of cholesterol metabolism. There are two salts produced by human liver - these are primary bile salts: cholic acid (CA), a trihydroxylated bile salt, and chenodeoxycholic acid (CDCA), a dihydroxy bile salt. Each of them can be conjugated with either taurine or glycine, making taurocholic acid (TCA), glycocholid acid (GCA), taurochenodeoxycholic acid (TDCA) and glycochenodeoxycholic acid (GDCA). Then the intestinal bacteria, by converting primary bile salts, to deoxycholic acid (DCA) and lithocholic acid (LCA), produce „secondary bile acids" - mostly reabsorbed in the distal intestine creating an enterohepatic circulation of bile salts [4].

The composition of bile salts in human bile is quite invariable; therefore, shifts in their proportions could be clinically significant. Each of primary bile salts, both CA and CDCA, constitute almost 40% of bile acid pool. Deoxycholic acid makes up the next 20% of bile acid composition, and the remaining percentage consists of the rest of bile salts. Current diagnostic techniques to distinguish benign from malignant biliary tract diseases are insufficient, while metabolic profiling of bile is a hopeful opportunity to simplify that process. Recent studies have shown that conjugated bile acids, but not free bile acids, stimulate cholangiocarcinoma (CCA) cell growth, and that an imbalance in the ratio of free to conjugated bile acids may play an important role in the tumorigenesis of CCA. [5]. According to Park et al. [6], total bile acid concentration, as well as DCA and LCA proportion to primary bile acids, were found to be lower in a group of patients with CCA compared to the patients with choledocholithiasis and control groups. The concentration of bile salts in CCA cases was almost 8 times lower than in stone control cases and 39 times lower than in healthy cases. The vast majority of individual bile salts was less often detected, except one, which level was significantly increased: glycoursodeoxycholic acid. The block of bile outflow and bile acids intestinal conversion impediment cannot be the sole reason for these observations. Strom et al. [7] found similar bile acids profile alteration in CCA patients, even after the removal of biliary tract obstruction. These conclusions were consistent with results reported by Sang et al. [8], who iincluded in their research pancreatic cancer (PC) cases. Interestingly, the average concentration of bile acids was the highest in PC patients compared to healthy and CCA patients, and that can be explained by the fact, that the increase of biliary bile acids intensifies pancreatobiliary reflux, which is one of the main triggers of chronic pancreatitis, leading to PC [9]. Moreover, in the Sang et al. [8] study, GCA was proposed as a potential positive and TDCA potential negative bile acid biomarker of CCA. In another research, by using magnetic resonance spectroscopy, Hashim Abdalla et al. [10] found, that taurine- and glycine-conjugated bile acids were significantly elevated in bile from patients with cholangiocarcinoma compared with bile from patients with benign diseases such as sphincter of Oddi dysfunction (SOD), choledocholithiasis and primary sclerosis cholangitis (PSC). This difference was significantly visible in the CCA bile compared with bile from SOD and PSC patients. [10]. This increased accumulation of conjugated bile acids in bile ducts (also serum and liver) is clearly distinct, as well as lower amounts of unconjugated and secondary bile acids returning from the intestines [11]. Every day, a perspective of metabolic profiling of bile is closer to implement into the standard process of diagnosis and it may improve CCA detectability, but presently there is no full cohesiveness in the various research conclusions.

Another study revealed a significant decrease in the mean amounts of glycocholic and taurocholic acids in the bile of patients with gallstone disease compared with that in the controls [12]. The concentration of conjugated bile acids, easily soluble in aquatic environment, was connected with the fluctuation of lithogenicity index (CSI) in the study group. The lower amount of hydrophilic bile acids, the more increased was CSI. There were no differences in the concentrations of the other bile acids examined (GDCA, glycodeoxycholic, TDCA and taurodeoxycholic acid), cholesterol and phospholipids. These conclusions give various therapeutic possibilities, such as the use of UDCA [13] or omega ω−3 polyunsaturated fatty acids (PUFAs) in gallstone prevention [14]. There is a hope, that studies on bile composition will result in new drugs, that can more efficiently prevent or treat gallstone disease.

PHOSPHATIDYLOCHOLINE/ CHOLESTEROL

Phosphatidylcholine (PtC), the major choline-containing phospholipid in human cells is crucial for membrane structure, lipoprotein metabolism and signal transduction. It forms mixed micelles with bile acids and cholesterol. According to Ijare et al. [15] study, the concentration of PtC was decreased in CCA patients compared to the control group (with benign biliary tract stricture) with the difference being statistically significant, which was a confirmation regarding previous research [10, 16]. A reduced PtC concentration was also detected in PC patients compared to the control group (non-cancer patients) [17]. In another study, PtC and lysophosphatidylcholine (LPC) profiles were similar in patients with or without PSC, thus these chemical compounds do not have diagnostic importance in this disease. Biliary LPC/PtC ratios and ratios of biliary PC to bilirubin, PtC to cholesterol, PtC to protein, and PtC to bile acids showed no intergroup differences [18, 19]. The samples of bile in this study were collected during ERCP. These conclusions were not confirmed in the latest research from Vienna, in which different study methods were used. The authors verified utility of a phosphorus-31 magnetic resonance spectroscopy (31P-MRSI) protocol for the quantification of PtC in the human gallbladder. They demonstrated significantly lower PtC content for the PSC group, and for the female subgroup of the primary biliary cholangitis (PBC) compared to the control group [20]. These results indicate that PtC is an interesting potential biomarker, potentially to be used in some specific benign hepatic and biliary diseases in the future.

PROTEINS, AMINO ACIDS AND PEPTIDES

There is an immense amount of proteins present in bile, but because of the very high concentrations of interfering substances, especially lipids and bile salts, a proteomic analysis is challenging. This has limited the number of studies of bile proteome to very few, comparatively to these concerning lipids.

Most of human bile proteins originate from plasma by a ‘sieving’ mechanism, thus we can observe a clear inverse relationship between the biliary and serum concentrations of proteins and their molecular weight (i.e: albumin, haptoglobin etc.). Even so, there are many proteins, which appear to enter bile by other mechanisms and their concentration in bile does not correlate with the aforementioned mechanism (i.e: secretory IgA, IgM, haemoglobin, caeruloplasmin etc.) [21]. The first big-scale study of bile proteomics was performed in 2004 and led to the discovery of 87 proteins in the bile of healthy patients [22]. Later, more recent research based on advanced technology revealed up to 2552 bile proteins [23]. Son et al. [24] with the use of the liquid chromatography mass spectrometry, identified a total of 245 proteins in the bile of extrahepatic CCA patients and control patients. The concentration of 14 proteins was increased in CCA patients, in some cases increased significantly [24]. In another small research, a concentration of 63 proteins was significantly increased in CCA bile compared to normal bile. Alpha-1-antitrypsin was one of the overexpressed proteins, which was especially often detected; immunohistochemical analysis revealed it in 50% of the samples, while immunoblotting detected overexpression in 70% of the samples [25]. In another studies, 18 proteins were identified as being more abundant and 30 proteins less abundant in the patients with malignancy [26], 104 proteins overexpressed in malignant samples [27] or featured SSP411 protein (sensitivity of 90.0% and specificity of 83.3% at a cutoff value of 0.63), TPD52 and DNAJB1 or ADAMTS4 as potential bile biomarkers for CCA [28-30]. Yamaguchi et al. [31] in their latest research proposed biliary WFA-sialylated MUC1 as a perspective biomarker, with a cut-off value, above which the sensitivity in discriminating CCA was greater (82.2%), than that of cytology (23.6%). Other potentially perspective biomarkers, which should be considered useful, are phoshpo-smad 3 protein (hepatocellular carcinoma - HCC) [32], MAD2L1 (CCA) [33], S100A9 (PSC) [34] or CEAM6 (CCA) [35]. Proteomic analysis of bile is a promising method for the diagnosis of biliary tract diseases, especially malignant ones. However, there are still many obstacles inhibiting from implementing it into standard methods of diagnostics. The complexity of bile and the presence of molecules interfering with protein analysis, differences in the bile-protein profiles of different types of tumors (for example intra- and extrahepatic cholangiocarcinoma [36]) and inconsistent results from various recent studies are the main problems. Table I summarizes the studies on proteins as potential biliary tract diseases biomarkers.

FATTY ACIDS

Fatty acids such as: palmitic, palmitoleic, stearic, oleic and linoleic acids, are an integral part of every molecule of phospholipids present in bile - PtC and lecithin [37]. The study of their fatty acids composition could help clarify, whether different combinations of them can be conducive to gallstone formation in the human biliary tract. Generally, gallstone patients have higher molar percentages of cholesterol and larger fractions of DCA and LCA than healthy people [38]. According to the study of Berr et al. [39], there is a tight correlation between phospholipid fatty acids (positive: oleic, arachidonic; negative: linoleic, palmitoleic) and the relative amount of cholesterol. Their research supports a hypothesis that secondary bile acids contribute to supersaturation of bile mainly by changing the fatty acid pattern of the secreted phospholipids, which increases the molar ratio of cholesterol/phospholipids in bile [39]. In another study, patients were supplementing marine fish oil with n-3 PUFAs. A change in biliary phospholipids fatty acids composition was observed and a decrease in the molar ratio of cholesterol to phospholipids (-19%). As a consequence the cholesterol saturation index was significantly reduced (-25%), which reduced a nucleation of cholesterol crystals [40].

Free fatty acids (FFA) constitute another molecular element of bile. Different proportions of them can be a stimulus to gallstone formation. Compared to the bile of controls, the bile from patients with gallstones had higher total free fatty acid content, more palmitic acid, more stearic acid, more linoleic acid and arachidonic acid [41]. Xiang et al. [42] suggest that pancreaticobiliary reflux induced an increase in FFAs and triglycerides in the gallbladder bile is a cause of gallstone formation.

BILIARY MICROBIOME

Bile has been considered for a long time as a sterile liquid [43]. However, a development of new diagnostic techniques including mainly metagenomic techniques, has revealed

Table I. Studies on proteins as potential biliary tract diseases biomarkers

References	Biliary protein	Potential cut-off value	Disease	Other
Kristiansen et al. [22]	87 proteins	None	Healthy patients	-
Barbhuiya et al. [23]	2552 proteins	None	Healthy patients	-
Son et al. [24]	14 proteins increased in CCA	None	Cholangiocarcinoma	In the study, there were identified a total of 245 proteins in the bile of CCA and control patients, but the concentration of only 14 of them was increased.
Laohaviroj et al. [25]	63 proteins, especially alpha-1-antitrypsin, increased in CCA	Cut-off value for alpha-1-antitrypsin <173.4 µg/g, with sensitivity of 80% and specificity of 75%	Cholangiocarcinoma	Serum levels of oxidized alpha-1-antitrypsin were also significantly higher in groups of patients with heavy O. viverrini infection, advanced periductal fibrosis (APF), and CCA when compared with healthy controls.
Navanethaan et al. [26]	18 proteins more abundant and 30 proteins less abundant in CCA and PC	None	Cholangiocarcinoma, pancreatic cancer	A total of 459 proteins were quantified in the malignant and benign groups.
Lukic et al. [27]	104 proteins overexpressed in CCA and PC	None	Cholangiocarcinoma, Pancreatic cancer	A total of 1267 proteins were identified in malignant and nonmalignant samples.
Shen et al. [28]	SSP441 protein	Cut-off value for SSP441: 0,63 with sensitivity of 90.0% and specificity of 83.3%	Cholangiocarcinoma	-
Ren et al. [29]	TPD52 and DNAJB1 proteins	None	Cholangiocarcinoma	-
Voigtländer et al. [30]	ADAMTS4 peptide	None	Cholangiocarcinoma	-
Yamaguchi et al. [31]	WFA-Sialylated MUC1 protein	The cutoff value for WFA-sialylated MUC1: 10.5 with the sensitivity 82.2 %	Cholangiocarcinoma	-
Nakamura et al. [32]	Phospho-Smad 3 protein	None	Hepatocellular carcinoma	-
Gao et al. [33]	MAD2L1 protein	None	Cholangiocarcinoma	-
L. Reinhard et al. [34]	S100A9 protein	None	PSC	The analyzed proteome consisted of a total of 379 non-redundant biliary proteins; 21% were of unknown function and 24% had .been previously described in serum.
Farina et al. [35]	CEAM6 protein	Distinguishing malignant from benign biliary stenoses with a receiver operating characteristic (ROC) area under the curve (AUC) of 0.92 (specificity 83% and sensitivity 93%)	Biliary tract cancers	-

very rich bacterial community in the biliary tree. Recent studies suggest interesting associations between specific microbiological patterns and diseases such as PSC, CCA and other biliary tract pathologies, but further research is required to prove these relationships. The understanding of the associations between the microbiota and the host may enable to create new strategies for the prevention and treatment of biliary tract diseases.

Numerous studies showed that a microbial factor could be entangled in the pathogenesis of gallstone formation. Maki et al. [44] proved that the presence of β-glucuronidase (bacterial enzyme) in bile could hydrolyze the bilirubin glucuronide into bilirubin and glucuronic acid, which with calcium molecules, could precipitate and form calcium bilirubinate crystals. In different studies β-glucuronidase, but also other bacterial enzymes and components (such as bile acid hydrolases, phospholipases or glycocalyx) have been identified and associated with the formation of pigmented gallstones [45-48]. The importance of these enzymes in the pathogenesis of pigmented and mixed gallstones has been shown by genomic techniques including PCR-based amplification, where the genes encoding β-glucuronidase and bacterial sequences were identified in the gallstones [49]. A presence of microbiota in cholesterol gallstones was proven much later; formation of these type of gallstones has been traditionally considered as a result of mainly metabolic imbalance. However, also in cholesterol gallstones specific bacterial DNA was found [50, 51]. This research suggests that bile microbiota may be an additional factor in the formation of every type of gallstone.

The term „biliary tract cancers" refers to malignant tumors of the bile duct, such as extrahepatic CCA or tumors concerning the gallbladder or ampulla of Vater. Altogether, they are consociated with poor prognosis and very low survival rates, and the crucial factor affecting prognosis is a diagnosis at late stages. Identification of risk factors, such as a microbiome predisposition could maintain a big role in predicting such cases. Biliary microbiome recognition in malignant tumors is still in the initial phase. According to a study of Avilés-Jiménez et al. [52], microbiota in extrahepatic cholangiocarcinoma noticeably varies from microbial composition of healthy people. Proteobacteria dominated in all bile samples, and *Fusobacterium*, *Prevotella*, *Actinomyces*, *Novosphingobium* and *Helicobacter pylori* were increased [52]. Other research showed increased concentration of Stenotrophomonas [53], *Klebsiella pneumoniae* [54] or *Gemmatimonadetes*, *Nitrospirae*, *Chloroflexi*, *Latescibacteria* and *Planctomycetes* (compared with patients with choledocholithiasis) [55]. An interesting fact is, that a comparison of *Opisthorchis viverrini* associated vs. non-associated groups showed abundance of specific enteric bacteria (*Bifidobacteriaceae*, *Enterobacteriaceae* and *Enterococcaceae*). Functional analysis of cholangiocarcinoma microbiomes revealed the bigger potential for producing ammonia and bile acids in *Opisthorchis viverrini* associated tissues, which naturally associates the altered microbiota with carcinogenesis [53]. In another study, *Fusobacterium nucleatum*, *Escherichia coli* and *Enterobacter spp*. were the predominant bacteria in the bile of patients with gallbladder carcinoma [56]. Li et al.[57] in the newest research, divided potential bacterial biomarkers into the perihilar CCA group and distal CCA group [57]. The most abundant three species of perihilar CCA were *Pseudomonas*, *Sphingomonas* and *Halomonas*, and of distal CCA were *Streptococcus*, *Prevotella* and *Halomonas*. Concluding these various biliary microbiome studies, there is still a lack of cohesiveness and unambiguous construals in these findings. Despite that, because of the great potential of this subject, this should be a trigger to induce more biliary microbiota analyses. While the presence of specific bacteria in bile of biliary tract cancer patients is known, scientists still are not able to prove, if is it an effect or a cause of the disease. We are waiting for coherent conclusions concerning this subject and surely some more studies must be conducted. Undoubtedly there is a great necessity for new methods of prediction and the diagnosis of biliary tract cancers.

Primary biliary cholangitis (PBC) is a chronic autoimmune disease affecting the small bile ducts. The most common hypothesis proposes that, in genetically predisposed humans, an exaggerated immune response is produced against self-antigens present in the biliary tract. It has been proposed that molecular mimicry between host antigens and microbes may act as a possible trigger [58]. The biliary microbiota could play a crucial role in disease development, but several studies concerning this subject is very limited. According to Hiramatsu et al. [59], in 75% of PBC cases were identified specific Gram-positive bacteria (coccus morphology), while these cocci were positive in only 5% in cholecystolithiasis. Primary sclerosis cholangitis is another autoimmunological disease, affecting bile ducts and leading eventually to fibrosis. Considering the strong association with IBD, it is possible, that a primary intestinal dysbiosis causing inflammation and consequent exposure of cholangiocytes to cytokines and microbial products could initiate the pathogenesis [60]. In a study of Pereira et al. [61] the bacterial communities of non-PSC subjects and early-stage PSC patients were similar, but *Streptococcus* abundance was positively correlated with an increase in disease severity. These findings suggest that the etiology of PSC is not associated with changes in the bile microbial composition, but the genus *Streptococcus* may play a pathogenic role in the progression of the disease. Moreover, the PSC-connected biliary microbiota increases accordingly to disease duration. As was shown in Miyabe et al. [62] study, various bacteria, mainly *Fusobacteria*, were in greater amount during every following ERCP-adjusted bile analysis.

NUCLEIC ACIDS

Every cell, both typical healthy cells of human tissue, as well as every tumor cells contain their DNA and RNA. The detection of tumor-specific genetic aberrations with use of cell-free DNA (cfDNA), floating in a biliary fluid, could be an alternative for specific serum biomarkers, which lack sensitivity and specificity. Moreover, tracking molecules of cfDNA in bile and blood was investigated as a potential diagnostic tool for pancreaticobiliary cancers, potentially replacing traditional tissue biopsies. Driescher et al. [63] found that 96% of pathogenic mutations identified by tissue sequencing could be detected in cfDNA from corresponding bile samples, yielding a sensitivity and specificity of 96.2% and 100%, respectively. Another study showed that liquid biopsy of bile might help us to diagnose gallbladder cancer even with higher sensitivity and positive predict value compared to cytology with ERCP. The sensitivity of cfDNA (58.3%) was higher than that of cytology (45.8%). In addition, the mutation accordance rate between gallbladder carcinoma tissue DNA and bile cfDNA was high at 85.7% of patients [64]. Bile cfDNA was revealed to consist of mainly long DNA fragments, which was not coherent with the findings in plasma cfDNA. These differences may be attributed to DNA restriction endonuclease variety or activity differences between the bile and plasma. As a result, bile cfDNA analysis could be even more precise and sensitive than the study of cell-free nucleic acids in plasma. According to Shen et al. [65], a vast majority of gene mutational variants can be detected in cfDNA of bile (of the 19 variants detected in the tumor samples, 18 were also detected in bile cfDNA). The investigation of specific genetic tumor markers in cfDNA, despite its usefulness, is a future step, which still has not been implemented into the clinical routine.

Another molecule considered to be promising diagnostic biomarker for cancer, is microRNA (miRNA), a small, single-stranded RNA particle, which plays the crucial role in the post-transcriptional regulation of gene expression. Analysis of bile miRNA, through cloning and using PCR methods, showed that 10 of the 667 miRNAs found in bile, were significantly more highly expressed in the group of patients with biliary tract cancer, than in the group with benign diseases. A decent long-term stability of miRNA in bile and localization in the biliary epithelia, makes some of these molecules perspective tumor biomarkers, especially miR-9 and miR-145 [51]. Li et al. [67] have made a novel biliary vesicle miRNA-based panel for CCA diagnosis that demonstrated a sensitivity of 67% and specificity of 96%. According to Voigtländer et al. [68], patients with PSC and cholangiocarcinoma have different miRNA profiles in serum and bile. The analyzed miRNAs (miRNA-412, miRNA-640, miRNA-1537 and miRNA-3189) were significantly elevated in the bile of patients with PSC compared to patients with malignant disease. Interestingly, the aforementioned miRNAs showed higher levels in the PSC vs. CCA group, but also higher levels in CCA complicating the PSC vs. PSC group. Etiopathogenesis of CCA in PSC compared to CC without chronic biliary disease can play a main role in these fluctuations, but this assumption needs more research to be confirmed [68]. MicroRNAs have recently emerged as a valuable class of diagnostic markers, but they have to be addressed in future studies to prove their usefulness.

CLINICAL APPLICATION PERSPECTIVES

Antibiotic prophylaxis for ERCP patients is considered a standard practice in cases at risk of incomplete biliary drainage. Biliary microbiome examination can be helpful for targeted antibiotic therapy selection. It could be particularly useful in patients with a medical history of previous ERCP procedures, as those patients may have modified microbiome composition.

Another promising use for the bile elements clinical assessment, are CCA/gallbladder carcinoma biomarkers, mainly proteins and nucleic acids, which provide a most decent level of sensitivity and specificity. Biomarkers could be utilized to detect a biliary tract cancer, including its recurrence, when the biopsy is non-diagnostic. The combined assessment of biliary and serum biomarkers, such as in Yamaguchi et al. research [31] (biliary WFA-sialylated MUC1 and serum CA19-9) can provide even better results. Further, an increased concentration of free and specific conjugated fatty acids in bile can be an effective predictor for recurrent choledocholithiasis, and particular miRNAs or higher activity of biliary S100A9 can be helpful in PSC detection in clinical scenario of indetermined bile strictures. Taken together there is a great scope of possible biliary markers, which can become important in biliary tract diseases diagnostics.

CONCLUSIONS

Analysis of bile composition and its use in the improvement of disease diagnosis and treatment is a fledgling brand of medicine. Every year the gaps in our knowledge are slowly being filled in, but many more studies have to be made. This review provides an insight into various components of bile and their potential utilization in diagnostic process. The perspective of discovering an optimal CCA biomarker or specific for various biliary tract diseases bile composition, is approaching with each subsequent scientific research. Perhaps it is only a matter of time, when analysis of bile acids concentration, increased amount of alfa-1-antitrypsin in bile or specific biliary microbiome, will be an essential stage in the detection of hepatobiliary cancers, but at first we have to overcome some crucial obstacles, which still make it difficult and uneconomical.

Conflicts of interest: None to declare.

Authors' contribution: P.B.P and A.P. conceived the study, selected the literature and analyzed the data. A.P. and P.N. revised the manuscript. All authors agreed with the final version of the manuscript.

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