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Posted Date: 23 December 2024

doi: 10.20944/preprints202412.1978.v1

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Article

GC-IMS Serum VOCs Detection: A Potent Aid for Cholangiocarcinoma Diagnosis

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Abstract: Background: Early diagnosis of cholangiocarcinoma (CCA) is still unreliable, resulting in poor patient outcomes. Volatile organic compounds (VOCs), emitted by the body, could help detect disease-related metabolic changes, aiding diagnosis. **Objective:** This study aims to assess if Gas chromatography ion mobility spectroscopy (GC-IMS) detection of serum VOCs can differentiate CCA from benign biliary diseases (BBD) and evaluate its diagnostic effectiveness. **Method:** Serum samples from 46 CCA and 46 BBD patients were collected. GC-IMS detected VOCs in the serum groups, and PCA was performed to analyze group differences. Utilize the Receiver Operating Characteristic (ROC) curve to assess the diagnostic efficacy of VOCs in CCA and analyze the correlation between VOCs and pathological characteristics. **Result:** Using GC-IMS, we detected 27 VOCs in two serum samples, including 23 known and 4 unknown. A PCA 3D scatter plot highlights distinct VOC patterns and clustering for CCA patients versus BBD. Statistical analysis reveals significant differences in 14 substances between the BBD and CCA groups, with 8 VOCs exhibiting upregulation and 6 demonstrating downregulation in the CCA group. ROC analysis reveals that (E)-2-nonenal exhibits the highest diagnostic efficiency for CCA, with an AUC value of 0.761 (95%CI: 0.662 - 0.860). **Conclusion:** Serum VOC analysis could aid the diagnosis and surveillance of CCA.

Keywords: Cholangiocarcinoma; GC-IMS; volatile organic compounds; identify; serum

Introduction

Cholangiocarcinoma (CCA) is a diverse malignant tumor originating from the biliary system, comprising approximately 15% of primary liver cancers and 3% of gastrointestinal cancers[1-4]. Early stage CCA has no specific symptoms. Common auxiliary exams in clinical practice, such as magnetic resonance cholangiopancreatography (MRCP), endoscopic retrograde cholangiopancreatography (ERCP), fluorescence in situ hybridization (FISH) analysis, and bile brush cytology, often have limitations, leading to false negatives and misdiagnoses[5-7]. A gold standard for tumor diagnosis is pathological tissue biopsy, but it's highly invasive and unsuitable for early cancer screening[8, 9]. Minimally invasive methods like tumor marker detection (e.g., CA19-9 and CEA) exist, but current markers lack the specificity and sensitivity needed for early CCA screening and diagnosis, in addition, there is no clear cause for the onset of CCA, leads to most patients being diagnosed late, missing the best treatment window[10, 11]. Its aggressive nature and treatment difficulties lead to a high mortality rate, making up 2% of global cancer deaths[12]. The five-year survival rate is 7% to 20%, with a high rate of tumor recurrence after surgery[13]. Thus, creating reliable and effective screening and diagnostic tools is essential.

Volatile organic compounds (VOCs) are defined as organic substances that are present in the vapor phase at ambient room temperature. It not only contaminates the living environment but also poses a significant threat to human life and health. VOCs are categorized as endogenous or exogenous. Exogenous VOCs come from the environment and aren't metabolized by the body, while

endogenous VOCs are produced by human cells during metabolism and oxidative stress, being released through tissues, fluids, or exhalation[14], these compounds indicate changes in metabolism and are linked to oxidative stress and inflammation, making them valuable biomarkers for assessing health status[15]. Currently, several studies indicate that VOCs possess distinct differential value in relation to multiple cancer types, including hepatocellular carcinoma[16], lung cancer[17], breast cancer[18] and colorectal cancer[19]. These compounds can be identified in a range of biological matrices, including tissues, urine, blood. Studies have shown that the most common biomarkers for urinary VOCs are hexanal, dimethyl disulfide and phenol[20]. Suggesting that cancer cells release VOCs into bodily fluids. Subsequently, these compounds are excreted via the pulmonary system and can be detected in exhaled air[21]. Consequently, the investigation of cancer-specific VOCs in biological fluids represents a promising avenue of research that may facilitate the identification of metabolic alterations associated with tumors, thereby enhancing tumor diagnosis.

Gas chromatography ion mobility spectroscopy (GC-IMS) is an emerging desktop technology used for sensitive, rapid, and selective detection of volatile organic compounds [22]. Studies show that GC-IMS effectively analyzes metabolites in urine, breath, serum, plasma, bile, and feces, separating VOC molecules for qualitative and quantitative analysis [23]. The objective of this study is to assess the efficacy of GC-IMS in detecting serum VOCs for differentiating between CCA and benign biliary diseases (BBD).

2. Materials and Methods

2.1. Study Population

We used Power and Sample Size, an online software application, to evaluate the sample size of this study (<http://powerandsamplesize.com/calculators>). Relevant parameters compare two means: two-sample, two-sided equality, power of test, $1-\beta = 0.9$ and type I error rate $\alpha = 0.05$ were selected. At least 25 samples are required for each group of cancer patients and benign control patients. The study involved 92 eligible patients from the Third Affiliated Provincial Hospital of Shandong University between April 2023 and January 2024, comprising 46 CCA patients and 46 BBD patients. The inclusion criteria for the cancer group are as follows: 1) Participants must have a first-time diagnosis of cholangiocarcinoma; 2) Participants must have no prior history of other malignant tumors; 3) Participants must not have a history of receiving anti-cancer treatments, including radiotherapy and chemotherapy; 4) Participants must not be undergoing radical resection surgery. Patients were included in Group BBD if BBD was verified based on imaging or pathology and other diseases of the liver and digestive tract were excluded.

2.2. Sample Preparation

Blood samples were obtained from the Laboratory Department at the Third Affiliated Hospital of Shandong University, centrifuged at 3000 g for 10 min at 4°C to extract serum, which was then aliquoted into 1.5 mL sterile tubes and stored at -80°C.

2.3. Analysis of volatile organic compounds in serum

To detect VOCs in serum with GC-IMS (GAS Dortmund, Germany), incubate 0.2 mL of serum at 37 °C for 10 min in a headspace vial, then extract 1 mL of gas. Use nitrogen as the carrier gas, beginning at 2 mL/min for 2 min and ramping up to 150 mL/min over 10 min. Maintain the IMS drift gas at 150 mL/min, with a drift tube temperature of 45 °C and a specific column temperature for gas chromatography.

2.4. Statistical Analysis

Analyze the data with IBM SPSS statistics 27.0.1 and Graphpad prism 10. For categorical data, the chi-square test is used and reported as frequency (percentage). For continuous data, the t-test or

Mann-Whitney U test is applied, presented as mean \pm standard deviation or median (interquartile range).

3. Results

3.1. Clinical characteristics of patients

Ninety-two serum samples were collected from confirmed patients (46 with CCA and 46 with BBD). Statistical analysis revealed that there were no remarkable differences in gender, AFP, WBC, and ALT levels between the two groups. Nevertheless, disparities in age, CEA, CA19-9, ALP, GGT, and AST were detectable between CCA and BBD. More detailed clinical characteristics are listed in Table 1.

Table 1. Clinical characteristics of all patients.

	BBD	CCA	<i>p</i>
Age#	55.13\pm17.712	70\pm10.75	.001
Male/Female	24/22	28/18	.406
AFP*	1.515(1.157-2.512)	1.64(1.052-2.837)	.799
CEA*	1.590(0.815-2.282)	2.825(1.775-4.742)	.001
CA199*	18.45(8.625-38.55)	278(98.975-854.5)	.001
WBC*	6.095(5.452-7.020)	6.980(4.720-8.275)	.689
ALP*	77(58.25-135)	221.95(161.5-519.75)	.001
GGT*	51.6(18-178)	215(127.75-385)	.001
ALT*	25.1(14.975-66.175)	37.3(21.775-70.475)	.161
AST*	22.1(15.75-36.025)	41.15(27.325-52.775)	.001

#Data represents mean \pm standard deviation; *Data represents the median (interquartile range). Abbreviation: CCA, cholangiocarcinoma; BBD, benign biliary diseases; AFP, Alpha-fetoprotein; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; WBC white blood cell; ALP alkaline phosphatase; GGT gamma-glutamyl transferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

3.2. VOC Profile Analysis in CCA and BBD Patients

Figure 1A shows that VOC signal peaks in serum samples are characterized by retention index, migration time, and peak intensity. Figures 1B and 1C illustrate the differences in peak intensity between CCA and BBD samples in both 3D and 2D spectra, respectively, as indicated by color intensity. From this analysis, a total of 51 volatile organic compound (VOC) signal peaks were identified from the serum samples (Figure 1D). Subsequently, 23 defined VOC substances along with 4 unidentified substances were analyzed using VOCal software (version 0.1.1) for further examination. A PCA 3D scatter plot reveals distinct clusters, indicating differing VOC profiles between CCA patients and those with BBD (Figure 1E).

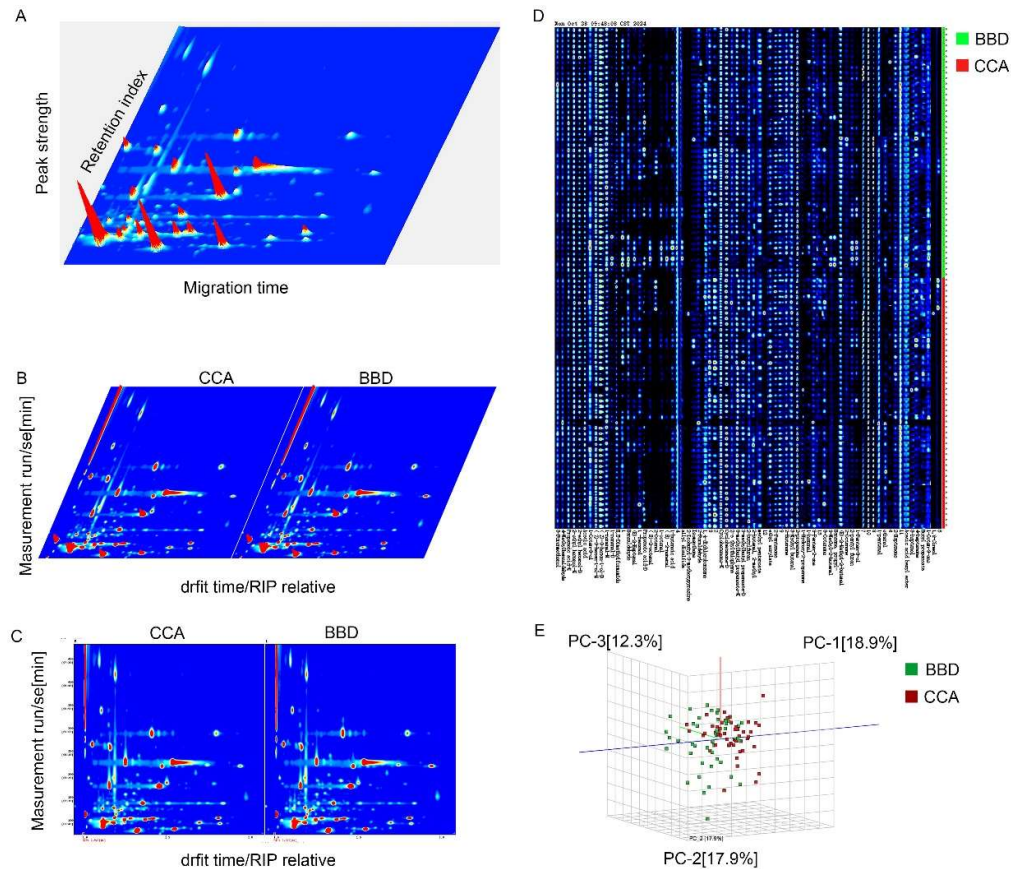


Figure 1. VOCs profile detected in CCA and BBD. (A) A 3D signal model is actually generated with three dimensions represent peak strength, retention index and migration time respectively. (B) 3D and (C) 2D map shows the difference in the VOCs between a CCA sample and a BBD sample, can intuitively observe the migration time and retention index of different VOCs. (D) The VOCs signal peaks detected in all serum samples with red representing a higher concentration of the substance and blue representing a lower concentration. (E) Principal component analysis (PCA) shows an obvious difference between CCA and BBD.

3.5. Vocs biomarkers in serum and pathological parameters

We investigated the association between serum VOC alterations and CCA progression by analyzing ten crucial VOCs against diverse pathological parameters. Table 4 illustrates the p-values for each VOC and distinct pathological factors. After conducting the Mann-Whitney U test, it was revealed that 1-pentanal and Unk4 had statistical disparities in relation to jaundice, where the median of the non-jaundice group was greater than that of the jaundice group. 3-methylbutyl propanoate-M, 3-methylbutyl propanoate-D, and ethyl acrylate manifested significant differences among the pathological types of cancer, accompanied by a decreasing trend in adenocarcinoma. Detailed data is in Supplementary Table 1.

Table 4. The *p*-values between each VOC and different pathological factors.

VOCs	Age	Gender	Areas	Differentiation	Jaundice	Pathological types
1-nonanal-M	0.318	0.212	0.966	0.847	0.170	0.725
1-nonanal-D	0.506	0.071	0.942	0.989	0.329	0.891
1-pentanal	0.233	0.589	0.158	0.280	0.039	0.055
1-octanal	0.894	0.552	0.811	0.503	0.667	0.560
2-butenal,2-methyl	0.381	0.906	0.650	0.201	0.254	0.631
3-methylbutyl propanoate-M	0.203	0.831	0.233	0.639	0.313	0.004
3-methylbutyl propanoate-D	0.866	0.884	0.128	0.295	0.763	0.036
(E) -2-nonenal	0.398	0.239	0.823	0.639	0.644	0.891
allyl disulfide	0.721	0.595	0.870	0.853	0.555	0.132
ethyl acrylate	0.730	0.464	0.322	0.846	0.987	0.038
Unk2	0.750	0.457	0.694	0.221	0.245	0.369
Unk3	0.508	0.719	0.444	0.800	0.508	0.458
Unk4	0.135	0.550	0.333	0.943	0.017	0.515
Unk12	0.903	0.606	0.877	0.659	0.861	0.492

Data are the median (interquartile range). *p*-value <0.05 indicates statistical significance (Mann-Whitney U test) .

3.3. Quantitative Analysis of VOCs in serum sample

Quantification used the internal standard method based on VOC peak volume. Specifically, 10 mL of 4-methyl-2-pentanol at 15 mL/L was added to each sample. Figure 2 shows 14 VOC expression differences between CCA and BBD patients., including 8 that are upregulated in CCA patients (3-methylbutyl propanoate-M, 3-methylbutyl propanoate-D, 1-pentanal, 2-butenal, 2-methyl, ethyl acrylate and three unidentified gases Unk2, Unk3 and Unk12) and 6 VOCs downregulated in CCA (1-nonanal-D, 1-nonanal-M, E)-2-nonenal, 1-octanal, diallyl disulfide and Unk4). No significant differences were found in the other 13 VOCs between CCA and BBD, as shown in Supplementary Figure 1. Detailed data is in Table 2.

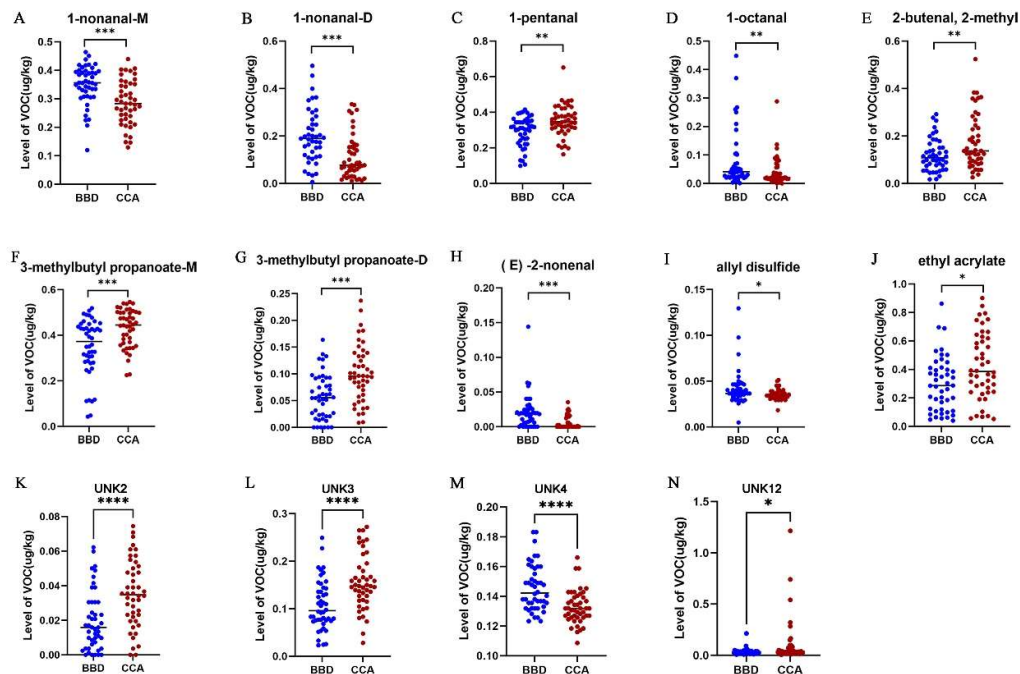


Figure 2. Quantitative analysis of VOCs. The levels of 1-nonanal-M (A), 1-nonanal-D (B), 1-pentanal (C), 1-octanal (D), 2-butenal 2-methyl (E), 3-methylbutyl propanoate-M (F), 3-methylbutyl propanoate-D (G), (E)-2-nonenal (H), allyl disulfide (I), ethyl acrylate (J), Unk2 (K), Unk3 (L), Unk4 (M), Unk12 (N). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (Mann–Whitney U test). Data represents the median (interquartile range).

Table 2. Quantitative analysis of VOCs.

VOCs	BBD	CCA	<i>p</i>
1-Octen-3-one	34(28-56)	32(27-46)	.322
1-pentanal	310(238-349)	346(312-400)	.003
1-Penten-3-ol	26(8-84)	38(7-58)	.758
1-octanal	42(23-78)	26(16-56)	.006
1-butanoic acid	17(5-21)	18(12-22)	.625
1-nonanal-M	356(309-397)	283(228-352)	<.001
1-nonanal-D	194(118-302)	77(41-156)	<.001
2-pentyl furan	63(42-98)	63(42-98)	.997
2-butenal, 2-methyl	108(70-155)	137(90-251)	.016
2-ethylthiophene	74(66-102)	71(52-95)	.206
2-ethyl hexanol-M	302(273-318)	290(250-315)	.131
2-ethyl hexanol-D	136(106-162)	118(79-159)	.079

3-methylbutyl propanoate-M	373(282-440)	450(362-500)	<.001
3-methylbutyl propanoate-D	56(21-92)	96(60-134)	<.001
3-Methyl-2-butenal	40(35-46)	37(33-44)	.131
(E) -2-nonenal	18(6-25)	0(0-15)	<.001
(E)-2-Heptenal	37(16-67)	26(17-54)	.517
(E)-2-octenal	32(16-45)	25(0-42)	.058
allyl disulfide	37(34-46)	34(32-37)	.017
Benzene, propyl-	18(13-25)	18(16-24)	.681
Benzaldehyde	26(22-35)	23(20-37)	.277
ethyl acrylate	286(116-411)	385(242-621)	.012
Propanoic acid-D	15(0-22)	5(0-17)	.078
Unk2	16(5.5-30)	35(21-50)	<.001
Unk3	96(75-143)	148(118-140)	<.001
Unk4	142(133-159)	132(156-140)	<.001
Unk12	31(23-38)	35(27-80)	.047

Data are the median (interquartile range). *p*-values are from Mann-Whitney U test. Unk2、Unk3、Unk4 and Unk12 was an unknown substance.

3.4. Diagnostic performance of ROC analysis for Vocs

ROC analysis showed that VOCs have high specificity (80.4%~100%) for diagnosing CCA, but their sensitivity is low (0%~60.9%). The compounds with AUC values exceeding 0.75 are 1-nonanal-D, (E)-2-nonanal, and Unk4, with (E)-2-nonanal having the highest AUC of 0.761 (95% CI: 0.662-0.860), 56.5% sensitivity, and 80.4% specificity (Table 3).

Table 3. The AUC, sensitivity and specificity of VOCs for CCA diagnosis.

VOCs	AUC (95%CI)	Sensitivity (%)	Specificity (%)	<i>p</i>
1-nonanal-M	0.730(0.626 - 0.834)	60.9	84.8	.000
1-nonanal-D	0.757(0.657 - 0.857)	32.6	89.1	.000
1-pentanal	0.318(0.210 - 0.427)	0	97.8	.003
1-octanal	0.666(0.554 - 0.778)	4.3	100	.006
2-butenal,2-methyl	0.355(0.243 - 0.467)	0	97.8	.016

3-methylbutylpropanoate-M	0.288(0.184- 0.392)	0	97.8	.000
3-methylbutyl propanoate-D	0.266(0.164 - 0.367)	2.2	87	.000
(E) -2-nonenal	0.761(0.662 - 0.860)	56.5	80.4	.000
allyl disulfide	0.644(0.528 - 0.760)	2.2	97.8	.017
ethyl acrylate	0.348(0.236- 0.460)	0	97.8	.012
Unk2	0.268(0.164- 0.372)	4.3	89.1	.000
Unk3	0.261(0.158-0.363)	0	97.8	.000
Unk4	0.750(0.652-0.849)	2.2	100	.000
Unk12	0.380(0.264 - 0.495)	2.2	100	.047

Data are the median (interquartile range). p-value <0.05 indicates statistical significance (Mann-Whitney U test) . AUC, area under the curve; CI, confidence interval; VOCs, volatile organic compounds.

4. Discussion

Cholangiocarcinoma has a high degree of malignancy and a low survival rate for patients, but its occurrence has no significant signs. The difficulty of early diagnosis is also well known. If early and accurate diagnosis can be made, early intervention measures can be taken to improve survival rates. Recently, the use of VOCs for diagnosing different cancers has attracted increased academic attention. Gui et al[24] used GC-IMS to successfully distinguish VOCs in bile between perihilar cholangiocarcinoma (PHCCA) and BBD. Similarly, Navaneetan U's research showed that bile VOCs could differentiate between CCA and primary sclerosing cholangitis (PSC) stenosis[25]. However, the difficult and distressing process of obtaining bile makes it unsuitable for early screening and diagnosis in routine physical exams.

In metabolomics research, serum is the second most used sample after urine due to its easy collection and low trauma. VOCs in serum, transported via the bloodstream and exhaled, may indicate early metabolic changes in tumor cells[26]. Thus, analyzing serum metabolites as biomarkers for cholangiocarcinoma is a sensible strategy. Sana et al [27] used solid-phase microextraction and gas chromatography/mass spectrometry to study VOCs in the serum of pregnant women with gestational diabetes. They identified 2-propanol as a possible biomarker for cognitive impairment in these women. Liu et al[28] showed that serum VOCs can effectively distinguish esophageal cancer patients from healthy individuals. Wu et al[29] found that HDL in serum mediated the relationship between co-exposure to VOCs and kidney stone formation. Therefore, in this study, we investigated the efficacy of VOCs in serum for diagnosing CCA. Our study is the first to identify significant changes in serum headspace VOCs in CCA and BBD patients. PCA scatter plots show a clear distinction between the two, indicating that CCA patients typically have distinct VOC profiles compared to BBD patients.

Using GC-IMS, we identified 27 substances in two serum sets, comprising 10 aldehydes, 3 esters, 3 alcohols, 2 acids, 2 heterocycles, 1 sulfur compound, 1 ketone, 1 hydrocarbon, and 4 unknowns. Notably, 14 VOCs differed significantly, with 8 being upregulated in CCA (3-methylbutyl propanoate-M, 3-methylbutyl propanoate-D, 1-pentanal, 2-butenal, 2-methyl-, ethyl acrylate, Unk2, Unk3 and Unk12) and six substances in CCA exhibit significant downregulation. (1-nonenal-D, 1-nonenal-M, E)-2-nonenal, 1-octanal, diallyl disulfide and Unk4). Aldehydes are the main type of these substances. E)-2-nonenal, an unsaturated aldehyde from lipid peroxidation, can signal proteins via ROS and is linked to oxidative processes. The exact method is not yet fully understood,

but its presence might indicate abnormal oxidative metabolism in cells, possibly related to a cancer-promoting environment[30]. Aldehyde dehydrogenase serves as a crucial catalyst within the human body, facilitating the oxidation of aldehydes to carboxylic acids[31], a carboxylic acid participates in the synthesis of intracellular lipids and provides materials for the cell membrane[32]. Due to the heightened metabolic activity of tumor cells, there is an upregulation in the activity of aldehyde dehydrogenase, which catalyzes the oxidation of a majority of aldehyde compounds into carboxylic acids, thereby facilitating tumor cell proliferation. This biochemical process might explain the notable drop in aldehyde levels in the CCA group versus the benign control group. Monedeiro et al.'s study indicates that (E)-2-nonenal could differentiate oral cancer from oral inflammatory diseases[33]. Our research indicates it can differentiate between cancer and benign disease groups.

Ethanol acrylate is an acrylic monomer utilized in the synthesis of various polymers and copolymers. The International Agency for Research on Cancer (IARC) categorizes it as a Group 2B carcinogen, indicating it is possibly carcinogenic to human[34]. Walker AM et al[35] conducted a retrospective study that revealed a significantly elevated risk of colon cancer mortality among workers exposed to ethyl acrylate. Likewise, our study found a notable rise in its levels among cholangiocarcinoma patients.

Furthermore, within the CCA cohort, a notable reduction was observed in compounds such as diallyl disulfide, a primary organic sulfur constituent found in garlic[36]. Recent research has provided substantial evidence indicating that it demonstrates anti-tumor activity against a variety of tumor cell types, including breast cancer [37], prostate cancer [38], lung cancer [39], gastric cancer [40], colorectal cancer [41], hepatocellular carcinoma cell lines[42]. Xia L et al[43] demonstrated that diallyl disulfide can inhibit the expression and activity of Rac1 by suppressing the PI3K/Akt signaling pathway, thereby reducing the invasion and migration capabilities of epithelial-mesenchymal transition (EMT) and colon cancer cells, ultimately exhibiting anti-colon cancer effects. Kim H et al[41] demonstrated that diallyl disulfide can inhibit the progression of colon cancer by downregulating the anti-apoptotic protein Bcl-2, in association with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Xiong T et al[44] demonstrated through both in vivo and in vitro experiments that diallyl disulfide significantly inhibits the proliferation, invasion, and migration of breast cancer cells. In our study, we found that the concentration of diallyl disulfide in the cholangiocarcinoma group was significantly lower than that in the control group, corroborating findings from prior research. While the resistance of diallyl disulfide to cholangiocarcinoma has not been previously documented, the differential expression of this compound holds substantial implications for differentiating cholangiocarcinoma from benign biliary diseases.

To assess the diagnostic efficacy of VOCs for CCA, we performed a ROC analysis on 14 key VOCs. The analysis revealed that these VOCs exhibited high specificity, ranging from 80% to 100%, while their sensitivity was relatively low, ranging from 0% to 60%. Among them, (E)-2-nominal has the best AUC value with 56.5% sensitivity and 80.4% specificity at 0.761, respectively. This observation contrasts with the high sensitivity yet low specificity associated with CA19-9. To enhance diagnostic efficiency, it may be beneficial to integrate the detection methods of VOCs with CA19-9. However, the efficacy of such a combined diagnostic approach requires further investigation and exploration.

Although VOCs generally exhibit good performance in differentiating CCA from BBD, not all individual VOCs are correlated with pathological features. In the present study, through the application of the Mann Whitney U test, significant associations were identified between specific VOCs and pathological parameters. We observed that 1-pentanal and Unk4 presented statistically significant differences in terms of jaundice, with the median of the non-jaundice group exceeding that of the jaundice group. The concentrations of 3-methylbutyl propanoate-M, 3-methylbutyl propanoate-D, and ethyl acrylate exhibited remarkable differences among diverse cancer pathological types, and a notably downward regulation was especially detectable in adenocarcinoma patients. These substances demonstrate a remarkable capacity to differentiate between patients with CCA and those with BBD.

However, this observation is based on a limited patient cohort and necessitates validation with larger, independent datasets for external confirmation.

5. Conclusion:

In this study, using GC-IMS, we identified 14 VOCs associated with cholangiocarcinoma, which have advantages in distinguishing cholangiocarcinoma patients from benign biliary disease patients, thereby offering a viable approach for CCA detection. The volatile organic compounds in serum are mainly aldehydes, which are of great significance for the differential diagnosis of biliary malignancies. In addition, this study first discovered a correlation between serum volatile organic compounds and pathological parameters in CCA, and analyzed its diagnostic efficiency using ROC. The findings present empirical evidence supporting the application of VOC analysis in CCA, which is anticipated to significantly influence future clinical diagnosis and monitoring of CCA, and has extremely broad clinical application prospects. However, more scientific research is needed to explore the mechanisms of VOCs production and metabolism.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1: 13 VOCs with no significant difference between CCA and BBD. Table S1: VOCs and different pathological factors.

Author contributions: Conceptualization: Xuewei Zhuang; Data curation: Jing He; Formal analysis: Jing He, Qi Liu; Investigation: Jing He, Xiaoyue Zhao; Methodology: Jing He; Resources: Jing He, Xuewei Zhuan; Software: Jing He; Supervision: Jing He, Xin Li; Validation: Jing He, Xiaoyue Zhao; Visualization: Jing He, Xin Li; Writing – original draft: Jing He; Writing – review & editing: Jing He, Xuewei Zhuang.

Funding: This work was supported by grants from Shandong Provincial Nature Foundation (Grant No. ZR2020MH321); Shandong Provincial Key Research and Development Program (Grant No.2016GSF201169) and Research on Key Technologies of Molecular POCT Systems in the Field of Infectious Diseases (SLSYKYB2022010).

Institutional Review Board Statement: This study was conducted in accordance with the Helsinki Declaration and has been approved by the Ethics Committee of the Third Hospital of Shandong University (Ethics Review Number: KYLL-2023084).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author(s).

Acknowledgments: Gratitude is extended to Professor Liu Yili from Shandong University for his invaluable assistance in statistical analysis.

Conflicts of Interest: The authors declare no conflict of interest.

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