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Review

Volatile organic compounds for the detection of hepatocellular carcinoma—A scoping review

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ABSTRACT

Hepatocellular carcinoma (HCC) is an increasingly common and the second leading cause of cancer mortality worldwide with a 5-year survival rate about 12%. Less than 20% of HCC patients are eligible to curative treatment owing to the late presentation. Clearly, there is a need for a readily accessible, early screening tool. This scoping review critically appraises and synthesizes the current published knowledge about the use of exhaled volatile organic compounds (VOCs) as a potential noninvasive means for HCC detection aiming to advance this nascent field. A systematic electronic search was conducted. The search strategy included all studies published until the 24th of March 2023 using a combination of relevant keywords. The search yielded 9 publications using the Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines. Two of the studies described *in vitro* experiments, and seven clinical studies were conducted on small groups of patients. Overall, 42 headspace gases were analyzed in the *in vitro* studies. Combined, the clinical studies included 420 HCC patients and 630 controls. The studies reported potential role for a combination of VOCs in the diagnosis of HCC. However, there is lack of consensus. Although there appears to be promise in VOCs research associated with HCC, there is no single volatile biomarker in exhaled breath attributed to HCC and data from extracted studies indicates a lack of standardization. Large multicentre population studies are required to verify the existence of VOCs linked to HCC.

1. Introduction

Liver cancer is one of the leading causes of cancer death worldwide [1,2]. According to the Global Cancer Statistics 2020, worldwide primary liver cancer has been diagnosed in over 920,000 people, represented a third of cancer-related death in 2020 [3], and accounted for the fifth cause of cancer-related mortality in males in the United States in 2019 [4]. The incidence is 9.5 per 100,000 with 30,200 new cases and a mortality rate of 8.7 cases per 100,000 in the United States in 2019 [4]. The vast majority of primary liver cancers, nearing 80%, is hepatocellular carcinoma (HCC), with growing global burden and an incidence that has tripled during the past 40 years [2]. HCC most commonly arises in the background of liver cirrhosis, and 80%–90% of patients are found to have cirrhotic changes at the time of diagnosis [5]. The etiology of cirrhosis varies depending on geographic location; however, most prevalent risk factors are hepatitis B virus and hepatitis C virus infection, excessive chronic consumption of alcohol, and diabetes or obesity-related non-alcoholic steatohepatitis (NASH) [6]. Up to one-third of patients with established cirrhosis will be diagnosed with HCC during their lifetime, with an annual incidence rate of 1%–8% [7]. It is crucial to early detect liver parenchyma growths as patients with Barcelona Clinic Classification (BCLC) stage 0 have a 70%–90% chance of 5-year survival. However,

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HCC develops insidiously, and frequently, it is diagnosed late. Life expectancy dramatically drops to only 4–6 months with the progression of the disease to BCLC stage 4 [6].

Given the dismal prognosis in advanced cases, several surveillance programs are recommended by national and international bodies such as the American Association for the Study of Liver Diseases, European Association for the Study of the Liver, and National Institute for Health and Care Excellence in the UK [8–10]. These guidelines universally support a regime of 6 monthly ultrasound scan screening. Combination with serum Alpha-Fetoprotein at a cut-off ≥20 ng/mL increases the sensitivity of detection of hepatic lesions [11]; however, at the best, the sensitivity remains as low as 47.7% [12].

Considering the rising HCC incidence, research efforts have been focussing on developing new noninvasive, cost- and time-effective, sensitive screening tests. Recently, numerous pieces of literature published on successfully profiling volatile organic compounds (VOCs) in malignancies, such as lung [13,14], breast, and colorectal [15], suggest that there is a scope for building similar diagnostic tools in liver disease. This approach has been explored in liver cirrhosis [16]; however, data about volatilome specific for HCC are generally scarce [17–22]. In this scoping review, we aim to compile and critically review existing knowledge related to the utility of VOCs for the detection of HCC.

2. Methodology

This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines [23].

Two independent authors searched electronic databases PubMed, Medline, OVID Embase, and Web of science for articles published before 24th of March 2023. The same search has been performed by an experienced medical librarian from the Royal College of Surgeon of Edinburgh library. Combinations of the following key words were used for searching HCC, hepatocellular carcinoma, volatile organic compounds. Keywords were searched in “all fields” and Medical Subject Headings Term sections. No restriction on language was imposed. Animal studies were excluded as the review focused on human HCC markers. All pertinent publications, excluding conference papers, were analyzed. Additionally, bibliographies of identified studies were searched for relevant publications. Subsequently, candidate articles were scrutinized by two independent investigators according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses protocol. A total of 4075 records were identified. Following exclusion of duplicates, rejection of irrelevant papers after review of titles and abstracts, and implementation of the exclusion criterion of animal studies and conference abstracts, nine publications remained for analysis (enumerated in Table 1). Studies selection flow diagram is illustrated in Fig. 1.

3. Results

Overall, 9 publications are included in this review. Both Amal et al. [17] and Mochalski et al. [20] generally utilized gas chromatography–mass spectrometry (GC–MS) to explore the VOCs released by *in-vitro* HCC cell cultures. Amal et al. focused on exploring characteristic VOC changes that reflect the metastatic potential of HCC cell lines obtained by *in-vivo* clonal selection [17]. The group utilized both GC–MS and an array of nanomaterial sensors to compare VOC profiles of 4 low metastatic potential HCC cell lines, a high metastatic potential cell line, and a normal human immortalized hepatocyte cell line [17]. They identified nine VOCs that can differentiate groups and found methane-sulfonyl chloride to be elevated in HCC compared to normal cell cultures, whereas 2,3 di-hydro-benzofuran is elevated in high metastatic potential HCC compared to low metastatic potential HCC [17]. On the other hand, Mochalski et al. [20] applied GC–MS and head-space needle trap device extraction to profile the VOCs metabolized by the HepG2 liver cell line. They identified 12 metabolites released by HepG2 cells, namely 2-pentanone, 3-heptanone, 2-heptanone, 3-octane, 2-nonanone, dimethyl sulfide, ethyl methyl sulfide, 3-methyl thiophene, 2-methyl-1-(methyl thio)-propane, 2-methyl-5-(methylthio) furan, 2-heptene, and n-propyl acetate [20].

On the other hand, the clinical studies generally included diverse patients with comparable demographic characteristics. Qin et al. compared the VOCs released from HCC patients comorbid with hepatitis B and cirrhosis ($n = 30$) to controls of patients with cirrhosis ($n = 27$) and healthy volunteers ($n = 36$) [22]. The group utilized GC–MS with solid-phase microextraction to analyze breath samples and identified 3 VOCs (3-hydroxy-2-butanone, styrene and decane) and highlighted significantly different levels of 3-hydroxy-2-butanone between the study groups [22]. Qin et al. built a model based on the 3 identified VOCs, which showed a sensitivity of 86.7% and a specificity of 91.7% in distinguishing HCC from healthy controls [22]. When applying the model to breath samples of cirrhosis patients, 33.3% were falsely diagnosed with cancer [22].

O'Hara et al. investigated the role of breath VOCs limonene, methanol, and 2-pentanone in distinguishing between liver cirrhosis ($n = 21$), HCC ($n = 10$), and controls ($n = 30$) [21] They used a proton transfer reaction quad mass spectrometer and identified significantly lower levels of limonene in patients with HCC than in those without HCC [21]. Conversely, Ferrandino et al. applied thermal desorption GC–MS to measure VOCs in exhaled breath samples of 40 controls, 32 cirrhotic patients, and 12 HCC patients and found significantly increased breath limonene in both cirrhosis and cirrhosis-induced HCC compared to controls [18].

Miller-Atkins et al. utilized selective ion flow tube mass spectrometry (SIFT-MS) to measure 22 VOCs in breath samples from patients with HCC ($n = 112$), colorectal cancer liver metastases ($n = 51$), cirrhosis ($n = 30$), and controls comprising pulmonary hypertension patients ($n = 49$) and individuals with no liver disease ($n = 54$) [19]. The group highlighted the VOCs acetone, acetaldehyde, and dimethyl sulfide best in differentiating cirrhosis and HCC patients, whereas E-2-nonene, ethane, and benzene were the best distinguished HCC and controls [19]. Furthermore, Miller-Atkins et al. built a random-forest machine-learning predictive model based on VOCs and demographic variables with an 85% classification accuracy. They demonstrated improved sensitivity in detecting HCC compared to Alpha-Fetoprotein (73% versus 53%, respectively) [19].

In 2022, Sukaram et al. profiled the VOCs in HCC patients ($n = 97$) and controls ($n = 111$, including healthy volunteers $n = 33$ and cirrhosis patients $n = 78$) using untargeted GC–MS and support vector machine algorithm for data analysis [24]. They identified a combination of 6 VOCs (acetone, 1,4-pentadiene, methylene chloride, benzene, phenol, and allyl methyl sulfide) with an accuracy of 79.6%, in the training set, however, the accuracy dropped to 55.4% accuracy in the test set [24]. They additionally followed up a subgroup of HCC patients after treatment ($n = 34$), including percutaneous local ablative therapy ($n = 14$) and transarterial chemoembolization ($n = 20$) for 1–2 months and highlighted associated reduction in acetone level in patients who responded [24].

Moreover, in 2023, Sukaram et al. compared the VOCs profiles of HCC ($n = 124$), cirrhosis patients ($n = 124$), and normal volunteers ($n = 95$) using thermal desorption and gas chromatography–field asymmetric ion mobility spectrometry (GC-FAIMS) [25]. The group utilized eXtreme Gradient Boosting (XGBoost) algorithm and identified 9 VOCs (acetone monomer, ethanol, acetone dimer, acetonitrile, benzene, toluene, 1,4-pentadiene, isopropyl alcohol, and dimethyl sulphide) that differentiate HCC from cirrhosis and healthy controls with 70.0% sensitivity, 88.6% specificity, 76.2% positive predictive value (PPV), 84.8% negative predictive value (NPV), and 82.1% accuracy [25]. Additionally, they identified a combination of ethanol, acetone dimer,

Table 1
Summary of included studies.

Study	Type of study	Number of subjects and etiology	Analysis method	Identified VOCs
Amal et al. (2012) [17]	*In-vitro*	36 cell cultures from 6 cell lines (1 high metastatic potential; 4 low metastatic potential; and 1 normal liver cells)	GC–MS and nanomaterial sensors	Methane-sulfonyl chloride (elevated in HCC compared to normal cell cultures) 2,3 di-hydro-benzofuran (elevated in high metastatic potential HCC compared to low metastatic potential HCC)
Mochalski et al. (2013) [20]	*In-vitro*	Six cultivation experiments of HepG2 cell line	GC–MS and head-space needle trap device extraction	2-pentanone, 3-heptanone, 2-heptanone, 3-octanone, 2-nonanone, dimethyl sulfide, ethyl methyl sulfide, 3-methyl thiophene, 2-methyl-1-(methylthio)- propane, 2-methyl-5-(methylthio) furan, 2-heptene and n-propyl acetate
Tao Qin et al. (2010) [22]	*In-vivo*	30 HCC patients (co-morbid with hepatitis B and cirrhosis). 27 cirrhotic patients due to chronic hepatitis B. 36 controls (healthy volunteers).	GC–MS with solid-phase microextraction	3-Hydroxy-2-butanone, styrene, and decane (3-hydroxy-2-butanone was found to have the best diagnostic value in distinguishing HCC from normal controls and a model including the 3 VOCs has a sensitivity of 86.7% and a specificity of 91.7% in differentiating HCC from controls)
O'Hara et al. (2016) [21]	*In-vivo*	10 HCC patients (various etiologies), 21 cirrhotic patients and 30 controls	Proton transfer reaction quad mass spectrometer	Limonene (level is significantly lower in patients with HCC compared to those without HCC)
Ferrandino et al. (2020) [18]	*In-vivo*	12 HCC patients (various etiologies) 32 cirrhotic patients 40 controls	thermal desorption GC–MS	Limonene (level is significantly increased in both cirrhosis and cirrhosis-induced HCC compared to controls)
Miller-Atkins et al. (2020) [19]	*In-vivo*	112 HCC patients (various etiologies) 30 cirrhotic patients 54 controls	Selected ion flow tube mass spectrometry	2-propanol, acetaldehyde, acetone, acetonitrile, acrylonitrile, benzene, carbon disulphide, dimethyl sulphide, ethanol, isoprene, pentane, 1-decene, 1-heptene, 1-nonene, 1-octene, 3-methylhexane-(E)-2-nonene, ammonia, ethane, hydrogen sulphide, triethylamine, and trimethylamine.
Sukaram et al. (2022) [24]	*In-vivo*	97 HCC patients and 111 controls	GC–MS	• The combination of acetone, 1,4-pentadiene, methylene chloride, benzene, phenol and allyl methyl sulfide for detection of HCC (accuracy reduced to 55.4% in the test set) • Acetone level reduction cor relates with treatment response.
Sukaram et al. (2023) [25]	*In-vivo*	124 HCC patients (including a subgroup of 38 patients who were progressively followed up for a month after chemoembolization $n = 22$ or radiofrequency/microwave ablation $n = 16$ to check for treatment response), 124 cirrhosis patients, and 95 healthy volunteers	Thermal desorption and gas chromatography–field asymmetric ion mobility spectrometry.	• A combination of 9 VOCs (acetone monomer, ethanol, acetone dimer, acetonitrile, benzene, toluene, 1,4-pentadiene, isopropyl alcohol and dimethyl sulphide) for differentiating HCC, cirrhosis and healthy controls. • A combination of 5 VOCs (ethanol, acetone dimer, benzene, 1,4-pentadiene, and isopropyl alcohol) for differentiating HCC stages. • Acetone dimer is slightly better than AFP for detection of early HCC from cirrhosis (AUC: 0.775 vs. 0.714, $p = 0.001$). • Acetone dimer can differentiate responders and non-responders to HCC treatment (95.7% sensitivity, 73.3% specificity, and 86.8% accuracy). • Isopropyl alcohol is independently associated with the survival of HCC patients (adjusted hazard ratio of 7.23 (95% CI: 1.36–38.54), $p = 0.020$).
Nazir and Abbas (2023) [26]	*In-vivo*	HCC ($n = 35$) and normal individuals ($n = 30$).	GC–MS untargeted analysis and validated by targeted electrochemical electrodes using thiol-modified gold nanoparticles biosensors.	Phenol 2,2 methylene bis [6-(1,1-dimethyl ethyl)-4-methyl]in breath of HCC patients.

Abbreviations: AFP, Alpha-Fetoprotein; AUC, area under the curve; CI, confidence interval; GC–MS, gas chromatography–mass spectrometry; HCC, hepatocellular carcinoma; VOC, volatile organic compound.

benzene, 1,4-pentadiene, and isopropyl alcohol for differentiating early (BCLC stages 0, A) from advanced (BCLC stages B, C) HCC with 78.6% sensitivity, 88.9% specificity, 72.7% PPV, 91.7% NPV, and 82.6% accuracy [25]. Furthermore, Sukaram et al. prospectively followed up a subgroup of HCC patients treated with transarterial chemoembolization ($n = 22$) or percutaneous local ablative therapy ($n = 16$) for a month and identified acetone dimer as a potential marker for treatment response [25].

Finally, Nazir and Abbas utilized untargeted GC–MS to compare VOCs in HCC ($n = 35$) and normal individuals ($n = 30$) [26]. They identified significant difference in phenol 2,2 methylene bis [6-(1,1-dimethyl ethyl)-4- methyl] VOC with concentrations at least 2100 PPM in the breath of HCC patients and confirmed their finding by targeted gold nanoparticle biosensors [26].

Tables 1 and 2 summarize all VOCs found in the included studies. Table 3 summarizes demographics data.

4. Discussion

VOCs mixtures emanated from various cells differ due to differences in cell membrane structures [17]. The fundamental premise in VOCs research is that tumorigenesis causes gene and protein changes, which

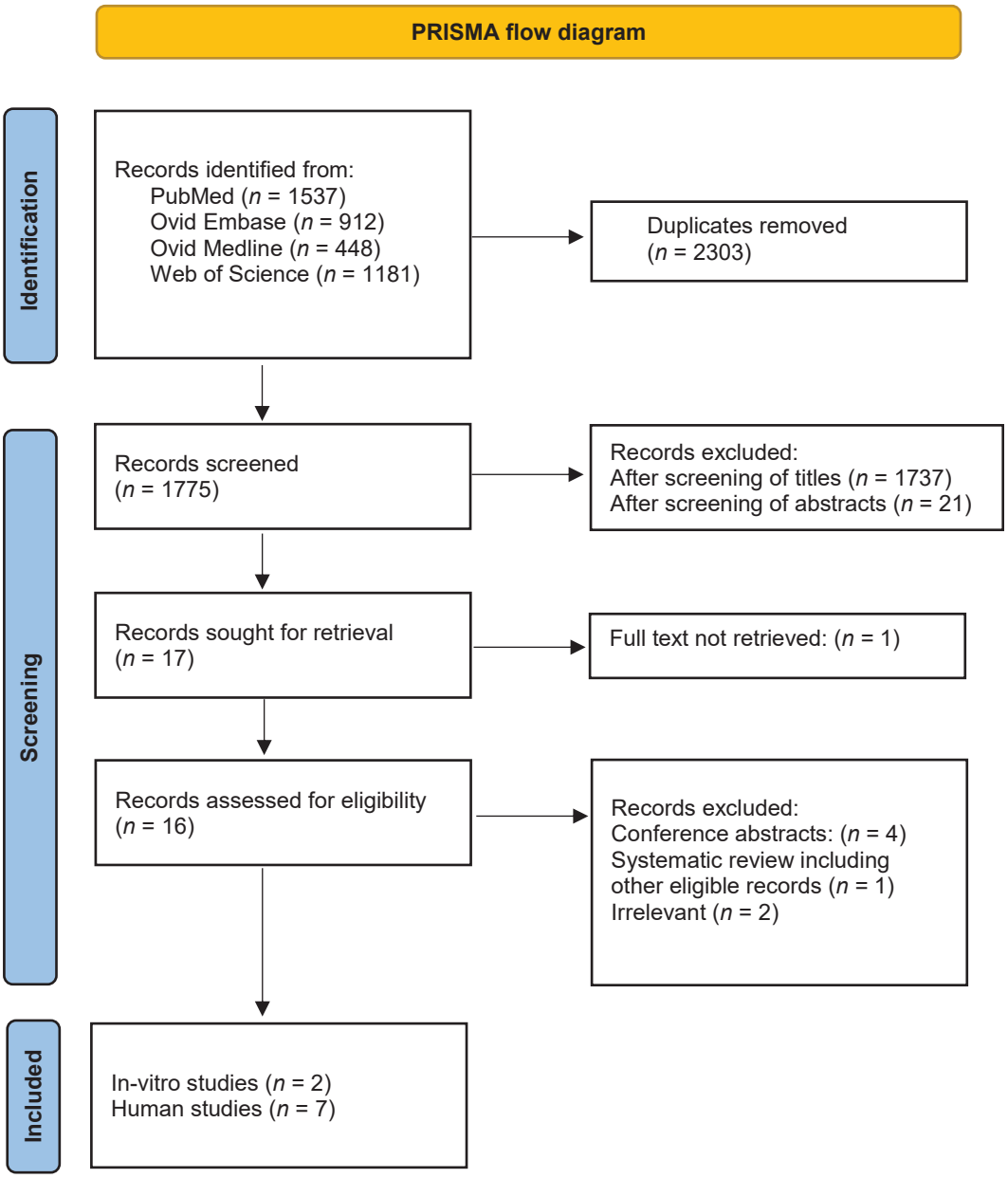

Fig. 1. PRISMA flow diagram. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-analyses.

lead to oxidative stress injury and peroxidation of the cell membrane lipids with a subsequent change in the resulting VOCs [17]. Overall, the published *in-vitro* VOCs research in HCC is highly variable with different cell lines, analytical methods, and statistical analysis, which are counterproductive for comparing results and generalization. Amal et al. and Mochalski et al. analyzed headspace gases emitted by cultured HCC cell lines, but the HCC cell lines are dissimilar (Table 1). HCC typically arises in a background of liver cirrhosis [27] and portal hypertension of various severities that can profoundly alter exhaled VOCs *in-vivo* [28]. Generally, *in-vitro* experiments exclude confounding factors associated with a patient's characteristics, such as comorbidities, medications, smoking, etc. Therefore, the presence of detected substances is contributed purely to unperturbed metabolic pathways. However, the metabolic activity of isolated cell lines in tissue cultures is an oversimplification that by no means fully represents the entire dynamism of *in-vivo* metabolism, given the apparent lack of modulatory factors, intersystems feedback loops, and compensatory mechanisms. Therefore, the results of *in-vitro* research do not necessarily reflect the actual findings in *in-vivo* studies.

The main drawbacks of the *in-vivo* clinical HCC VOCs research are the lack of standardization, relatively small sample size, and absence of external validation. Both O'Hara et al. [21] and Ferrandino et al. [18] investigated the role of breath limonene as a potential marker for HCC and reported contradictory results (Table 1). Limonene is an exometabolite of dietary origin that cannot be synthesized in the body and naturally occurs in citrus fruits and many vegetables [21]. Furthermore, it is widely used in industry to impart a citrus flavor and is an important ingredient in several perfumes and air fresheners [21]. Ingested limonene is rapidly cleared in normal individuals [21]; however, it tends to accumulate to a variable extent in adipose tissue in patients with liver disease owing to its highly lipophilic nature and the downregulation of the hepatic metabolizing enzymes CYP2C9 and CYP2C19 (Fig. 2) [29]. Due to such variability in the level of accumulated limonene, it can remain high for a few days after liver transplantation in some patients while continuing to be high for months in others [29].

Another important factor adding to the variability of the VOCs research is the analytical variability—different technologies utilized to

Table 2
Identified volatile organic compounds.

VOC	Class	Study	Type of study	*p*-value
Methane-sulfonyl chloride	Organosulfur compound	Amal et al. (2012) [17]	*In-vitro*	0.05 (+)
2,3 di-hydro-benzofuran	Coumaran	Amal et al. (2012) [17]	*In-vitro*	0.047 (+)[a]
Acetic acid	Organic acid	Amal et al. (2012) [17]	*In-vitro*	0.047 (+)[a]
Ethanol	Alcohol	Amal et al. (2012) [17] and Sukaram et al. (2023) [25]	*In-vitro* *In-vivo*	0.047 (+)[a] Not reported
Dimethyl sulphide	Organosulfur compound	Mochalski et al. (2013) [20] and Sukaram et al. (2023) [25]	*In-vitro* *In-vivo*	Not reported
Ethyl methyl sulphide	Organosulfur compound	Mochalski et al. (2023) [25]	*In-vitro*	Not reported
2-Pentanone	Ketone	Mochalski et al. (2023) [25] O'Hara et al. (2016) [21]	*In-vitro* *In-vivo*	>0.05
n-Propyl acetate	Ester	Mochalski et al. (2023) [25]	*In-vitro*	Not reported
3-methyl-thiophene	Organosulfur compound	Mochalski et al. (2023) [25]	*In-vitro*	Not reported
2-Heptene	Hydrocarbon	Mochalski et al. (2023) [25]	*In-vitro*	Not reported
2-methyl-1-(methylthio)-propane	Organosulfur compound	Mochalski et al. (2023) [25]	*In-vitro*	Not reported
3-Heptanone	Ketone	Mochalski et al. (2023) [25]	*In-vitro*	Not reported
2-Heptanone	Ketone	Mochalski et al. (2023) [25]	*In-vitro*	Not reported
2-methyl-5-(methylthio)-furan	Organosulfur compound	Mochalski et al. (2023) [25]	*In-vitro*	Not reported
3-Octanone	Ketone	Mochalski et al. (2023) [25]	*In-vitro*	Not reported
2-Nonanone	Ketone	Mochalski et al. (2023) [25]	*In-vitro*	Not reported
3-hydroxy-2-butanone	Ketone	Tao Qin et al. [22]	*In-vivo*	0.002[b]
Styrene	Hydrocarbon	Tao Qin et al. [22]	*In-vivo*	0.015[b]
Decane	Hydrocarbon	Tao Qin et al. [22]	*In-vivo*	0.076[b]
Limonene	Hydrocarbon	Ferrandino et al. [18] O'Hara et al. (2016) [21]	*In-vivo* *In-vivo*	0.37 0.015
Methanol	Alcohol	O'Hara et al. (2016) [21]	*In-vivo*	>0.05
Acetone	Ketone	Sukaram et al. (2022) [24] and Sukaram et al. (2023) [25]	*In-vivo*	Not reported
1,4-pentadiene	Hydrocarbon	Sukaram et al. (2022) [24] and Sukaram et al. (2023) [25]	*In-vivo*	Not reported
Methylene chloride	Organochlorine	Sukaram et al. (2022) [24]	*In-vivo*	Not reported
Benzene	Hydrocarbon	Sukaram et al. (2022) [24] and Sukaram et al. (2023) [25]	*In-vivo*	Not reported
Toluene	Hydrocarbon	Sukaram et al. (2022) [24]	*In-vivo*	Not reported
Phenol	Phenols	Sukaram et al. (2022) [24]	*In-vivo*	Not reported
Allyl methyl sulfide	Organosulfur	Sukaram et al. (2022) [24]	*In-vivo*	Not reported
Acetonitrile	organic nitrile	Sukaram et al. (2023) [25]	*In-vivo*	Not reported
isopropyl alcohol	Alcohol	Sukaram et al. (2023) [25]	*In-vivo*	Not reported
Phenol 2,2 methylene bis [6-(1,1-dimethyl ethyl)-4- methyl]	Phenol	Nazir and Abbas (2023) [26]	*In-vivo*	Not reported

VOC, volatile organic compound.
[a] Hepatocellular carcinoma cell with high metastatic potential (HCC-HMP) vs normal cells.
[b] Hepatocirrhotic (HC) vs HCC patients.

analyze VOCs profiles in HCC research. GC–MS is the gold standard for VOCs determination and the method of choice for qualitative analysis of complex mixtures of gases [30]. However, it requires laborious sample preparation and a relatively lengthier analysis time; therefore, it not capable of doing real-time analysis or performing direct quantitative analysis and requires costly standard solutions for quantitative determinations [30]. GC–MS was utilized in the *in-vitro* studies and four *in-vivo* studies, whereas other *in-vivo* studies utilized SIFT-MS, proton transfer reaction quad mass spectrometer, and GC-FAIMS. Combining thermal desorption or solid-phase microextraction with GC–MS reduces the ability to analyze complex gas mixtures [30]. These techniques have selective affinity for certain VOCs, and as such, only a fraction of the sample is analyzed, which is especially counterproductive in untargeted profiling research [30].

On the other hand, direct injection mass spectrometry techniques, such as SIFT-MS and proton-transfer-reaction mass spectrometry (PTR-MS), allow performing mass-spectrometry quantitative analysis of complex gas mixtures without the need for prior chromatographic separation [30]. Therefore, these methods are capable of rapid quantitative analysis without needing pricy standards [30]. There is a growing interest in PTR-MS given its high sensitivity, low detection limit, rapid real-time

Table 3
Demographics of the *in-vivo* clinical studies.

Study	Group (*n*)	Age (mean ± SD); or median [range]	Sex (male/female)
Tao Qin et al. (2010) [22]	HCC patients ($n = 30$)	53.0 ± 12.40	26/4
	Cirrhotic patients ($n = 27$)	51.67 ± 11.25	18/9
	Controls ($n = 36$)	48.57 ± 10.97	24/12
Ferrandino et al. (2020) [18]	HCC patients ($n = 12$)	69.5 [55–79]	7/5
	Cirrhotic patients ($n = 32$)	56.5 [35–78]	20/12
	Controls ($n = 40$)	62.0 [34–81]	21/19
Miller-Atkins et al. (2020) [19]	HCC patients ($n = 112$)	66.7 [25–95]	84/28
	Cirrhotic patients ($n = 30$)	59.6 [37–79]	14/16
	Controls ($n = 54$)	58.8 [36–80]	14/35
Sukaram et al. (2022) [24]	HCC patients ($n = 97$)	61.2 ± 11.6	72/25
	Controls ($n = 111$)	60.2 ± 10.7	88/23
Sukaram et al. (2023) [25]	HCC patients ($n = 124$)	62.7 ± 12.6	60/64
	Cirrhosis patients ($n = 124$)	60.6 ± 9.2	62/62
	Controls ($n = 95$)	59.3 ± 9.1	47/48

Abbreviations: HCC, hepatocellular carcinoma; SD, standard deviation.

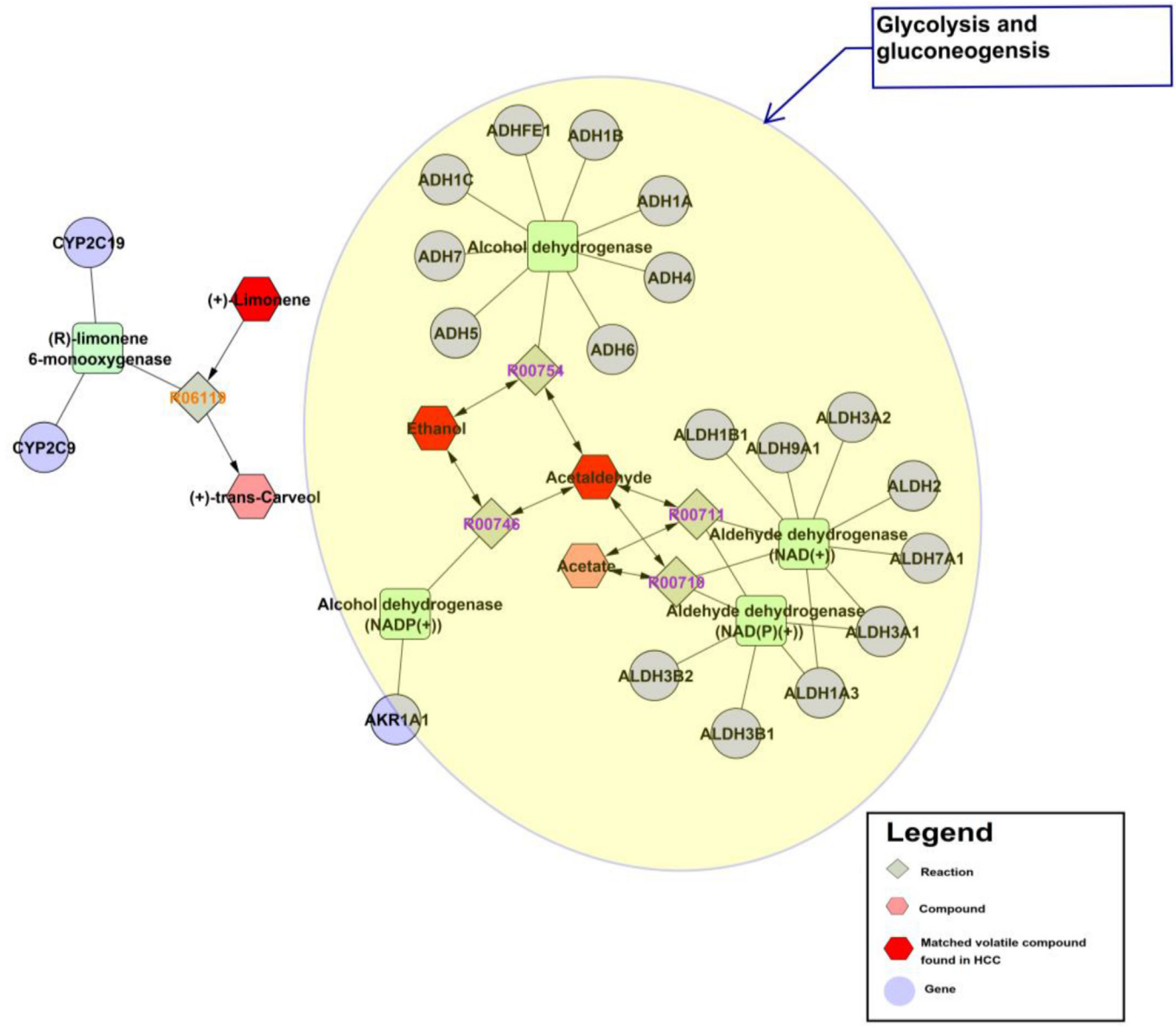

Fig. 2. Enrichment analysis of HCC VOCs. Pathway enrichment analysis of the VOVs identified in HCC *in-vitro* studies highlighted 3 VOCs: limonene, ethanol and acetaldehyde that are present in the Kyoto Encyclopedia of Genes and Genomes (KEGG). Limonene is metabolized by the enzyme limonene 6 monooxygenase under the control of hepatic CYP2C9 and CYP2C19. Acetaldehyde and ethanol participate in glycolysis and gluconeogenesis. HCC, hepatocellular carcinoma; VOC, volatile organic compound.

analysis, and no requirement for a carrier gas [30]. On the other hand, owing to the lack of separation in PTR-MS, it is impossible to differentiate isomers [30]. Additionally, PTR-MS can only detect VOCs with proton affinity; therefore, short-chain alkanes cannot be detected [30].

Furthermore, it is important to avoid the confounding effect of the coexisting conditions and their severity on VOCs [28]. Given that 80% of HCC cases rise on a background of cirrhosis, it is important to accurately profile its VOCs to avoid false positive results [28].

Nonetheless, the studies serve an exemplar for metabolomics ability to help unravel the potential underlying pathophysiology of HCC. Statistically significant acetaldehyde level difference was noted, among others, by Miller-Atkins et al. [19] when comparing HCC patients to individuals with cirrhosis (Fig. 2). Endogenous acetaldehyde causes alterations to the DNA strands' structure and function, leading to carcinogenic potential [31]. In healthy cells, acetaldehyde is metabolized to acetate by acetaldehyde dehydrogenase (ALDH) [32]. However, it has been demonstrated that the risk of oncogenic transformation rises when ALDH capacity is diminished, particularly ALDH2, and the risk of oncogenic transformation rises [33]. Furthermore, other VOC compounds highlighted by this review were linked to cancer, such as 3-hydroxy-2-butanone in lung cancer [34]. Endogenous styrene production is also found to relate to cell toxicity [35]. These findings reinforce the conceptual validity of the utility of VOCs as a diagnostic tool. However, further work is required to profile consistent biomarkers for HCC.

5. Conclusions and future directions

This scoping review demonstrated a lack of unified methodology in the included studies. Although no single volatile organic compound was found to be related to HCC, some light was shed on glucose metabolic pathways as a potential source of cancerous VOCs. Large population studies are required to explore the potential VOCs related

to HCC. Ideally, these studies should include patients with cirrhosis as controls.

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Author contributions

SM and AP drafted the manuscript. AP, OS and SM did the literature search. SM, IA, AM and MD critical appraisal and editing. MB general oversight and manuscript final approval. AP drafted the initial introduction, methods and results section. SM wrote the results, discussion, and introduction and critically reviewed the manuscript. MB conceptualized the study and contributed, with AM, MD, IA, OS to the critical review of the final manuscript. All authors read and approved the final manuscript.

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Declaration of competing interest

The authors declare that they have no competing interests.

Data available statement

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Ethics statement

NA.

Informed consent

NA.

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