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REVIEW ARTICLE

Breath biopsy, a novel technology to identify head and neck squamous cell carcinoma: A systematic review

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Abstract

Head and neck cancers are a heterogeneous group of neoplasms, which together comprise the sixth most common cancer globally. Breath biopsies are a non-invasive clinical investigation that detect volatile organic compounds (VOCs) in exhaled breath. This systematic review examines current applications of breath biopsy for the diagnosis of head and neck squamous cell carcinoma (HNSCC), including data on efficacy and utility, and speculates on the future uses of this non-invasive detection method. Medline, PubMed, Web of Science, Cochrane and Scopus, as well as the grey literature were searched using a search strategy developed to identify relevant studies on the role of breath biopsy in the diagnosis of HNSCC. All included studies were subject to a thorough methodological quality assessment. The initial search generated a total of 1443 articles, 20 of which were eligible for review. A total of 660 HNSCC samples were investigated across the included studies. 3,7-dimethylundecane and benzaldehyde were among several VOCs to be significantly correlated with the presence of HNSCC compared to healthy controls. We show that current breath biopsy methods have high accuracy, specificity and sensitivity for identifying HNSCC. However, further studies are needed given the reported poor quality of the included studies.

KEYWORDS
breath biopsy, diagnosis, head and neck cancer, volatile organic compound

1 | INTRODUCTION

Head and neck cancers collectively form the sixth most common type of cancer globally, with both incidence and mortality rates continuing on an upward trend (Johnson et al., 2019a, 2020; Patterson et al., 2020). The burden of disease is not only significant in terms of morbidity and mortality, but also weighs heavily economically (Patterson et al., 2020) and in terms of the quality of life of patients (Johnson et al., 2020; Min Ang et al., 2019). Head and neck cancer includes any malignant neoplasm affecting the lips and oral cavity, any pharyngeal region, the larynx and the major salivary glands: the majority are squamous cell carcinoma (HNSCC; Johnson et al., 2019a, 2020). The anatomical sites most commonly affected are the tongue, accounting for approximately 25%–40%, and the floor of the mouth, accounting for approximately 15%–20% (Johnson et al., 2019a). Common risk factors for HNSCC include consumption of tobacco and/or areca nut, excessive alcohol consumption and viral infections, especially with so-called high-risk genotypes of Human Papilloma Virus or Epstein–Barr Virus (Johnson et al., 2019a, 2020; Pai & Westra, 2009). Both the risk and incidence of HNSCC increases with

age and are higher in males (Johnson et al., 2019a, 2019b, 2020). There is a strong socio-economic gradient, which is to some extent site specific: oral cancer is more common in lower socioeconomic status (SES) communities worldwide; HPV-related cancers, nowadays regarded as a sexually transmitted disease, are more common in males and in higher SES groups (Gupta & Johnson, 2014a). In many South and Southeast Asian communities, the chewing of areca nut in numerous forms, often in the form of 'betel quid', which may or may not contain smokeless also known as chewing tobacco, are culturally specific risk factors (Gupta & Johnson, 2014a, 2014b). Aside from these, poor oral hygiene, diets poor in antioxidants, exposure to environmental pollution and UV radiation (Johnson et al., 2019a, 2020; Pai & Westra, 2009) as well as a genetic diseases such as Fanconi anaemia and dyskeratosis congenita, and complex syndromes like Plummer-Vinson syndrome are closely correlated with the development of HNSCC (Johnson et al., 2019a).

HNSCCs are most commonly diagnosed at late stages (stage III or IV) due to poorly accessible anatomical sites and a frequent lack of clear symptoms and signs. Visually accessible sites such as the floor of the mouth, or clearly ulcerated lesions, carry a lower association with advanced stage at diagnosis due to their prominence and obvious symptoms (Kerdpon & Sriplung, 2001): However less obvious lesions such as nasopharyngeal, oropharyngeal and hypopharyngeal tumours are often more insidious as their anatomical location makes them more difficult to examine visually (Kerdpon & Sriplung, 2001). HNSCC are staged using the tumour-node-metastasis system (Johnson et al., 2020). In 2017, the 8th edition of the Cancer Staging Manual released by the American Joint Commission on Cancer was updated so that staging now takes into account the depth of tumour invasion, extracapsular nodal extension and HPV association (Chow, 2020; Johnson et al., 2020). Staging of tumours is inversely associated with prognosis, advanced stage III and IV disease carrying a 5-year survival of less than 50%, whereas that of early stage I disease is approximately 80% (Chow, 2020). Overall survival (OS) rates also vary depending on the tumour subsite, with a p16-positive oropharyngeal carcinoma having the highest 10-year OS (87%), followed by the oral cavity (69%) and larynx (67%), p16-negative oropharyngeal tumours (56%) and hypopharyngeal tumours (51%; Du et al., 2019).

While radiological approaches such as Computerised Tomography, Magnetic Resonance Imaging and Positron Emission Tomography are useful for staging and monitoring HNSCC (Guenette, 2021; Leroy et al., 2019), diagnosis is achieved through clinical and histopathological assessment (Chow, 2020; Johnson et al., 2020). Biopsy procedures are varied and can include cup forceps, incisional or punch biopsy, excisional biopsy, brush biopsy or fine needle aspiration, largely depending on the location of the tumour (Idrees et al., 2022; Johnson et al., 2020). Delays in diagnosis and treatment worsen the prognosis (Schutte et al., 2020). The majority of biopsy methods are invasive, involving traumatic removal of tissue (Schache et al., 2021). Furthermore, incisional biopsies have been shown to shed cancer cells into the circulation (Kusukawa et al., 2000) with increased risk of metastatic lesions (van Schaijik et al., 2019). While incisional biopsies are still the gold standard in diagnosing HNSCC, it is preferable to have a minimally invasive adjunctive identifying tool in patients with a high risk of developing metastatic disease. Combined with the link between late diagnosis and poor prognosis, there is a need to develop more effective, non-invasive methods for detecting HNSCC at an early stage. This includes the analysis of saliva for biomarkers of Oral Squamous Cell Carcinomata (OSCC; Cristaldi et al., 2019) and HNSCC (Kusampudi & Konduru, 2021), such as circulating tumour DNA, extracellular vesicles, microRNAs and circulating tumour cells (Cristaldi et al., 2019; Kusampudi & Konduru, 2021).

Recently, there has been growing interest in the application of breath biopsies to diagnose HNSCC. Conventional breath biopsies involve taking a sample of exhaled breath and analysing this with gas chromatography–mass spectrometry (GC–MS) or heat desorption to determine volatile compounds present (Abderrahman, 2019). It is a non-invasive and relatively simple means of analysis, and thus of potential diagnosis for many disorders: it can provide valuable information on metabolic changes in the body due to disease; it represents a promising method of diagnosis for numerous diseases, including inflammatory diseases (Abderrahman, 2019), infectious diseases (Belizário et al., 2021) and cancer (van der Schee et al., 2018). Breath biopsies detect volatile organic compounds (VOCs), found in exhaled air and can act as biomarkers for disease (Mertz, 2020). VOCs are produced in numerous ways, including from microbial dysbiosis of oral or respiratory tract or gut microflora, or through tissue metabolism, which may be distinctive from malignant tissues (Belizário et al., 2021; van der Schee et al., 2018). Over 1800 VOCs have already been discovered leading to establishment of the *volatolome*, with over 800 of these detectable in expired air (de Lacy et al., 2014): this suggests that it is likely that many more disease-specific VOCs remain to be documented. Through the use of electronic noses (e-Nose), VOCs can be detected in the breath in a quick and non-invasive manner (van der Schee et al., 2018). The e-Nose has shown promising potential for use as an auxiliary test for the screening (de Leon-Martínez et al., 2020) and diagnosis of breast (Phillips et al., 2010; Yang et al., 2021) and lung cancers (Amann et al., 2011; D'Amico et al., 2010). In multiple breast cancer studies, results showed that e-noses are able to discriminate healthy individuals from cancer patients with high accuracy (de Leon-Martínez et al., 2020; Phillips et al., 2010). Tirzīte et al. focused on the use of e-Nose for detecting lung cancer and found that it was not only able to distinguish patients with lung cancer from healthy controls, but could even differentiate lung cancer patients from those with other respiratory diseases such as Chronic Obstructive Pulmonary Disease (Tirzīte et al., 2017). e-Nose technology has recently been paired with artificial intelligence, allowing such devices to be trained on an extant dataset so that patterns in the breath composition of new suspected cancer patients can be quickly identified (Tirzīte et al., 2017; Van de Goor et al., 2018; Waltman et al., 2020; Yang et al., 2021). There are numerous on-going trials evaluating different types of e-Noses for identifying patients with different types of cancer (Abderrahman, 2019; D'Amico et al., 2010).

This systematic review examines current applications of breath biopsy for identifying HNSCC, including data on efficacy and utility, and speculates on the future uses of this non-invasive detection method.

2 | METHODS

This systematic review was performed and reported according to the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA; Moher et al., 2009; Page et al., 2021). The article selection flow chart is presented in Figure 1.

2.1 | PICO statement

For this article, the population of interest was patients with squamous cell carcinoma of the head and neck, the intervention was diagnosis or detection by breath biopsy, the control group was breath biopsies in normal, healthy subjects, and the outcome was to determine the efficacy (by means of accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value), and application of breath biopsies for diagnosis of HNSCC.

2.2 | Data sources and search strategy

Electronic databases were searched by two authors (R.K. and B.V.S.). Databases were Medline, PubMed, Web of Science, Cochrane and Scopus, as well as the grey literature (the first 10 pages of Google Scholar, ProQuest Central and WHO-IRIS using the search term 'Head and neck cancer breath biopsy'). The search strategies were developed using the specific Medical Subject Headings (MeSH) terms in Table 1. References were checked from bibliographies in relevant articles and included in the systematic review if not identified initially. The search strategy included all studies published up to January 2022.

2.3 | Study selection and data extraction

Initial studies identified through database searching were screened according to title and abstract against the inclusion criteria. These

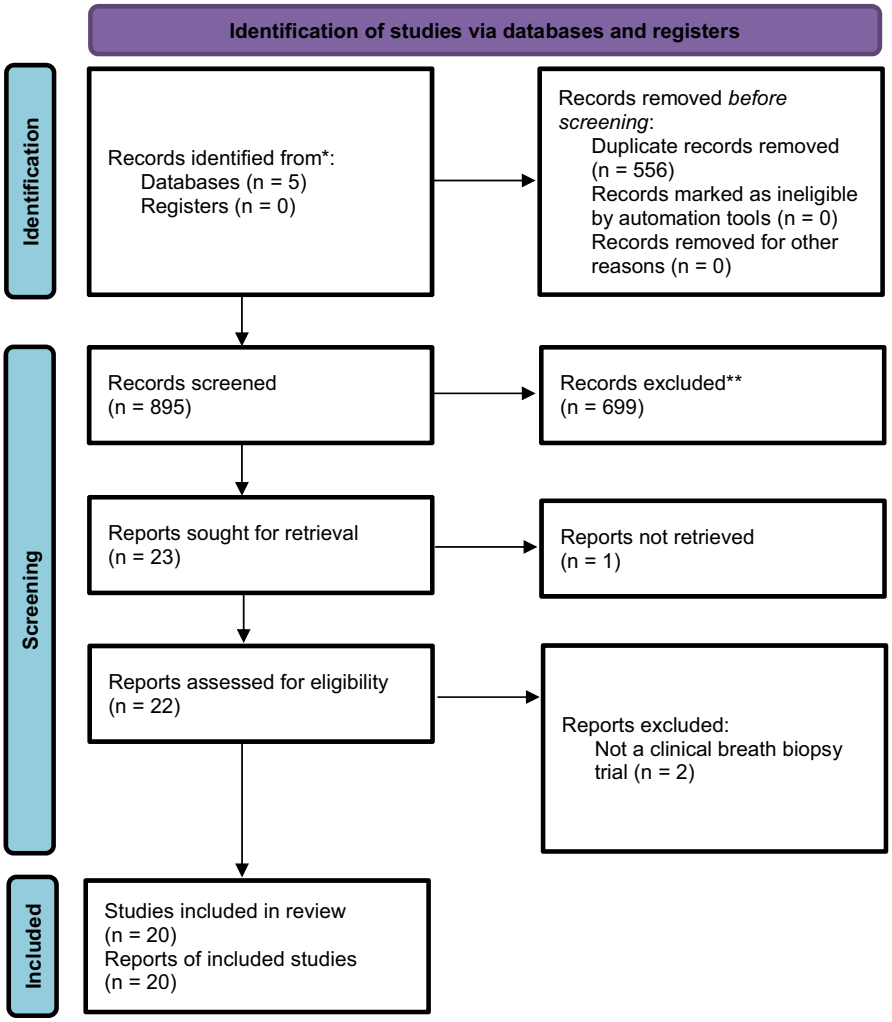

FIGURE 1 Article selection flow chart for the systematic review following the PRISMA guidelines (Page et al., 2021)

TABLE 1 Medical subject headings terms for the search strategy

No.	Search strategy
1	Head and neck or oral or gingiva or buccal or tongue or oropharyngeal or cheek or lip or lingual or floor of the mouth or tonsils or retromolar or palate or mandible or maxilla
2	Squamous cell carcinoma or SCC or HNSCC or OSCC or epithelial dysplasia or precancer or cancer or erythroplakia or lesion or malignancy or neoplasm
3	Breath biopsy or breath analysis or breath screen or breath test or volatile organic compound or VOC or gas chromatography or GCMS or GC–MS or E-nose or Enose or E nose or electronic nose

were restricted to English language, breath biopsy methods, and lesions or tumours in the head and neck region. Review articles, case reports, non-English articles, animal studies or studies using cell lines were excluded. All studies considered eligible were included for full-text evaluation. Disagreements between the two reviewers were resolved through discussion, and through consultation with the third reviewer (O.K.). Then, data were extracted from each included study using a standardized form which included author, publication year, study design and setting, population, sample size, patient age and gender, head and neck subsite, control group, intervention, key outcomes and study limitations.

2.4 | Quality assessment

Evaluation of the quality of studies included in this review was undertaken using the Newcastle-Ottawa Quality Assessment Scale (NOS; Wells et al., 2014). The categories assessed for each study were the selection of the cohort, the comparability of the study group and control group and the manner in which the outcome was achieved (Wells et al., 2014). A total score for each study was obtained by summing up the individual scores across the categories assessed (Wells et al., 2014).

Quality of evidence for each included article was assessed via the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) tool (Guyatt et al., 2011). Each article was assessed for factors that reduced quality of evidence, such as risk of bias and imprecision, and factors that increased the quality of evidence, such as magnitude of effect (Guyatt et al., 2011). The grading for each factor and the overall grading are defined in terms of 'High', 'Moderate', 'Low' and 'Very Low' (Guyatt et al., 2011). If only one factor reduced the quality of evidence, we gave the article an overall score of 'Moderate'. If two to three factors reduced the quality of evidence, an overall score of 'Low' was given. Four to five factors that reduced the quality of evidence would result in an overall score of 'Very Low' (Guyatt et al., 2011).

Assessment of the studies via the NOS and GRADE tool were carried out by two authors (R.K. and B.V.S.) and disagreements were resolved through discussion and consultation with the third reviewer (O.K.).

3 | RESULTS

The initial search generated a total of 1443 articles from all databases (Medline ($n = 30$), PubMed ($n = 261$), Web of Science ($n = 240$), Cochrane ($n = 85$) and Scopus ($n = 827$)). After removing 556 duplicates, 895 articles were deemed eligible for screening. These were assessed against the inclusion and exclusion criteria. Twenty-three articles were eligible for full text assessment: one was excluded due to inaccessibility and two did not meet the inclusion criteria. A final total of 20 articles were eligible for review.

The year of publication ranged from 2008 to 2021. A total of 660 HNSCC samples were investigated across all studies. The average age, taken from 15 articles with available data, was 60.83 years (range 21–89), and the male to female ratio was 3.27. The head and neck subsites studied include the tongue, alveolar process, floor of mouth, gingiva, palate, buccal and labial mucosa, oropharynx, larynx, hypopharynx, nasopharynx, nasal cavity and non-specified oral cavity. Thirteen articles were retrospective cohort studies with four articles having a prospective cohort design and three articles having a cross-sectional design. A summary of patient demographics and extracted data are available in Table 2.

Seven studies used portable e-Nose devices (CyranoseTM (Fielding et al., 2020), DiagNoseTM (Leunis et al., 2014) or AeonoseTM (Mohamed et al., 2021; Van De Goor et al., 2017, 2019, 2020; van Hooren et al., 2016)) to collect and analyse the VOC composition of exhaled air (Fielding et al., 2020; Leunis et al., 2014; Mohamed et al., 2021; Van De Goor et al., 2017, 2019, 2020; van Hooren et al., 2016). The remaining 13 studies created their own methodologies for breath collection (Bouza et al., 2017; Chandran et al., 2019; Chernov et al., 2020; Dharmawardana, Goddard, Woods, Watson, Butler, et al., 2020a; Dharmawardana, Goddard, Woods, Watson, Ooi, & Yazbeck, 2020b; García et al., 2014; Gruber et al., 2014; Hakim et al., 2011; Hartwig et al., 2017; Lang et al., 2016; Mentel et al., 2021; Schmutzhard et al., 2008; Witt et al., 2012). Analysis of the collected breath in these studies was carried out either through Gas Chromatography–Mass Spectrometry (GC–MS; Bouza et al., 2017; García et al., 2014; Gruber et al., 2014; Hakim et al., 2011; Hartwig et al., 2017; Mentel et al., 2021), Selected Ion Flow-Tube-Mass Spectrometry (SIFT-MS; Chandran et al., 2019; Dharmawardana, Goddard, Woods, Watson, Butler, et al., 2020a; Dharmawardana, Goddard, Woods, Watson, Ooi, & Yazbeck, 2020b), Proton Transfer Reaction-Mass Spectrometry (PTR-MS; Schmutzhard et al., 2008) or via custom-built gas analysis systems (Chernov et al., 2020; Hakim et al., 2011; Lang et al., 2016; Witt et al., 2012).

Three studies identified a total of 31 unique VOCs as potential biomarkers for HNSCC (Bouza et al., 2017; García et al., 2014;

TABLE 2 Summary of patient demographics and extracted data

References	Population	Sample size	Age mean (range)	Gender M:F	Head and neck subsite	Control	Gas analysis method	Key outcomes
Bouza et al. (2017)	OSCC: Ex-smokers ($n = 25$) Active smokers ($n = 1$)	26	62.5 (36–83)	15:11	Tongue ($n = 9$) FoM ($n = 7$) Gingiva ($n = 2$) Palate ($n = 2$) Buccal mucosa ($n = 3$) Oropharynx ($n = 3$)	Healthy controls: Active smokers ($n = 14$) Ex-smokers ($n = 3$) Non-smokers ($n = 9$)	SPME/GC–MS	Benzaldehyde and 3,7-dimethylunde associated with tumour size. Benzaldehyde and butyl acetate associated with histological degree of differentiation. Benzaldehyde associated with recurrence
Chandran et al. (2019)	SCC of the larynx, hypopharynx, oropharynx and oral cavity prior to treatment	23	61.5	20:3	OC ($n = 4$) Oropharynx ($n = 8$) Larynx ($n = 8$) Hypopharynx ($n = 3$)	Healthy volunteers ($n = 21$)	SIFT-MS SIM scans for 91 specific VOCs	Median concentration of hydrogen cyanide was significantly higher in the HNSCC group ($p < 0.05$) Sensitivity 91% Specificity 76% PPV 80.4% NPV 88.6% AUC 0.801
Chernov et al. (2020)	HNSCCs of the oral cavity, oropharynx, larynx, tongue, or lung	9	Not stated	Not stated	Tongue ($n = 4$) OC ($n = 4$) Mucous membrane of alveolar process of mandible ($n = 1$)	No confirmed malignancy ($n = 23$)	Custom-built gas analysis system	Accuracy 81.8% Sensitivity 90.7% Specificity 61.4%
Dharmawardana, Goddard, Woods, Watson, Butler, et al. (2020a)	SCC of the oral cavity, oropharynx, or larynx.	74	56.5 (33–88)	59:15	OC Oropharynx Larynx	Healthy adults ($n = 61$) Room air samples	Quintron Breath-Tracker and SIFT-MS	CH4:H2 ratio was higher in HNSCC cohort ($p = 0.044$) and an increase was correlated with increased T-stage ($p = 0.0259$)
Dharmawardana, Goddard, Woods, Watson, Ooi, and Yazbeck (2020b)	SCC in the mucosa of the oral cavity, oropharynx or larynx	50	58 (33–88)	43:7	OC ($n = 16$) Oropharynx ($n = 23$) Larynx ($n = 15$)	Healthy controls ($n = 50$) Room air samples	SIFT-MS	Accuracy 83% Sensitivity 80% Specificity 86% AUC 0.821
Fielding et al. (2020)	In-situ or invasive SCC of the bronchial and laryngeal and supraglottic regions	22	62.27	19:3	Larynx ($n = 22$)	Healthy controls ($n = 13$)	E-nose Cyranose 320	Sensitivity 100% Specificity 85%

(Continues)

TABLE 2 (Continued)

References	Population	Sample size	Age mean (range)	Gender M:F	Head and neck subsite	Control	Gas analysis method	Key outcomes
García et al. (2014)	Laryngeal carcinoma	11	61 (50–82)	10:1	Larynx ($n = 11$)	Healthy smokers ($n = 5$) Healthy non-smokers ($n = 5$)	SPME/GCMS	Potential marker compounds of laryngeal carcinoma: Ethanol, 2-butanone, 2,3-butanediol, 9-tetradecen-1-ol, octene derivative, cycloheptane derivative, cyclononane derivative. Ethanol and 2 butanone are most significant in diagnosing T3 laryngeal cancer
Gruber et al. (2014)	Benign or malignant head and neck lesions	22	62	19:3	Larynx ($n = 12$) Pharynx ($n = 10$)	Healthy controls ($n = 19$)	GC–MS and breath print analysis	*HNSCC versus healthy:* Accuracy 83% Sensitivity 77% Specificity 90% *HNSCC versus benign:* Accuracy 84% Sensitivity 77% Specificity 90% Ethanol, 2-propanenitrile and undecane distinguished between HNSCC and health, HNSCC and benign, and benign and health
Hakim et al. (2011)	Head and neck cancer	22	60	19:3	Subsites not stated	Healthy subjects ($n = 40$) Lung cancer ($n = 25$)	GC–MS and/or Nanoscale Artificial Nose (NA-NOSE)	*HNC versus Healthy:* Accuracy 95% Sensitivity 100% Specificity 92% *HNC versus LC:* Accuracy 100% Sensitivity 100% Specificity 100% NA-NOSE superior to GC–MS in separating head and neck cancer, healthy and lung cancer states

TABLE 2 (Continued)

References	Population	Sample size	Age mean (range)	Gender M:F	Head and neck subsite	Control	Gas analysis method	Key outcomes
Hartwig et al. (2017)	Primary OSCC	10	59.5 (48–76)	7:3	Tongue ($n = 2$) FoM ($n = 6$) Planum buccale ($n = 1$) Oropharynx ($n = 1$)	Healthy subjects ($n = 4$) Room air filled Mylar bags	Capillary GC–MS	Three compounds decreased following surgery: Dimethyl disulfide (DDS), Decamethylcyclopentasiloxane (D5) and p-xylene (PX) Sensitivity 90% Specificity 80%
Lang et al. (2016)	HNSCC	3	Not stated	Not stated	Not stated	Healthy donors ($n = 4$)	Custom membrane-type surface stress sensor e-nose	Membrane-type surface stress sensor distinguished HNSCC pre-surgery from post-surgery and healthy controls
Leunis et al. (2014)	HNSCC	36	59 (41–78)	25:11	Oropharynx ($n = 18$) Hypopharynx ($n = 8$) Larynx ($n = 10$)	Benign controls, age and smoking matched ($n = 23$)	E-nose (DiagNose)	Sensitivity 90% Specificity 80% AUC 0.85
Mentel et al. (2021)	OSCC	35	67.2 (49–89)	24:11	Gum ($n = 9$) Tongue ($n = 9$) FOM ($n = 9$) Palate ($n = 4$) Overlapping lip ($n = 1$) Buccal mucosa ($n = 3$)	Healthy controls ($n = 50$) Room air samples	BreathSpec (GC–MS)	Accuracy 90% (positive drift) Accuracy 86% (negative drift)
Mohamed et al. (2021)	Histologically confirmed OSCC patients with no other previous or current diagnosis or any treatment for current tumour other than OSCC	49	55.6 M 52.2 F Range (21–82)	24:25	Lower buccal ($n = 13$) Lower labial ($n = 12$) Tongue ($n = 7$) Palate ($n = 4$) Other ($n = 6$) Missing data ($n = 7$)	Non-cancer control patients ($n = 35$)	E-nose (Aeonose) with a disposable mouthpiece and a HEPA filter	Accuracy 81% Sensitivity 88% Specificity 71% NPV 81% PPV 81% AUC 0.86

(Continues)

TABLE 2 (Continued)

References	Population	Sample size	Age mean (range)	Gender M:F	Head and neck subsite	Control	Gas analysis method	Key outcomes
Schmutzhard et al. (2008)	Upper aerodigestive tract SCC	22	Not stated	Not stated	Not stated	*Low risk group:* healthy controls *High risk group:* chronic laryngitis	PTR-MS	42 VOCs were significantly different between SCC and controls. Isoprene was increased in carcinoma group compared to the control ($p = 0.00$).
van de Goor et al. (2020)	HNSCC without tracheostomy, treatment for current tumour, or history of other cancers	37	Not stated	Not stated	Oral Cavity ($n = 37$)	Healthy controls ($n = 72$)	E-nose (Aeonose) with HEPA filter	Accuracy 72% Sensitivity 84% Specificity 67% Area under curve 0.85
van de Goor et al. (2019)	Locoregional recurrent and/or second or third primary HNSCC	20	69	17:3	OC ($n = 7$) Oropharynx ($n = 4$) Hypopharynx ($n = 1$) Larynx ($n = 7$) Regional Lymph Node ($n = 1$)	Curative HNSCC with no recurrent disease ($n = 20$)	E-nose (Aeonose)	Sensitivity 85% Specificity 80% Accuracy 83% AUC 0.85 PPV 81% NPV 84%
Van De Goor et al. (2017)	Primary HNSCC	100	64	74:26	OC ($n = 28$) Oropharynx ($n = 23$) Nasopharynx/nasal cavity ($n = 4$) Hypopharynx ($n = 11$) Larynx ($n = 34$)	Bladder cancer ($n = 40$) Colon cancer ($n = 28$)	E-nose (Aeonose)	*HNSCC versus colon cancer:* Accuracy 84% Sensitivity 80% Specificity 86% AUC 0.83 *HNSCC versus bladder cancer:* Accuracy 84% Sensitivity 80% Specificity 86% AUC 0.85
van Hooren et al. (2016)	HNSCC	52	63	43:9	OC ($n = 15$) Oropharynx ($n = 13$) Nasopharynx/nasal cavity ($n = 2$) Hypopharynx ($n = 3$) Larynx ($n = 19$)	Lung carcinoma ($n = 32$)	E-nose (Aeonose)	Accuracy 93% Sensitivity 96% Specificity 88% AUC 0.98
Witt et al. (2012)	Primary HNC	10	Not stated	10:0	Not stated	Matched controls ($n = 13$)	Metal oxide gas sensor	Accuracy 82.3%

Abbreviations: AUC, area under curve; D5, decamethylcyclopentasiloxane; DDS, dimethyl disulfide; FoM, floor of mouth; GS-MS, gas chromatography – mass spectroscopy; HNSCC, head and neck squamous cell carcinoma; NPV, negative predictive value; OC, oral cavity; OSCC, oral squamous cell carcinoma; PPV, positive predictive value; PX, p-xylene; SIFT, selected ion flow-tube; SIM, selective ion mode; SPME, solid phase microextraction; VOC, volatile organic compound.

Hartwig et al., 2017). Bouza et al. (2017) identified sixteen VOCs as possible biomarkers for HNSCC (Bouza et al., 2017). Eight of these VOC biomarkers were uniquely identified in exhaled breath, eight were identified in air extracted from the oral cavity without exhalation and one was of unspecified origin (Bouza et al., 2017). Of these VOCs, benzaldehyde was shown to have significant correlations to tumour size, histological degree of differentiation and tumour recurrence; 3,7-dimethylundecane was significantly correlated with tumour size, and butyl acetate was significantly correlated to histological degree of differentiation (Bouza et al., 2017). In the study by Hartwig et al. (2017), the levels of eight VOCs were noted to differ significantly between healthy patients and HNSCC patients (Hartwig et al., 2017). In the same study, an additional three VOCs which were present in HNSCC patients were found to be completely absent in some of the same patients after curative surgery (Hartwig et al., 2017). García et al. (2014) also found that ethanol and 2-butanone were the most significant biomarkers for identifying laryngeal SCC (García et al., 2014). A summary of all potential VOC biomarkers reported is given in Table 3.

Results of the efficacy of breath biopsy show that there is high accuracy, sensitivity and specificity in diagnosing OSCC. Data from 15 studies (Chandran et al., 2019; Chernov et al., 2020; Dharmawardana, Goddard, Woods, Watson, Ooi, & Yazbeck, 2020b; Fielding et al., 2020; Gruber et al., 2014; Hakim et al., 2011; Hartwig et al., 2017; Leunis et al., 2014; Mentel et al., 2021; Mohamed et al., 2021; Van De Goor et al., 2017, 2018, 2019, 2020; van Hooren et al., 2016; Witt et al., 2012) was used to calculate a weighted average of accuracy, sensitivity, specificity, Negative Predictive Value (NPV), PPV and Area Under Curve (AUC) with respect to sample size. Across the 13 articles which reported on sensitivity and specificity, an average specificity of 85.7% and sensitivity of 82.7% was shown (Chandran et al., 2019; Chernov et al., 2020; Dharmawardana, Goddard, Woods, Watson, Ooi, & Yazbeck, 2020b; Fielding et al., 2020; Gruber et al., 2014; Hakim et al., 2011; Hartwig et al., 2017; Leunis et al., 2014; Mentel et al., 2021; Mohamed et al., 2021; Van De Goor et al., 2017, 2019, 2020; van Hooren et al., 2016; Witt et al., 2012). From three articles, an average NPV of 83.6% and PPV of 80.9% was shown (Chandran et al., 2019; Mohamed et al., 2021; van de Goor et al., 2019). Similarly, average accuracy across 11 studies was 84.9% (Chernov et al., 2020; Dharmawardana, Goddard, Woods, Watson, Ooi, & Yazbeck, 2020b; Gruber et al., 2014; Hakim et al., 2011; Mentel et al., 2021; Mohamed et al., 2021; Van De Goor et al., 2017, 2019, 2020; van Hooren et al., 2016; Witt et al., 2012) and the average AUC from eight articles was 0.85 (Chandran et al., 2019; Dharmawardana, Goddard, Woods, Watson, Ooi, & Yazbeck, 2020b; Leunis et al., 2014; Mohamed et al., 2021; Van De Goor et al., 2017, 2019, 2020; van Hooren et al., 2016).

Quality assessment of included studies was performed using NOS and GRADE. Overall, NOS showed that the quality of studies was poor, with the majority (12) of studies scoring 6/9. Five studies scored higher, with 7/9, and three studies scored lower, with two at 4/9 and one at 2/9, as shown in Table 4a. GRADE analysis showed similar poor quality of evidence, with the majority (14) of studies scoring low, two scoring moderately and four scoring very low, as shown in Table 4b.

4 | DISCUSSION

The use of breath biopsy in diagnosing HNSCC has only begun recently and there is limited evidence on the topic. Most relevant studies were conducted and published within the last 5 years.

Available data show that breath biopsies can diagnose HNSCC with high accuracy, sensitivity and specificity (Chandran et al., 2019, Chernov et al., 2020, Dharmawardana, Goddard, Woods, Watson, Ooi, & Yazbeck, 2020b, Fielding et al., 2020, Gruber et al., 2014, Hakim et al., 2011, Hartwig et al., 2017, Leunis et al., 2014, Mentel et al., 2021, Mohamed et al., 2021, Van De Goor et al., 2017, 2018, 2019, 2020, van Hooren et al., 2016, Witt et al., 2012). In comparison, studies on the use of breath biopsies in diagnosing breast and lung cancers have shown similar or higher levels of accuracy, sensitivity and specificity (de Leon-Martínez et al., 2020; Van de Goor et al., 2018; Yang et al., 2021). According to two studies on diagnosing breast cancer (de Leon-Martínez et al., 2020; Yang et al., 2021), breath biopsies displayed close to 100% accuracy and specificity (de Leon-Martínez et al., 2020; Yang et al., 2021). The sensitivity of the breath biopsies for diagnosing breast cancer reported in these studies was also high, with de leon Martinez et al. reporting a sensitivity of 100% (de Leon-Martínez et al., 2020) and Yang et al. reporting a sensitivity of 86% (Yang et al., 2021). In the scope of lung cancer, Van de Goor et al. (2018) reported an accuracy of 83% (Van de Goor et al., 2018), which is similar to the average we calculated from the HNSCC studies included in our review (Chernov et al., 2020, Dharmawardana, Goddard, Woods, Watson, Ooi, & Yazbeck, 2020b, Gruber et al., 2014, Hakim et al., 2011, Mentel et al., 2021, Mohamed et al., 2021, Van De Goor et al., 2017, 2019, 2020, van Hooren et al., 2016, Witt et al., 2012). Sensitivity and specificity for diagnosing lung cancer were also similar, at 83% and 84%, respectively (Bouza et al., 2017; Chandran et al., 2019; Chernov et al., 2020; Dharmawardana, Goddard, Woods, Watson, Ooi, & Yazbeck, 2020b; Fielding et al., 2020; Gruber et al., 2014; Hakim et al., 2011; Hartwig et al., 2017; Leunis et al., 2014; Mentel et al., 2021; Mohamed et al., 2021; Van De Goor et al., 2017, 2018, 2019, 2020; van Hooren et al., 2016; Witt et al., 2012; Yang et al., 2021). Some studies reported relatively low sensitivities of 77%, suggesting breath biopsy methods using breath print analysis for detecting HNSCC may require refinement before implementation in practice (Gruber et al., 2014).

In the studies which focused on the VOC composition of the breath of HNSCC patients, the only common compound reported is toluene (Bouza et al., 2017; Hartwig et al., 2017). There was no overlap in the VOCs identified by García et al. (2014) and any other study. Explanations for this limited concordance may lie in the populations selected: Hartwig et al. (2017) included only current

TABLE 3 Summary of VOCs identified as potential biomarkers for HNSCC

References	Potential VOC biomarkers for OSCC	Relation to pathological variables	VOC level after curative surgery compared to before curative surgery
Bouza et al. (2017)	Undecane	None identified	NA
	Dodecane	None identified	NA
	Decanal	None identified	NA
	3,7-dimethyl undecane	Tumour size	NA
	4,5-dimethyl nonane	None identified	NA
	1-octene	None identified	NA
	Hexadecane	None identified	NA
	Benzaldehyde	Tumour size, Histological degree of differentiation, Tumour recurrence	NA
	Styrene	None identified	NA
	Nonanal	None identified	NA
	Decanal	None identified	NA
	Benzyl alcohol	None identified	NA
	Dodecanal	None identified	NA
	2-ethyl-1-hexanol	None identified	NA
	Toluene	None identified	NA
	Butyl acetate	Histological degree of differentiation	NA
Hartwig et al. (2017)	Dibutylhydroxytoluene	None identified	Decreased ($n = 3$), Increased ($n = 6$), Increased greater than 8.4-fold ($n = 1$)
	Dimethyl disulfide	None identified	Completely absent ($n = 5$), Decreased ($n = 4$), Increased ($n = 1$)
	Decamethylcyclopentasiloxane	None identified	Completely absent ($n = 2$), Decreased ($n = 4$), No change ($n = 2$), Increased ($n = 2$)
	Methyl ethyl ketone	None identified	Decreased ($n = 3$), Increased ($n = 3$), Increased greater than 8.4-fold ($n = 4$)
	N-heptane	None identified	Decreased ($n = 3$), No change ($n = 1$), Increased ($n = 1$), Increased greater than 8.4-fold ($n = 5$)
	P-xylene	None identified	Decreased to zero ($n = 3$), Decreased ($n = 3$), No change ($n = 3$), Increased ($n = 1$)
	1-Heptene	None identified	Decreased ($n = 3$), No change ($n = 2$), Increased ($n = 1$), Increased greater than 8.4-fold ($n = 4$)
	Toluene	None identified	Decreased ($n = 2$), No change ($n = 1$), Increased ($n = 3$), Increased greater than 8.4-fold ($n = 4$)
García et al. (2014)	Ethanol	Most significant in diagnosing T3 laryngeal cancer	NA
	3-butanone	Most significant in diagnosing T3 laryngeal cancer	NA
	2,3-butandiol	None identified	NA
	9-tetradecen-1-ol	None identified	NA
	Octene derivative	None identified	NA
	Cycloheptane derivative	None identified	NA
	Cyclononane derivative	None identified	NA

smokers with a smoking history of more than 10 years and who consumed more than 10 cigarettes a day (Hartwig et al., 2017), whereas Bouza et al. (2017) included non-smokers and ex-smokers (Bouza et al., 2017). The analytical methods employed by these two studies also differed. García et al. (2014) did not specify the smoking history of their study group and their method of gas analysis was the same

TABLE 4 Quality assessment of included articles using Newcastle-Ottawa Quality Assessment Scale (Wells et al., 2014) (A) and GRADE (Guyatt et al., 2011) (B)

(A)

	Selection				Comparability	Outcome			
References	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Demonstration that outcome was not present at start of study	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Was follow-up long enough for outcomes to occur	Adequacy of follow up of cohorts	Total score
Bouza et al. (2017)	*	*	*	0	*	*	0	*	6
Chandran et al. (2019)	*	*	*	0	*	*	0	*	6
Chernov et al. (2020)	*	*	*	0	*	*	0	*	6
Dharmawardana, Goddard, Woods, Watson, Butler, et al. (2020a)	*	*	*	0	*	*	0	*	6
Dharmawardana, Goddard, Woods, Watson, Ooi, and Yazbeck (2020b)	*	*	*	0	*	*	0	*	6
Fielding et al. (2020)	*	*	*	0	*	*	0	*	6
García et al. (2014)	0	*	0	0	*	*	0	*	4
Gruber et al. (2014)	*	*	*	0	*	*	0	*	6
Hakim et al. (2011)	*	*	*	0	**	*	0	*	7
Hartwig et al. (2017)	*	*	*	0	*	*	*	*	7
Lang et al. (2016)	*	*	*	*	*	*	*	0	7
Leunis et al. (2014)	*	*	*	0	*	*	0	*	6
Mentel et al. (2021)	0	*	0	0	*	*	*	0	4
Mohamed et al. (2021)	*	*	*	0	*	*	0	*	6
Schmutzhard et al. (2008)	*	*	*	0	*	*	0	*	6
Van De Goor et al. (2017)	*	*	*	0	*	*	*	*	7
van de Goor et al. (2019)	*	*	*	0	**	*	0	*	7
van de Goor et al. (2020)	*	*	*	0	*	*	0	*	6
van Hooren et al. (2016)	*	*	*	0	*	*	0	*	6
Witt et al. (2012)	0	0	0	0	*	*	0	0	2

(Continues)

TABLE 4 (Continued)

(B)

References	Factors that reduce quality of evidence					Overall grading
	Risk of bias	Imprecision	Inconsistency	Indirectness	Publication bias	
Bouza et al. (2017)	Low	High	Outcome measured is in different terms from other papers	Low	Bias detected	Low
Chandran et al. (2019)	Moderate	High	Low	Low	Undetected	Low
Chernov et al. (2020)	Moderate	High	Moderate	Low	Undetected	Low
Dharmawardana, Goddard, Woods, Watson, Butler, et al. (2020a)	Low	High	Outcome measured is in different terms from other papers	Low	Bias detected	Low
Dharmawardana, Goddard, Woods, Watson, Ooi, and Yazbeck (2020b)	High	High	Low	Low	Undetected	Low
Fielding et al. (2020)	Low	High	Moderate	Low	Bias detected	Low
García et al. (2014)	Moderate	High	Outcome measured is in different terms from other papers	Low	Undetected	Low
Gruber et al. (2014)	Moderate	High	Low	High	Undetected	Low
Hakim et al. (2011)	low	High	Moderate	Low	Undetected	Low
Hartwig et al. (2017)	High	High	Low	Low	Undetected	Low
Lang et al. (2016)	High	High	Outcome measured is in different terms from other papers	High	Bias detected	Very low
Leunis et al. (2014)	Low	High	Low	Moderate	Undetected	Low
Mentel et al. (2021)	Moderate	High	Low	Moderate	Undetected	Low
Mohamed et al. (2021)	Low	High	Moderate	Low	Undetected	Low
Schmutzhard et al. (2008)	Moderate	High	Outcome measured is in different terms from other papers	Low	Bias detected	Very low
Van De Goor et al. (2017)	Low	High	Low	Low	Undetected	Moderate
van de Goor et al. (2019)	Low	High	Low	Low	Undetected	Moderate
van de Goor et al. (2020)	Low	High	Moderate	Moderate	Bias detected	Very low
van Hooren et al. (2016)	Low	High	Moderate	Low	Undetected	Low
Witt et al. (2012)	High	High	Low	High	Bias detected	Very low

as that carried out by Bouza et al. (2017). In this case, the difference in their results could be due to their study focusing solely on carcinomata in the larynx (García et al., 2014).

The applications for breath biopsy are limited by the feasibility of use in practice, however as our results show there is high sensitivity and specificity for identifying HNSCC. Therefore, future uses of breath biopsy can include screening patients with risk factors or premalignant conditions, regular surveillance of suspicious lesions and assistance in diagnosis. This technology should be best implemented by dental and health professionals in a public hospital setting in areas where there are high cases of HNSCC due to epidemiological risk factors, such as smoking, alcohol and areca nut chewing (Johnson et al., 2019a, 2020; Pai & Westra, 2009). By this means, the greatest impact of breath biopsy in identifying HNSCC can be achieved.

The quality of evidence presented in the studies included in this review is low and insufficient to make a strong recommendation for clinical application. The most common limitation is small sample size, indicating a need for larger cohort studies. Another limitation of the included articles is a lack of follow-up and baseline testing. We have shown that breath biopsy is a suitable method for detection of HNSCC, however future studies would be suited to a long-term follow-up with baseline readings to examine the production of VOCs before tumour formation, during the course of HNSCC and following surgery, in order to gain a comprehensive view of VOC production throughout the whole disease process. Furthermore, there is a gap in the literature pertaining to the feasibility of incorporating E-nose technology in practice, therefore future research should focus on a cost/benefit analysis as well as the capability of public hospitals to acquire and implement this technology. Despite this, our results clearly indicate that breath biopsy is a feasible method of detecting HNSCC, such that larger studies are indicated. As we expand our knowledge on the volatolome of HNSCC and refine the technologies for collection and analyses of breath samples, the clinical application of e-noses and other breath biopsy methods is likely to increase. This study is also limited by the lack of microbial analysis, as the presence of specific microbes has been associated with VOC presence in breath (Belizário et al., 2021; van der Schee et al., 2018). Current physical methods of detecting HNSCC are limited by accessibility to many tumours (Kerdpon & Sriplung, 2001). Tissue biopsy is invasive (Schache et al., 2021) and has the potential to disseminate disease (Kusukawa et al., 2000). If future research is able to provide stronger evidence for the utility of breath biopsies, we believe that this technology could be used in screening campaigns that may improve rates of diagnosis of HNSCC at earlier stages, improve prognosis (Koch et al., 2011) and reduce the global burden of this disease (Johnson et al., 2019a, 2020; Patterson et al., 2020). There is a great potential for this technique to be linked with other diagnostic tools to improve HNSCC prognostic outcomes (Idrees et al., 2021, 2022). Future research in this area should focus on larger, long-term studies, as well as investigation into the capabilities of breath biopsy methods in detecting pre-cancerous lesions, such as oral epithelial dysplasia.

5 | CONCLUSION

Our systematic review of the current literature shows that breath biopsy has the potential to be an effective method of detecting HNSCC, providing a non-invasive alternative to conventional detection methods with equivocal specificity, sensitivity and accuracy. However, more research is needed to determine the extent of its usefulness. Further research in this area should focus on the practicality of breath biopsies in all settings and accessibility of E-nose and GC–MS equipment to medical facilities, including those with limited resources.

AUTHOR CONTRIBUTIONS

Rachel Kok: Data curation; formal analysis; writing – original draft; writing – review and editing. **Bede van Schaijik:** Data curation; formal analysis; writing – original draft; writing – review and editing. **Newell W. Johnson:** Writing – original draft; writing – review and editing. **Mohammed Imad Malki:** Writing – original draft; writing – review and editing. **Agnieszka Frydrych:** Supervision; writing – original draft; writing – review and editing. **Omar Kujan:** Conceptualization; data curation; formal analysis; methodology; project administration; supervision; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

Data are available upon request.

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