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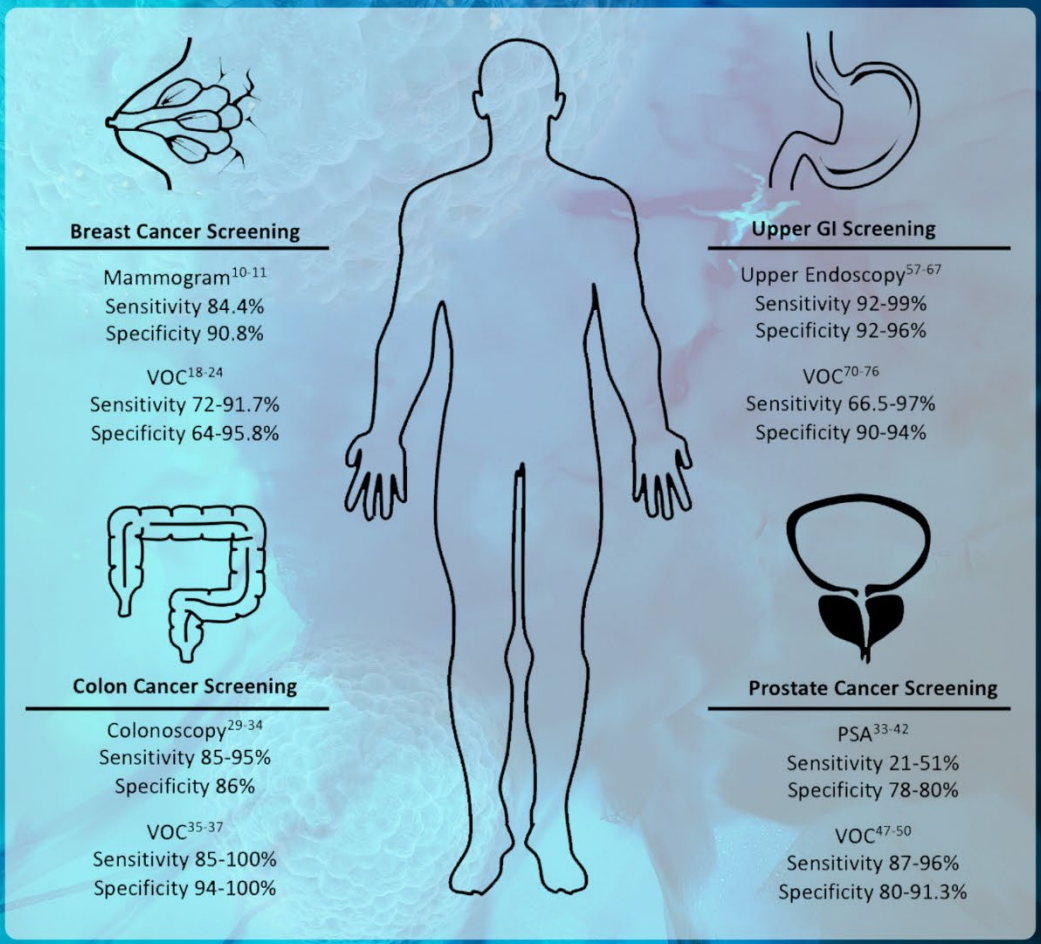

Volatile organic compounds: A promising new frontier for cancer screening

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REVIEW ARTICLE

Volatile organic compounds: A promising new frontier for cancer screening

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Abstract

The late onset of cancer symptoms can cause a significant delay in diagnosis, impacting patients' prognosis and quality of life, thus prompting a need for alternative screening and detection methods. Neoplastic processes cause distinct and immediate changes to the body's metabolism, creating unique patterns in the volatile organic compounds (VOCs) produced and released through exhaled breath. For this reason, VOC profiles have emerged as diagnostic indicators for several types of malignancies, facilitating early cancer detection. Both non-invasive and accessible, the analysis of breath VOCs for cancer screening and detection has gained recognition as a new frontier in cancer diagnostics. Using exhaled breath instead of gold-standard cancer detection and screening tools that are traditionally invasive and uncomfortable for the patient could be revolutionary in improving patient compliance. Further, compared to the gold-standard tools, breath testing is relatively inexpensive, and the method of analysis, storage, and transporting the samples is simplified. Several studies have demonstrated the accuracy of VOC analysis in detecting various types of cancer, including breast cancer, colon cancer, prostate cancer, gastric cancer, and melanoma. This article summarizes the evidence supporting VOC analysis for cancer screening and detection. It reviews the clinical utility, current limitations, and necessity for standardization across all VOC screening tools to ensure the standardization and reliability of measurements. The evidence supporting breath tests to detect cancer accurately is strong, demonstrating that VOC sampling improves patient outcomes and decreases the global burden of malignant conditions by detecting cancer earlier.

Keywords: Volatile organic compounds; Breath analysis; Cancer screening; Cancer diagnostics

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1. Introduction

There is an ever-expanding interest in developing tools for accurate screening and early cancer detection. Volatile organic compound (VOC) analysis has emerged as a promising

new technique with a wide range of clinical applications (Table 1).[1-7] VOCs are by-products of biochemical reactions and are defined as carbon-containing compounds detectable as a gas at room temperature.[1] Endogenous VOCs are generated within the human body as by-products of metabolic biochemical pathways.[1-6] Once produced, the VOCs diffuse into bodily fluids, tissues, and systemic circulation.[1-3] Consequently, they can be detected in the bloodstream and transported by the circulatory system. Some VOCs are released in exhaled breath, while others are secreted in urine and feces.[1,3-6] In contrast, exogenous VOCs are introduced into the body, including smoking, dietary intake, medications, and cytotoxic treatments.[2] Metabolic changes associated with the pathophysiology of several diseases and malignancies have been shown to trigger shifts in the VOCs produced by the human body.[1-5]

Recent efforts have focused on the identification of VOCs as disease biomarkers. The hallmarks of tumor biology and the neoplastic process include sustained proliferative signaling, uninhibited growth, angiogenesis, and reprogrammed energy metabolism, leading to invasion and metastasis.[6] Hypoxia, hyperproliferation, inflammation, and reactive oxygen species result in marked shifts in both the range and concentration of detectable VOCs.[1,5,6] These neoplastic processes cause measurable, distinct, and immediate changes to the human

Table 1. Emerging applications for VOC analysis

Application	Description
Environmental exposures[117-119]	• Health risk assessment and personal exposures to environmental VOCs • Exposure to cigarette smoke, tobacco, VOCs from e-cigarettes • Workplace exposures to hazardous VOCs, fumes, smoke, and inhaled particles • Environmental risk assessment of toxicity exposure
Oncology[9,120]	• Potential applications as a screening tool for several malignancies, including colon, lung, breast, ovarian, prostate, hepatobiliary, genitourinary, head and neck, cutaneous, and gastric cancers • Can be used to estimate the burden of disease • Monitor response to treatment • Surveillance testing for disease recurrence • Represents an innovative, accessible, inexpensive, and non-invasive diagnostic point-of-care tool
Benign disease[121-126]	• Non-invasive diagnosis of inflammatory bowel disease (Crohn's disease and ulcerative colitis) • Detection and clinical monitoring of benign respiratory conditions, including asthma, pulmonary hypertension, and chronic obstructive pulmonary disease (COPD). • Detection and monitoring severity of chronic kidney and liver disease • Diagnosis of Parkinson's disease and multiple sclerosis • Monitoring glycemic controls and sequela of diabetes mellitus
Perioperative medicine[126-131]	• Prediction and early detection of anastomotic leaks in esophageal, pancreatic, and colorectal surgery • Diagnosis of post-operative pneumonia • Predicting paralytic ileus • Intraoperative monitoring, analysis of anesthetic and sedation • Diagnosis and monitoring of sepsis • Response to nutritional interventions
Drug testing[134,135]	• Detection of marijuana metabolites in urine and in exhaled breath • Detection of impact and extent of use of tobacco products, monitor response to smoking cessation • Detection of alcohol consumption • Detection of illicit drug biomarkers in exhaled breath • Assessing compliance with medical treatments • Assessing absorption and metabolism of medical treatments as well as surveillance of adverse effects
Transplant[132,133,136-138]	• Analysis of VOCs for early detection and prediction of transplant rejection • Applications in lung and hepatobiliary transplant • Diagnosis of lung allograft dysfunction • Detection of exhaled ammonia for early diagnosis of hepatic encephalopathy and monitoring response to treatment • Diagnosis of graft-versus-host disease • Detection of post-transplant acute kidney injury and monitoring response to hemodialysis
Infections[21-23]	• Monitoring respiratory infections in at-risk populations, i.e., immunosuppression, post-transplant, cystic fibrosis, and pediatrics • Differentiation between viral and bacterial respiratory infections in cystic fibrosis and COPD • Diagnosis of human echinococcosis, an infectious disease caused by helminths • Diagnosis of tuberculosis and response to treatment • Diagnosis of pneumonia and response to treatment, i.e., *Pseudomonas* and *Aspergillosis*

Abbreviation: VOC: Volatile organic compound.

body's metabolism, creating unique patterns in the VOCs being produced and released. Unique VOC profiles have demonstrated diagnostic utility for several benign and malignant conditions, enabling prediction of disease burden and response to treatment.[1-6]

Conditions with similar pathophysiological processes often exhibit similar VOC patterns (i.e., ulcerative colitis, Crohn's disease, and irritable bowel syndrome are inflammatory gastrointestinal [GI] conditions that produce similar VOC spectrums).[1,3] As such, a single VOC cannot discriminate between such disease processes. Rather, patterns of several measured VOCs have been utilized to describe distinct profiles, which have been demonstrated in proof-of-concept clinical studies to be sensitive and specific for the diagnosis of several important diseases, including malignancies.[1-5] Thus, VOC profiles represent promising oncologic biomarkers.

Breath analysis of exhaled VOCs is emerging as a non-invasive method for early cancer diagnosis. Exhaled breath is non-invasively accessible, inexpensive to sample, associated with increased patient compliance, and yields samples that are easily analyzed, stored, and transported.[1-4] Serial testing is both safe and feasible. Exhaled breath VOC analysis has the potential to be widely implemented as a simple point-of-care tool providing concurrent screening for a wide range of cancers. In addition, this technology may facilitate treatment response monitoring and post-treatment cancer surveillance.

2. Breast cancer

With over 2.3 million cases and 685,000 deaths worldwide in 2020, breast cancer is the second most diagnosed malignancy.[7,8] It remains the leading cause of cancer-related death in women.[7-9] At present, mammography is the gold-standard modality for the early detection of breast cancer, detecting cancer 1.5 – 4 years before the disease becoming clinically detectable.[10]

The impact of early, effective screening has been well established. A seminal study by Tabár *et al.*[11] demonstrated that women aged 40 – 69 participating in breast cancer screening benefit significantly from earlier intervention with decreased morbidity and mortality, compared to women who did not participate in screening programs. Patients participating in organized mammography screening have a 60% lower risk of breast cancer-related mortality within 10 years of diagnosis. The importance of timely access and compliance with breast cancer screening is highlighted by the increased morbidity and mortality of breast cancer in developing countries, where a delayed diagnosis is associated with worse outcomes compared to high- and middle-income countries.[8]

When diagnosed early, breast cancer is often curable. Improved breast cancer screening and early detection are associated with improved prognosis and decreased health-care costs. Studies exploring barriers to breast cancer screening report a combination of social, geographic, and economic factors.[12-17] Social factors, well described in the literature, include health literacy, perceived physical and emotional discomfort associated with a breast examination and mammography and cultural and religious considerations.[13,15,17] Geographic and socioeconomic factors include disparities in access to screening services for breast cancer.[13-16] There is an ongoing global need to develop inexpensive screening tests that are safe, effective, and improve patient experience. Breath analysis represents a minimally invasive point-of-care tool allowing for early cancer detection that is inexpensive and accessible. This technology may increase compliance with screening and improve access to cancer care globally.

Analysis of over 1.8 million screening mammograms in the United States between 2004 and 2008 in women between 18 and 80 years of age reported a sensitivity of 84.4% and a specificity of 90.8%. The recall rate was 9.6%, with a positive predictive value of 4.3 (Figure 1).[10] In contrast, in a small case–control study, Phillips *et al.*[12] reported a 93.8% sensitivity and 84.6% specificity in predicting the presence of breast cancer with biopsy-proven breast cancer using a prediction model with five VOCs in exhaled breath (Figure 2). This small case–control study (n = 101, with 51 patients with breast cancer) supports the potential for accurate breast cancer diagnosis using a pattern of five exhaled VOCs (1-phenylethanone, 2.3-dihydro-1-phenyl-4(1H)-quinazolinone, 2-propanol, heptanal, and isopropyl myristate) identified through gas chromatography (GC)/mass spectroscopy (MS).[12]

Patterson *et al.*[18] analyzed 308 VOCs in 20 patients with breast cancer and 20 healthy controls, using aggregate low-dimensional summaries and compound quantity clustering to predict a diagnosis of breast cancer with a sensitivity of 72% and a specificity of 64%. Similarly, Phillips *et al.*[19] employed c-statistics to identify the predictive value of individual VOCs to identify potential breast cancer biomarkers in exhaled breath. Monte Carlo simulations were then used to select the chromatographic time slices that identified breast cancer with better-than-random accuracy. The VOCs with the highest predictive values were identified. A multivariate algorithm using a combination of >30 VOCs comparing 54 women with breast cancer and 204 cancer-free controls revealed a sensitivity of 78.5%, a specificity of 88.3%, and a c-statistic of 0.89.

Li *et al.*[20] validated a predictive model using four biomarkers for breast cancer in exhaled breath (hexanal,

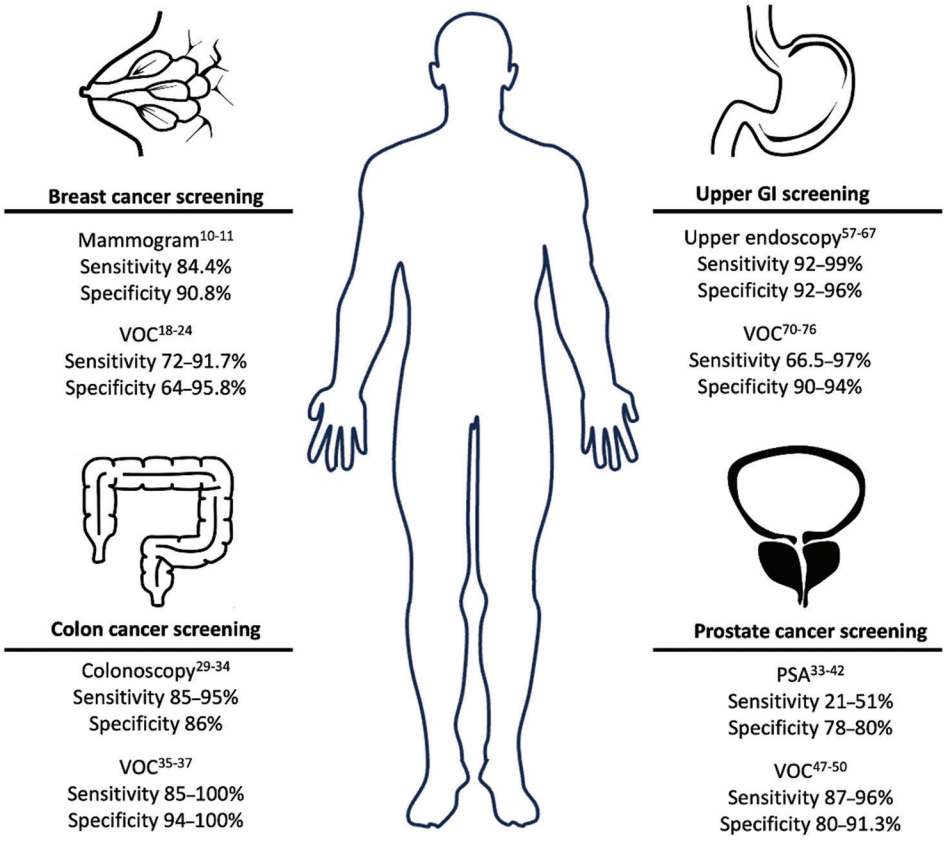

Figure 1. VOC for cancer screening and detection
Abbreviations: GI: Gastrointestinal; PSA: Prostate-specific antigen; VOC: Volatile organic compound.

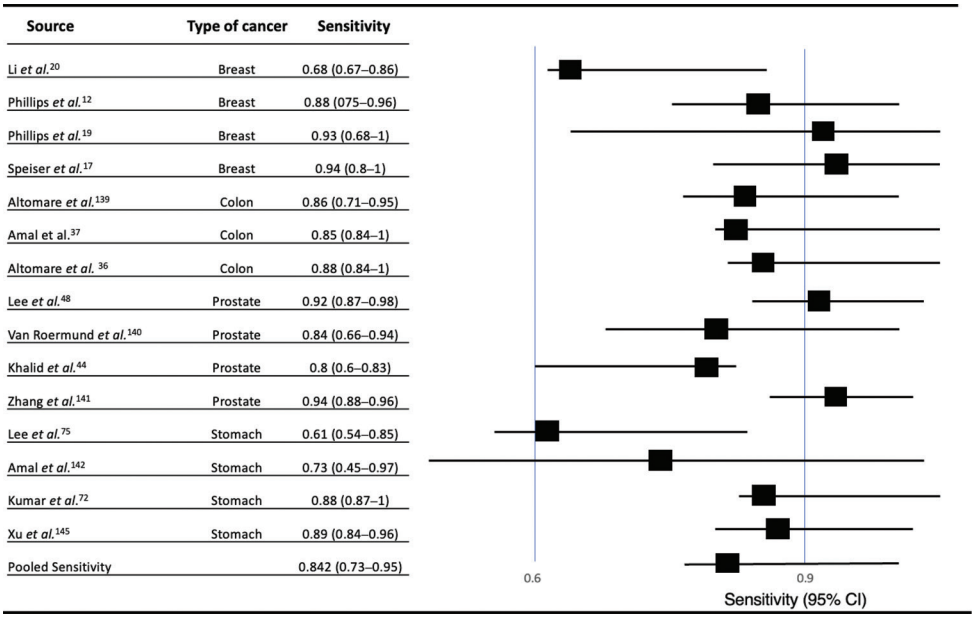

Figure 2. Forest plot of pooled sensitivity analysis

heptanal, octanal, and nonanal). A predictive model was developed using these four biomarkers to differentiate between 24 healthy controls, 17 patients with benign tumors, and 22 patients with breast cancer. Using this model, they reported an area under the curve (AUC) of 0.934, a sensitivity of 91.7%, and a specificity of 95.8%.

The biological significance of individual VOCs remains unclear. Some have been shown to be cancer biomarkers (i.e., heptanal),[21] while analogs of others have been shown to have anti-tumor properties (i.e., 2,3-dihydro-1-phenyl-4(1H)-quinazolinone).[22] Similarly, analogs of 1-phenyl-ethanone inhibit the invasion of human MCF-7/6 mammary carcinoma cells *in vitro*.[23]

Previous studies of patients with lung cancer suggest that the pattern of exhaled VOCs is associated with accelerated catabolism of otherwise benign metabolic by-products, which in turn has been correlated to the induction of cytochrome P450 enzymes.[24] The aromatase enzyme, which acts as an estrogen synthase, is a cytochrome P450 enzyme complex that acts as a catalyst for estrogen production. The induced cytochrome P450 activity associated with breast cancer may thus influence the pattern of VOCs in exhaled breath.[24] It is also likely that some VOCs found to be increased in breast cancer result from ongoing inflammatory oxidative stress, including lipid peroxidation of fatty acids.[24] Pentane, a lipid peroxidation reaction product, is well described as a VOC that is found in higher levels in patients with breast cancer.[18,24] Aldehydes, well described as increasing in the exhaled breath of patients with breast cancer, are also secondary reaction products of lipid peroxidation.

Strong evidence supports the use of exhaled VOCs to accurately detect breast cancer. While the individual molecules described may not be specific to breast cancer, it is the expression patterns that are diagnostic, leading to the description of validated unique "breathprints" that combine up to 30 exhaled VOCs. VOC analysis is intrinsically safe, painless, and inexpensive and may be superior in sensitivity and specificity when compared to screening mammograms. Confirmatory clinical studies in human populations are required to confirm these preliminary findings.

3. Colon cancer

Colon cancer is the third most common malignancy and remains a leading cause of cancer-related deaths worldwide.[25-28] There are over 1.9 million new diagnoses and 930,000 deaths annually from colon cancer.[26] The global burden of colon cancer is expected to increase by 60%, representing at least 2.2 million new cases and causing as many as 1.1 million deaths annually by 2030.[25-27]

It has been well established that early detection of colon cancer is associated with significantly better outcomes and lower health-care costs.[8,27-30] Colon cancer is typically diagnosed after the onset of symptoms through screening, colonoscopy, or stool testing. Unfortunately, over 86% of colon cancers in patients under 50 years old are symptomatic at diagnosis, associated with advanced disease and poor outcomes.[25-29] Indeed, despite strong recommendations for screening, global participation rates and compliance with screening remain as low as 1.9 – 54%.[30-34]

Colon cancer screening tools include stool-based testing to detect hemoglobin or DNA alterations suggestive of malignancy, direct visualization with endoscopy, and radiologic imaging. Stool-based screening includes immunochemical tests (fecal immunochemical test [FIT]), guaiac-based fecal occult blood tests (gFOBT), and multitarget fecal DNA testing.[34] Stool-based screening tests for colon cancer are more sensitive to the detection of cancer than pre-cancerous polyps.[30-33] FIT testing involves the evaluation of a single stool sample, with a specificity of 96.4% and a sensitivity of 73.8% for the detection of colon cancer.[29-31,33-34] It is only 23.8% sensitive for the detection of adenomas >10 mm and 7.6% for adenomas <10 mm.[29-32]

gFOBTs are 92.5% specific and 70% sensitive for the diagnosis of colon cancers, but the sensitivity is only 23.9% for adenomas >10 mm and <12.4% for smaller adenomas.[29-30] Multitarget stool DNA testing is more sensitive than other stool-based screening tests, with a sensitivity of 92.3% and a specificity of 89.8% for the detection of colon cancer.[29-34] It offers improved sensitivity for the detection of adenomas, which is reported at 42.4% for adenomas >10 mm and 17.2% for adenomas <10 mm.[29-34]

Any patient with an abnormal screening test currently requires a follow-up colonoscopy. A colonoscopy is both a diagnostic and therapeutic tool, allowing for the examination and treatment of the rectum, colon, and proximal terminal ileum. It is the definitive test for detecting pre-cancerous adenomas and CRC, with a specificity reported at 86%.[22,27,29-33] It is 75% sensitive for the detection of adenomas <5 mm, 85% for adenomas 6 – 9 mm, and 95% for adenomas >10 mm (Figure 1).[22,27,29-33] Disadvantages of colonoscopy as a screening test include patient discomfort, the inconvenience of bowel preparation, and the potential for procedural and sedation-related complications.[32] Procedural risks include the possibility of a perforated visceral organ, significant bleeding, and infection.[29-33]

Thus, there is an unmet need for a reliable, non-invasive screening test for colon cancer. The analysis of VOCs in exhaled breath has been applied to colon cancer, and while data remain sparse, studies have identified several potential metabolic biomarkers. Indeed, predictive models using combinations of 4 – 60 VOCs have been shown to be comparable to both colonoscopy and stool-based screening for the detection of colon cancer (Figure 2). However, while the individual molecules described may not be specific to colon cancer, the expression patterns are diagnostic, leading to the description of validated "breathprints," which combine clusters of exhaled VOCs.

Wang *et al.*,[35] using solid-phase microextraction-GC/MS, described increased levels of cyclohexanone, 2,2-dimethyldecane, dodecane, 4-ethyl-1-octyn-3-ol, ethylaniline, cyclooctylmethanol, trans-2-dodecen-1-ol, and 3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate ($P < 0.05$) in the exhaled breath of patients with colon cancer. The biological significance of these molecules remains unclear. The authors hypothesize that malignancy is associated with increased oxidative stress and lipid peroxidation, which may explain the patterns of VOC expressed in the exhaled breath of patients with colon cancer.[25] Phillips *et al.*[12,19,24] published several analyses of VOC patterns expressed in various malignancies, which further support the theory that alkanes and alkane-derivatives in exhaled air may be associated with increased cytochrome P450 activity and increased oxidative stress.

Altomare *et al.*[36] demonstrated the relationship between the presence of malignancy and expressed VOCs. They discovered that a combination of 11 VOCs was diagnostic for colon cancer, with a sensitivity of 100%, a specificity of 97.92%, and an overall accuracy of 98.75% (Figure 2). The same VOC pattern discriminated between patients with a history of colon cancer who had been disease free for over a year and healthy controls with a sensitivity of 100%, specificity of 90.91%, and accuracy of 94.25%.[26] In another similar study, 418 breath samples were collected from 65 patients with colon cancer, as well as 22 with adenomas, and 122 non-cancer control cases. Using GC-MS analysis to detect four compounds of interest (acetone, ethyl acetate, ethanol, and 4-methyl octane), patients with colon cancer were distinguishable from controls with 85% sensitivity, 94% specificity, and 91% accuracy (Figure 1).[37] Their model also distinguished between advanced and non-advanced adenomas with 88% sensitivity, 100% specificity, and 94% accuracy. As such, VOC analysis offers an advantage over stool-based screening in its ability to accurately detect pre-cancerous adenoma.[37]

Given the intrinsic limitations of stool-based screening, colonoscopy remains the gold standard for the detection of pre-cancerous adenomas and the recommended evaluation following an abnormal stool-based screening test. It is an invasive test associated with patient discomfort, procedural risks, and suboptimal compliance. In contrast, early detection of colon cancer through VOC analysis would allow for non-invasive, inexpensive, and accessible screening. VOC analysis may also be superior in sensitivity and specificity when compared to screening colonoscopy.

4. Prostate cancer

Despite being the second most prevalent malignancy in men worldwide, there are currently no reliable screening tools for prostate cancer.[38-40] Prostate cancer is the third-leading cause of new cancer cases diagnosed worldwide, with approximately 1.4 million new cases diagnosed in 2020.[39] At present, a combination of digital rectal examination, serum prostate-specific antigen (PSA), and trans-rectal ultrasound-guided prostate biopsy is employed for prostate cancer.[38-41]

Serum PSA, at a cut-off of 4 ng/mL, was integrated into screening programs in the United States of America in the 1990s.[38-44] However, due to a sensitivity as low as 21% for prostate cancer and 51% for high-grade cancers, the use of PSA for cancer screening is no longer recommended in most international screening guidelines (Figure 1).[40-46] Indeed, two large screening trials failed to demonstrate a significant decrease in prostate cancer-associated mortality using PSA-based screening tests.[46,47] Moreover, with a false-positive rate as high as 20%, PSA screening has been associated with significant overdiagnosis as well as subsequent unnecessary testing/biopsies.[38-46]

Liu *et al.*[47] utilized a combination of 86 VOCs in a cohort of 43 patients with a definite pathological diagnosis of prostate cancer and 64 controls, whereby their model accurately detected prostate cancer with a sensitivity of 87.0% and a specificity of 91.3% (AOC = 0.945). Specifically, furan-3-methanol, (e,e)-octadeca-2,4-dienal, 2-ethylhexan-1-ol, and 2-undecen-1-al were most specific in differentiating cancer specimens from controls, with AUCs >0.70. Similarly, Gao *et al.*[48] measured the VOC profile of the urine headspace through GC-MS in a test cohort of 108 patients with biopsy-confirmed prostate cancer positives and compared them to controls, creating a diagnostic model with 11 VOCs, which they subsequently validated with another group of test samples, which included 53 patients with prostate cancer compared to 22 healthy controls, with a resulting AUC of 0.86. Following cross-validation, the AUC for this model was 0.92 (sensitivity = 0.96; specificity = 0.80) (Figure 1).[48]

Despite these promising results, little is known about the mechanism of production of VOCs specific to prostate cancer, and a reliable VOC profile for prostate cancer has not yet been described. Peng *et al.*[49] examined the exhaled VOC profiles of healthy controls (n = 22) compared with patients with biopsy-confirmed malignancy (breast (n = 22), lung (n = 30), colorectal (n = 26), prostate (n = 18). They describe several VOCs that, when measured, showed no overlap between controls and patients with prostate cancer (toluene, 2-amino-5-isopropyl-8-methyl-1-azulenecarbonitrile, p-xylene, and 2,2-dimethyl-decane).[49] However, this study was limited by a small sample size, and their model failed to reach statistical significance for their VOC profile for prostate cancer. The molecule sarcosine has previously been proposed as a biomarker of aggressive tumor biology in prostate cancer, and interestingly, higher levels are reported in the urine of patients with biopsy-confirmed prostate cancer (n = 44) compared to healthy controls (n = 51).[50] However, the receiver operator characteristic (ROC) for sarcosine was modest (0.71 for urine sediments; 0.67 for supernatants), which limits its potential for clinical application.[50]

In vitro studies report elevated acetaldehyde dehydrogenase activity in tumor cells, a molecule that catalyzes the oxidation of exogenous and endogenous aldehyde substrates into carboxylic acids, which may explain the finding of decreased aldehyde levels in prostate cancer VOC profiles.[46,51-54] This finding is not, however, specific to prostate cancer and has been described in colon, gastric, and breast cancer as well.[54,55] Similarly, Liu *et al.*[47] describe 2-ethyl-1-hexanol in their prostate cancer VOC profile as a known metabolite of diethylhexyl phthalate with a role in the induction of apoptosis previously implicated in the VOC profile of several other malignancies.[46,53-55] Nevertheless, VOC for prostate cancer screening and detection is promising, with excellent sensitivity and specificity profiles in studies using VOCs detected in the headspace of urine samples (Figure 1). Given the current limitations of PSA screening, further study is warranted to explore the role of VOC analysis as a non-invasive test for prostate cancer screening.

5. Gastric cancer

Gastric cancer is the fifth most common cancer worldwide, with approximately 1.09 million new cases of gastric cancer diagnosed in 2020.[56] Globally, it is the fourth leading cancer cause of death, responsible for 7.7% of all cancer-related deaths in 2020.[56] The incidence and prevalence of gastric cancer have significant variability worldwide, with most new cases of gastric cancer occurring in Eastern Asia.[56] As a result, recommendations for gastric cancer screening are varied, with some countries adopting population-based screening, while other countries reserving screening investigations for specific high-risk subgroups dependent on endemic incidence. Early diagnosis and treatment of gastric cancer results in decreased mortality. However, early-stage gastric cancer is most often asymptomatic or with symptoms similar to gastritis.[56-58] Owing to the silent or ambiguous presentation of early gastric cancer, effective screening investigations are important to ensure early gastric cancer diagnosis.

The primary method of detecting gastric cancer in the early 20th century was X-ray imaging. With the ingestion of barium contrast media, the GI tract could be assessed for abnormalities with minimal risk to patients.[59] However, the sensitivity of the single-contrast barium examination in the diagnosis of gastric carcinoma ranged from 54%[60] to an average of 75%.[61] In the late 1960s, Japanese physicians developed the gas-barium double-contrast method of X-ray imaging, which involved the ingestion of CO_2 gas-producing granules in conjunction with a barium suspension.[60-63] This method permitted visualization of the enhanced barium-saturated mucous membrane of the stomach, which was subsequently inflated with air to assess its elasticity.[62] Over time, specific techniques involving rotating the patient and timings for spot films were developed to optimize the mucosal coating of the stomach and to permit assessment of the stomach in its entirety. Sensitivity with the double contrast barium study ranged from an average of 76 – 96%; however, it has been established as superior to conventional computed tomography in localizing the diagnosis.[60,61,64]

The flexible design of the fiberoptic endoscope created in 1957 allowed for the examination of the entire stomach, overcoming the limitations of its predecessor, the rigid gastroscope.[57,65] In the early 1960s, the utility of endoscopy in the diagnosis of gastric cancer was realized as it enabled direct visualization of the gastric mucosa and allowed for biopsies to be carried out on ulcers and other suspicious areas of the stomach.[62] The sensitivity of endoscopy in the diagnosis of gastric cancer ranged from 92% to 99%, and therefore, endoscopy rapidly gained popularity for its superior diagnostic accuracy as compared to the single contrast barium meal (Figure 1).[59-61] Ultimately, endoscopy was accepted as the superior test for gastric cancer screening due to its better accuracy.[57,58,66,67] Today, endoscopy and endoscopic biopsy are accepted as the gold standard for the diagnosis of gastric cancer.[64]

Helicobacter pylori infection was identified as a major risk factor for gastric cancer in the 1980s, resulting in

the identification and eradication of *H. pylori* as a key preventative measure.[68] Endoscopy made a significant impact on gastric cancers not only by increasing the detection of early gastric cancers by enabling biopsies but also by aiding in decreasing gastric cancer occurrence by allowing for the detection and implementation of eradication therapy against *H. pylori*.[65] The limitations of upper endoscopy are primarily related to its invasiveness and resultant potential patient discomfort, as well as the associated risks of the procedure, including bleeding or perforation. As with all technical procedures, the accuracy of endoscopy can vary based on the skill and experience of the endoscopist. Endoscopy remains the gold standard in the detection of gastric cancer as it allows for the collection of biopsies for histological examination and definitive diagnosis.[69]

Before 2010, the application of human breath analysis was largely limited to urea breath testing for *H. pylori* infection, a hydrogen breath test for small bowel bacterial overgrowth, and the concentration of exhaled nitric oxide for the investigation of asthma.[70] In 2013, exhaled breath metabolites in 18 patients with biopsy-proven esophagogastric cancers were analyzed and compared to the concentrations of the metabolites in a control group of 18 patients with biopsy-proven non-cancer diseases of the upper GI tract and 17 healthy controls.[71] The study identified a significant increase in the concentration of hexanoic acid in the exhaled breath of the esophagogastric cancer patients compared to patients in the positive control and healthy control groups. In addition, there were statistically significant increases in the concentrations of phenols and their derivatives, methyl phenol and ethyl phenol, in the exhaled breath of patients with esophagogastric cancer, compared with the positive control, and healthy control groups. It was thought that these differences in concentrations were due to increased protein catabolism in gut microbiota and the upregulation of tyrosine metabolism in patients with esophagogastric cancers.

In 2015, Kumar *et al.*[72] quantified exhaled breath VOCs from 210 patients with either esophagogastric adenocarcinoma, Barrett's esophagus, benign upper GI disease such as gastritis and gastric ulcer, or a normal upper GI tract. The study identified 29 exhaled molecules of interest, including 12 VOCs present at statistically significantly higher concentrations in patients with esophagogastric cancers. The AUC using these 12 molecules to discriminate patients with esophageal and gastric adenocarcinoma from those with non-malignant conditions as well as healthy controls was 0.92 and 0.98, respectively. The authors further proposed a predictive model that differentiates patients with gastric cancer from controls. The AUC for their model was 0.92, with a sensitivity of 89.3% (95% confidence interval [CI]: 77.0 – 95.7) and specificity of 83.7% (95% CI: 74.5 – 90.9). Interestingly, *H. pylori* status and proton-pump inhibitor independently predict exhaled ammonia concentrations, identifying a key limitation of VOC screening as it remains susceptible to interference from both endogenous and exogenous factors (Figure 1).

Similarly, a recent meta-analysis published in 2021 pooled the data from five studies exploring the role of exhaled VOCs in the diagnosis of GI cancer. These studies analyzed endogenous VOCs in exhaled breath of patients with biopsy-confirmed GI cancer.[73] The pooled data analysis suggests that VOCs can be used to differentiate between gastric cancer and non-malignant gastric conditions with sensitivity of 85% and specificity of 89%, with diagnostic odds ratio and AUC values reported as 41.30 and 0.93, respectively.[73] Durán-Acevedo *et al.*[74] compared breath samples from 14 patients with gastric cancer and 15 controls. Using a novel solid-state sensor in addition to GC-MS, a significantly higher concentration of six VOCs was identified in patients with gastric cancer, leading to a predictive model that identified patients with gastric cancer with a sensitivity of 100% and a specificity of 93%. Similarly, Lee *et al.*[75] determined that four VOCs (propanal, acetamide, isoprene, and 1,3 propanediol) exhibit a gradual increase in concentration from normal control to early and advanced gastric cancer (Figure 2). Analysis of the ROC curves for these four VOCs demonstrated that the AUC for gastric cancer prediction was highest (0.842) when three or more VOCs were measured in tandem.

Intraluminal gas has also been used for VOC analysis for gastric cancer diagnosis. Yang *et al.*[76] reported on using a combination of intraluminal and exhaled gas collecting during a prospective trial involving 259 patients undergoing endoscopy to discriminate between upper GI cancer and healthy controls. Intraluminal VOC analysis was better in discriminating upper GI cancer from benign controls when compared to exhaled VOC analysis (sensitivity: 91.23% vs. 81.75%, specificity: 90.65% vs. 88.46%, and AUC: 0.930 vs. 0.877). Gastric cancer could also be detected with both intraluminal and exhaled breath VOC analysis, which discriminated this patient population versus benign controls (sensitivity: 87.04% vs. 74%, specificity: 96.99% vs. 92.31%, and AUC: 0.983 vs. 0.889).

At this time, more research is required to identify specific and reliable VOC biomarkers associated with gastric cancer to improve its diagnostic accuracy. Although several models have been shown to differentiate between benign and malignant conditions, as well as discriminate between early

and late-stage gastric cancer, exhaled VOCs lack reliable sensitivity and specificity, limiting clinical applications. Nevertheless, since exhaled VOC analysis is non-invasive and does not require sedation or tissue sampling, it remains an exciting avenue for further research.

6. Skin and soft tissue malignancies

Melanoma is the fifth most common cancer in the United States.[9] Survival is directly dependent on the stage of diagnosis, with early detection leading to improved outcomes.[77] Aside from visual skin surveillance, there are no screening tests for melanoma. Presentation often results after the detection of a new or changed skin lesion.[78] The diagnosis of early melanoma is through the biopsy of worrisome skin lesions selected by a visual assessment, which in itself remains challenging even for experienced clinicians.[78-80] Indeed, clinical diagnosis by skilled dermatologists has been estimated to be approximately 70% sensitive, and sensitivity can be improved with clinical aids such as a dermatoscopy.[80,81] More recently, the accuracy of the visual inspection was assessed in a systematic review and meta-analysis of 49 studies with a total of 34,000 skin lesions, of which 2,500 were melanomas.[81] The sensitivity and specificity of visual inspection in this analysis were 92.4% (95% CI: 26.2 – 99.8%) and 79.7% (95% CI: 073.7 – 84.7%), respectively, where the wide CI reflects significant heterogeneity in diagnostic accuracy.[81]

Melanoma-related VOCs have been found to differ from normal skin VOC expression patterns in GC studies, leading to the proposal of melanoma-specific breathprints. On a cellular level, Preti *et al.*[82] employed solid-phase micro-extraction, GC-MS, and single-stranded DNA-coated nanotube sensors to compare VOCs from normal and malignant melanocytes to identify VOCs unique to melanoma cells, including dimethyldi- and trisulfide. Similarly, Santonico *et al.*[83] utilized a gas sensor array to discriminate between benign nevi and melanoma lesions with about 80% accuracy (n = 40). Abaffy *et al.*[84] propose a dozen potential VOCs potentially derived from melanoma *in vitro*; however, many have since been shown to be environmental contaminants. The use of *in vitro* models or small tissue samples may have contributed to environmental contaminants in these studies, and as of yet, there are no convincing, characteristic VOC profiles for the detection of melanoma, despite evidence that there is a distinct volatile metabolome emanating from melanoma cells that differ from that of normal skin.

Sarcomas are defined as malignant tumors of mesenchymal origin, comprising <1% of all adult malignancies.[1] Up to 80% of sarcoma originates from soft tissue, and 20% originates from bones.[85] There are no screening tests for sarcoma nor are there characteristic signs or symptoms to facilitate diagnosis.[85-87] The diagnosis is further challenged by the multitude of subtypes and the pathology expertise required to make a correct diagnosis.[86] On expert review, up to 40% of cases were considered incorrectly diagnosed.[86-88]

The most common presenting complaint for soft tissue sarcoma is a gradually enlarging, painless mass.[85] Diagnosis is confirmed with a tissue sample and histologic examination, and radiographic imaging is used to further define the etiology of the mass, the extent of the disease, and plan treatment options.[89-92] Diagnostic delays are common and are associated with worse outcomes.[86,90] Indeed, prompt diagnosis is relevant to prognosis, as the most common prognostic factors in sarcoma are tumor size, histologic grade, and pathologic stage.[91] Recently, a cross-sectional pilot study of 59 patients described the use of electronic nose microarray technology to identify patients with an underlying diagnosis of biopsy-confirmed soft tissue sarcoma using the profile of their exhaled volatile organic molecules, and their preliminary publication reports a c-statistic of 0.85, sensitivity of 83%, and specificity of 60%.[92] Further studies are needed to establish a reproducible breathprint specific to sarcoma and to the various histological subtypes, as there is a clear need for diagnostic tools to facilitate earlier detection and reduce diagnostic delays for these malignancies.

7. Discussion

The analysis of VOCs is a promising approach for screening and diagnosing multiple tumor types. It has been shown to be non-invasive, relevant in many cancer subtypes, and has the potential to be inexpensive.[93,94] There are also several possible applications for VOC testing in cancer care. This technique could be used to triage patients with non-specific symptoms and expedite/guide the referral process from primary care to subspecialty care, improving compliance with screening and potentially decreasing the proportion of inappropriate referrals. If proven to be effective, this non-invasive technology has the potential to increase the uptake of screening, given the non-invasive nature of the test and the ease with which the samples are obtained. Moreover, given that several VOCs have been shown to reliably fluctuate based on tumor burden, there is potential for a role in monitoring response to therapy and surveillance for disease recurrence.[95-97] However, the analysis of VOCs has yet to be incorporated into clinical practice, and the approach faces several challenges to widespread implementation.

7.1. Standardization

The results of VOC testing are sensitive to the method of sample collection, patient physiology, and test environment. When measuring VOCs in exhaled breath, multiple respiratory factors may influence the concentration of certain molecules within the breath sample. There are, for example, international recommendations for the standardized measurement of nitric oxide within breath samples published by the American Thoracic Society and the European Respiratory Society, which in turn have supported the adoption of this metric as a diagnostic tool in clinical practice.[1,98] A similar framework must be proposed and implemented across all VOC screening tools to ensure the standardization and reliability of the measurements. Quality control measures will need to be implemented with established guidelines for calibration procedures, and accurate quantification analyses of VOC samples will be required to ensure reliable, high-quality, reproducible testing. The detection limits of instruments used in clinical settings for trace VOC measurements will need to be specified.

7.2. Limitations

There are several tumor factors that influence the sensitivity and specificity of VOC for cancer screening and detection, which in turn may impact the implementation of this technology in clinical practice. Tumor volume and stage, for example, are critical considerations when applying VOC analysis to cancer screening and detection. The sensitivity and specificity of VOC analysis for colorectal cancer detection have been shown to be affected by tumor size, whereby larger tumors release higher VOC concentrations, increasing the accuracy of VOC analysis.[97] VOC analysis has been shown to have a higher accuracy in detecting early-stage breast cancer compared to advanced-stage cancer, and as such, the impact of tumor volume may also vary by cancer type.[99,100] Larger tumors produce more volatile compounds, leading to a more complex VOC profile and making it more difficult to detect specific biomarkers.[100] The accuracy of VOC analysis for cancer detection can also be influenced by other factors related to tumor volumes, such as necrosis and inflammation. A study by Angioli *et al.*[101] reported that necrosis in ovarian cancer could affect the VOC profile and lead to inaccurate results in cancer detection. Similarly, a study by Rodriguez-Miguelez *et al.*[102] discovered that inflammation in breast cancer can influence the VOC signature and lead to false positives.

In addition, some malignancies may be better candidates for screening through VOC analysis. While Haick *et al.*[103] confirmed that VOC analysis could be highly sensitive and specific in detecting breast cancer, and Dragonieri *et al.*[104] demonstrated a VOC analysis protocol highly accurate for the detection of lung cancer, a study by Ge *et al.*[105] discovered that VOC analysis had low sensitivity in detecting pancreatic cancer. Moreover, the histologic subtype of the cancer may also influence the VOC profile of the malignancy, in turn affecting the sensitivity and specificity of the diagnostic test. VOC analysis has been shown to have higher accuracy in detecting adenocarcinomas than squamous cell carcinoma in lung cancer.[100] Similarly, the VOC profile of ovarian cancer varies by histological type, whereby serous tumors have a different VOC profile compared to endometrioid tumors.[106] The tumor's location within the affected organ may also impact the accuracy of the VOC analysis. The VOC profile of gastric cancer has been shown to vary by the location of the primary tumor within the stomach, whereby tumors located in the antrum produce a different VOC profile compared to those discovered in the body of the stomach.[107] Similarly, higher concentrations of VOCs are produced by lung tumors in the central airways compared to peripheral tumors.[108]

- The use of chemotherapy and radiation therapy will also influence the accuracy of VOC analysis, as adjuvant therapy changes VOC profiles produced by tumors. VOC analysis has shown lower accuracy in detecting oral cancer in patients who have received chemotherapy or radiation therapy.[109] The VOC profile of cancer has also been shown to change during adjuvant therapy, which may affect the accuracy of VOC analysis in detecting residual disease.[110,111]
- The metabolic activity of cancer cells is different at different stages of lung cancer, leading to different VOC profiles, and several recent studies have shown that the cancer stage influences the sensitivity and specificity of VOC analysis for cancer detection.[112] The VOC profiles of early-stage lung and colon cancer patients differ significantly from those of late-stage patients, and the sensitivity of VOC analysis for early-stage lung cancer detection is higher than that of late-stage cancer.[112-114] A study by Cao *et al.*[115] investigated the use of VOC analysis for early detection of gastric cancer and discovered that the sensitivity of the technique was higher for early-stage tumors than for advanced-stage tumors. Similarly, a study by Saorin *et al.*[116] reported that VOC analysis had higher accuracy in detecting early-stage ovarian cancer compared to advanced-stage cancer.
- The use of VOC analysis for cancer screening and detection is still in its early stages, and further research is needed to fully understand the impact of

tumor factors such as location, size, and histologic subtype on its accuracy. Larger tumor volumes and advanced-stage cancers may produce more complex VOC profiles, which can lead to false positives or reduced sensitivity of the technique. Moreover, the impact of adjuvant therapies such as chemotherapy, radiation, and immunotherapy on VOC release by tumor cells remains incompletely understood, further highlighting the potential limitations of VOC analysis for cancer screening and detection. Despite these challenges, VOC analysis remains a promising tool, and continued research will further refine its utility in clinical practice.

7.3. Integration into clinical practice

MS for VOC analysis is a standard technique used widely in several industries. The instrumentation has been validated over several years and is no longer in the development stage.[9] However, the accuracy and reliability of this technique for obtaining results in clinical practice have yet to be established. Furthermore, the reproducibility of the results in a clinical setting needs to be validated, reported, and optimized before implementation. Randomized control trials against the current standard of care for cancer screening and detection should be carried out before considering the integration of this tool into clinical practice. Indeed, it would be important to establish the repeatability of VOC measurements using the same analytical platform and the reproducibility of VOC measurements among instruments using different analytical platforms and/or different laboratories/centers.

7.4. Validation of VOCs as a diagnostic model

Formal trials are required to provide external validation against positive control groups and compare against current standards of care, ultimately validating the tool among the target population and the external environment where the VOC testing will ultimately take place. Validation studies are required to establish test thresholds for various cancers at various stages as well as to establish a differentiation between tumor subtypes and compare these metrics against a control test population. Subsequently, large, multicenter, blinded randomized control trials are required to validate this technology against the standard of care. Importantly, the studies will need to follow international standards for reporting using Standards for Reporting of Diagnostic Accuracy Studies guidelines.

8. Conclusion

There is strong evidence supporting the potential use of VOCs in exhaled breath for accurate cancer detection. Implementing VOC analysis for accessible screening and early detection of cancer could improve patient outcomes and decrease cancer-related deaths and the global disease burden. However, despite its immense potential, VOC analysis continues to face several implementation challenges before it can be integrated into clinical practice. Standardization of sample collection and analysis, clinical validation, and, ultimately, multicenter randomized control trials are necessary to establish the role of VOC analysis in cancer screening and detection.

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ACCSCIENCE PUBLISHING

Tumor Discovery

PERSPECTIVE ARTICLE

Vasculoendothelial dysfunction and bone health in obese children: A connection with cancers

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Abstract

Obesity in children, a growing global epidemic, is not merely characterized by excessive body fat but also by a cascade of metabolic and functional derangements with significant musculoskeletal consequences. One of the key underpinnings of these detrimental effects is vasculoendothelial dysfunction (VED), a multifaceted pathological process characterized by impaired endothelial cell function. The endothelium, lining the vasculature, plays a pivotal role in regulating vascular tone, inflammation, and coagulation, and its dysfunction in obese children leads to a plethora of downstream complications with profound implications for musculoskeletal and bone health. Diagnosing VED in obese children remains a challenge. Traditional cardiovascular risk factors such as hypertension and dyslipidemia are often detected long after the occurrence of endothelial dysfunction. In the context of bone sarcoma, the interaction between cancer cells and vascular endothelium in the bone microenvironment is critical. The bone is a highly vascularized tissue, and the vascular endothelium plays a role in regulating bone homeostasis. In the presence of cancer cells, altered bone microenvironment provides a milieu favorable to tumor growth and invasion. The relationship between the vascular endothelium and cancer is complex and can vary depending on the type of cancer and its microenvironment. Understanding the interactions between vascular endothelium and bone sarcoma is crucial for developing targeted therapies. New molecular and cellular mechanisms involved in these interactions are awaiting to be uncovered, shedding light on potential therapeutic strategies for cancer treatment. On a relevant note, emerging vasculoendothelial markers hold promise for early detection and intervention. Early identification of VED, through the detection of vasculoendothelial markers, opens doors for timely interventions aimed at preventing its detrimental effects on musculoskeletal and bone health in obese children.

Keywords: Childhood obesity; Vasculoendothelial; Vascular dysfunction; Inflammatory markers; Cancer; Bone

1. Introduction

Obesity in children, a growing global epidemic, is not merely characterized by excessive body fat but also by a cascade of metabolic and functional derangements with significant musculoskeletal and bone health consequences. One of the key underpinnings of these detrimental effects is vasculoendothelial dysfunction (VED), a multifaceted

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pathological process characterized by impaired endothelial cell function.[1]

The endothelium, lining the vasculature, plays a pivotal role in regulating vascular tone, inflammation, and coagulation. Emerging evidence suggests a dynamic interplay between obesity and endothelial dysfunction.[2] A recent study has reported impaired endothelial function in obese children, emphasizing the importance of vascular health assessment. Obesity influences endothelial function through obesity-related complications such as hypertension, dyslipidemia, diabetes, metabolic syndrome, and obstructive sleep apnea syndrome. An association between obesity and endothelial function has been demonstrated by means of anthropometric indices and imaging modalities.[3] In the context of obesity, metabolic complications (e.g., diabetes) and cardiovascular complications (e.g., atherosclerosis) are resulted from dysfunctional adipose tissue that generates a pro-inflammatory, hyperlipidemic, and insulin-resistant environment.[4]

Understanding the intricate relationship between obesity and vascular health is crucial for early intervention and prevention. Although a few studies have explored the relationships between VED and obesity, information regarding various vasculoendothelial and inflammatory markers in obese children is still lacking.

After critically examining the literature regarding various experimental methodologies and verification in children, we realize that there are no studies thus far regarding the correlation between vasculoendothelial and inflammatory markers in obese children and the implications of obesity for bone health and cancers. Thus, the present paper intends to offer a perspective of childhood obesity, focusing on vasculoendothelial and inflammatory markers in the context of obesity, as well as bone growth and cancers in children.

2. VED and bone health in obese children

2.1. Vascular endothelium

The vascular endothelium serves as a physical barrier between blood and tissues. It is also an endocrine structure that helps maintain cardiovascular homeostasis. Endothelial dysfunction occurs at an early stage in the development of atherosclerosis, leading to an increased risk of cardiovascular events. Vascular homeostasis is maintained by a balance between endothelium-derived relaxing and contracting factors. However, the disruption of this balance, mediated by inflammatory and traditional cardiovascular risk factors, renders the vasculature susceptible to atheroma formation.

2.2. VED

Fundamental and translational studies have led to a significant understanding of cellular and molecular alterations responsible for endothelial dysfunction. Unfortunately, progression of atherosclerosis in obese adults, who have been chronically affected by endothelial dysfunction, is still the dominant topic in literature. Despite technical difficulties and ethical concerns, the investigation of endothelial function in obese children stands as a necessary step to deepen our understanding of the initiation and progression of endothelial dysfunction, which is crucial to the development of treatment strategies.

2.2.1. VED and obesity

Emerging evidence suggests a dynamic interplay between obesity and endothelial dysfunction.[2] Accumulating evidence indicates that endothelial dysfunction is an independent predictor of cardiovascular events and may serve as an early indicator of cardiovascular risk in obese children.[5] Childhood obesity has also been associated with endothelial dysfunction although there has been relatively little research into the relationship between biomarkers of endothelial activation and either insulin resistance or inflammation in obese children.

Reactive oxygen species (ROS), which is generated during oxidative stress, contributes to endothelial dysfunction. ROS and ox-low-density lipoprotein (ox-LDL), a modified form of low-density lipoprotein (LDL), have been implicated in the pathogenesis of atherosclerosis. Oxidative modification of LDL renders it more atherogenic, promoting endothelial dysfunction and recruitment of inflammatory cells to the arterial wall, which might be the mechanisms underlying atherosclerotic processes in the context of excess adiposity.[5] Oxidative damage of LDL produces ox-LDL, which is implicated in endothelial dysfunction and atherosclerosis that are often observed in obese children; therefore, exploring the mechanistic relationship between ox-LDL and endothelial dysfunction would help with identifying potential therapeutic targets. It has been demonstrated that the impairment of endothelial nitric oxide synthase (eNOS) and the loss of nitric oxide (NO), a major vasodilator and anti-inflammatory agent, are hallmarks of obesity-induced endothelial dysfunction.

Although various studies have independently explored the impact of vasculoendothelial and inflammatory markers in the context of childhood obesity, a comprehensive understanding of their association is still lacking.

2.3. Vasculoendothelium and bone health

The endothelium is an integral part of bone tissue, performing physiological paracrine functions through

growth factors and chemokines release, and interacting with different cell types. Endothelial cells in bone have several functions, namely maintenance of vascular integrity, bone formation, and direct stimulation of osteoblasts/osteoclasts crosstalk.[6]

Alterations of the complex biochemical interactions between vasculature and bone cells dramatically affect bone metabolism and health and may lead to various clinical manifestations. Changes to the blood supply in the vascular networks surrounding bone cells very often could lead to inhibition of bone metabolism, resulting in decreased bone formation.

Obesity influences bone metabolism by stimulating pre-osteoblasts to differentiate into adipocytes rather than osteoblasts, thus filling the cavities of bone marrow with adipocytes rather than trabecular bone, consequently increasing bone fragility. The RANKL/RANK/OPG pathway is a signaling pathway that regulates the formation and activity of osteoclasts, which are cells responsible for breaking down bone tissue. Increased levels of pro-inflammatory cytokines due to obesity would dysregulate this pathway, leading to an increase in osteoclast formation and activity. Ultimately, this results in a decrease in bone density and an increased risk of fractures. Therefore, it is crucial to maintain a healthy weight through proper dietary intake and exercise, especially for obese children, to ensure good bone health.[7]

Obesity is related to inflammatory musculoskeletal diseases (i.e., osteoarthritis).[8] VED in obese children manifests in several ways, impacting various aspects of musculoskeletal function. Endothelial NO bioavailability is significantly reduced in obesity, leading to impaired vasodilation and decreased blood flow to muscles and causing diminished exercise capacity, muscle fatigue, and reduced exercise tolerance, which poses a hindrance to physical activity participation, thereby perpetuating the obesity cycle. Furthermore, VED promotes chronic low-grade inflammation, contributing to muscle weakness, pain, and impaired bone health, all of which increase the risk of musculoskeletal injuries and fractures. VED also promotes pro-thrombotic changes, increasing the risk of deep vein thrombosis and other musculoskeletal complications.[9]

Spatiotemporal interaction between endothelial cells and neighboring skeletal cells plays critical roles in development, homeostasis, and pathological bone destruction. The altered relationship between endothelium, vasculature, and bone tissue can result in pathologies such as avascular necrosis, osteopetrosis, rickets, osteoporosis, inflammatory bone loss, multiple myeloma, Paget's disease, and metastatic bone disease. Thus, a greater understanding of the role of bone vasculature in both normal development and pathological condition may lead to new therapeutic approaches for a range of bone conditions.

2.3.1. VED and its implications in cancer

VED is characterized by an imbalance in the production and release of various substances by endothelial cells, leading to altered vascular tone, increased inflammation, and impaired blood vessel function. While VED is commonly associated with cardiovascular diseases, emerging research has also highlighted its implications for cancer development and progression. VED can contribute to increased angiogenesis, which is a critical process for supplying tumors with nutrients and oxygen in cancer patients, facilitating their growth and progression. Endothelial dysfunction is often associated with chronic inflammation. Inflammation in the tumor microenvironment can promote cancer development and contribute to the evasion of immune surveillance. The dysfunctional endothelium may facilitate the recruitment of immune cells that support tumor growth. Dysfunction of the vascular endothelium can lead to increased permeability of blood vessels that might contribute to the intravasation and extravasation of cancer cells, facilitating their spread to distant sites and promoting metastasis.

Endothelial dysfunction can induce a procoagulant state and may contribute to cancer-associated thrombosis, a common complication in cancer patients. Cancer cells can influence the endothelium through the release of various factors that may further exacerbate VED and create a microenvironment conducive to tumor growth.

Markers of VED, such as elevated levels of circulating endothelial cells, von Willebrand factor (vWF), and adhesion molecules, have been investigated for their diagnostic and prognostic value in cancer. These markers may serve as indicators of the vascular changes associated with tumor development.

Targeting VED has become an area of interest in cancer therapeutics. Anti-angiogenic therapies are conceptualized to disrupt the formation of new blood vessels so as to impede tumor growth. In addition, drugs targeting inflammation and endothelial function are being explored in cancer treatment.

Understanding the intricate relationship between VED and cancer is essential for developing targeted therapeutic strategies and improving patient outcomes. The molecular and cellular mechanisms underlying this relationship are currently under exploration in ongoing research.

2.3.2. Vascular endothelium and bone sarcomas

In the context of bone sarcoma, the interaction between cancer cells and the vascular endothelium in the bone microenvironment plays a critical role.[10] Osteosarcoma is the

most common tumor in children and adolescents, followed by chondrosarcoma. The bone is a highly vascularized tissue, and the vascular endothelium plays a role in regulating bone homeostasis. In the presence of cancer cells, changes in the bone microenvironment can favor tumor growth and invasion. Angiogenesis is essential for tumor growth and metastasis, and vascular endothelium is involved in angiogenesis by releasing growth factors and signaling molecules that promote the formation of new blood vessels.

The immune response plays a complex role in cancer, with immune cells potentially either suppressing or promoting tumor growth. The vascular endothelium is involved in the recruitment of immune cells to the tumor site. Furthermore, vascular endothelium influences the migration of immune cells to the tumor microenvironment, affecting the balance between anti-tumor and pro-tumor immune responses.

The relationship between the vascular endothelium and cancer is complex and can vary depending on the type of cancer and its microenvironment. The molecular and cellular mechanisms underlying the interactions between the vascular endothelium and cancer are currently under exploration in ongoing research, with a view to uncovering potential new therapeutic strategies for cancer treatment.

2.4. Markers of endothelial function

Various biomarkers of endothelial function and integrity include C-reactive protein, endothelial adhesion molecules, E-selectin, cytokines, CD62+, ox-LDL, asymmetric dimethylarginine, and endocan. Novel biomarkers of endothelial dysfunction include endoglin, annexin V+, matrix metalloproteinases, ANGPTL2, serum homocysteine, hepatokines, cellular adhesion molecules (CAMs; including E-selectin, intercellular adhesion molecule 1 [ICAM-1, pentraxin-3, vascular cell adhesion molecule 1 [VCAM-1), and various sonographic parameters (liver ultrasound and carotid intima-media thickness parameters).[11-14]

2.4.1. Markers of endothelial dysfunction

Elevated levels of sICAM-1 have been implicated in obesity-related inflammation and could be a potential indicator of endothelial dysfunction and cardiovascular risk.[14] VCAM and E-selectin, which are endothelial adhesion molecules, contribute to the inflammatory cascade by facilitating the recruitment of immune cells to adipose tissue, thereby exacerbating chronic low-grade inflammation associated with obesity.[15]

Advances in spatial omics such as spatial transcriptomics, epigenomics, proteomics, and metabolomics, and their integrations at various biological scales, may help decipher the previously unappreciated pathophysiological mechanisms in bone and refine classifications of certain diseases, facilitating precise and individualized medical treatment for patients.[15]

Exploration of the dynamics of these adhesion molecules in the context of obesity and the musculoskeletal would provide valuable insights into the pathophysiological mechanisms underlying obesity-related vascular complications. Emerging evidence underscores the importance of unveiling further skeletal-vascular interactions, which are instrumental to enhancing treatment for patients suffering from cancer as well as other bone-destructive diseases.

2.4.2. Markers of vasculoendothelial and bone growth

Several markers are associated with vasculoendothelial and bone growth that reflect the dynamic processes involved in angiogenesis, bone formation, and remodeling. These markers are often used in research and clinical settings to assess the status of blood vessels and bone tissue. Common markers for vasculoendothelium and bone growth include vasculoendothelial growth factor, endothelial cell markers (CD31 [PECAM-1, vWF), bone formation markers (osteocalcin, bone alkaline phosphatase), bone resorption markers (tartrate-resistant acid phosphatase, C-terminal telopeptide of type I collagen), transforming growth factor-beta, fibroblast growth factor, platelet-derived growth factor, and angiopoietins.[16]

These markers can be measured from blood or tissue samples to assess the activity of endothelial cells, osteoblasts, and osteoclasts. Changes in the levels of these markers can provide insights into the status of vasculoendothelium, bone growth, angiogenesis, bone formation, and remodeling in cancer and may have implications for cancer diagnosis, prognosis, and/or response to therapeutic interventions.

3. Conclusion

At present, there are no specific studies addressing the possible link between VED and bone health in obese children, as well as investigating the diagnostic utility of vasculoendothelial and bone growth markers in the context of cancer. However, it is known that the seemingly disparate research domains of obesity, cardiovascular health, bone health, and cancer often intersect, offering a distinct direction to advance research progress. Early identification of VED, through measurement of vasculoendothelial markers, opens doors for timely interventions aimed at preventing its detrimental effects on musculoskeletal health in obese children. Lifestyle modifications, including dietary changes and increased physical activity, are crucial first-line interventions, promoting NO bioavailability and reducing inflammation.[16] In addition, specific therapeutic strategies targeting endothelial function, such as anti-inflammatory medications and antioxidants, might hold

promise in the future. Understanding the interactions between the vascular endothelium and bone sarcoma is crucial for developing targeted therapies. At present, anti-angiogenic therapies are being conceptualized and designed, with the aim of disrupting blood supply to tumors by targeting vascular endothelium, to inhibit tumor growth and metastasis. By proactively addressing VED and its musculoskeletal consequences, we can empower obese children to lead healthier, more active lives.

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ACCSCIENCE PUBLISHING

Tumor Discovery

ORIGINAL RESEARCH ARTICLE

Clinicohematological profile and immunophenotypic patterns of childhood acute leukemia: Prognostic correlation

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Abstract

Acute leukemia (AL) presents a heterogeneous molecular profile, requiring precise diagnostic categorization and subcategorization. The present study aims to estimate the clinicohematological profile and immunophenotypic pattern of childhood AL while conducting prognostic assessments. This cross-sectional study analyzed a total of 68 samples of AL collected from January 2019 to June 2021. The male-to-female ratio was 4.6:1, with a mean age of 6.6 ± 3.4 years. Total leukocyte count (TLC) was significantly increased in all types of AL (P = 0.03). The median value (interquartile range) of TLC ($\times 10^6$/dL) was 8,450 (4,100 – 27,950), with blast counts in peripheral smears at 59 (24 – 80), and in bone marrow aspirates (BMAs) at 95 (75 – 98). There was a significant association ($P < 0.001$) and a strong association (C = 0.9110) between the morphology of BMA with immunophenotype. Based on immunophenotype, AL was categorized into four groups: B-cell acute lymphoblastic leukemia (B-ALL) (51.5%), T-cell acute lymphoblastic leukemia (T-ALL) (10.3%), AML (22%), and mixed phenotype AL (MPAL) (16.2%). Furthermore, eight subgroups were identified: B lineage, Ia-Common-B-ALL (88.6%) and Ib-Pre-B-ALL (11.4%); T-lineage, IIa-Cortical T-ALL (71.4%) and IIb-Pre-T-ALL (28.6%); AML subgroups, IIIa-M2 (73.93%) and III-M4 (26.7%); and MPAL subgroups, IVa-aberrant expression of myeloid antigens in B-ALL (90.9%), and IVb-aberrant expression of lymphoid markers in AML (9.1%). A poor prognostic immunophenotype (T-ALL, AML) was significantly (P = 0.023) more prevalent in deceased patients with AL. The highest mortality rate was observed in AML (86.4%), followed by T-ALL (57.2%). The most common immunophenotype observed was Common-B-ALL in childhood AL, and a poor prognostic immunophenotype (T-ALL and AML) with the highest mortality rate was found in AML. Thus, knowledge about clinicohematological and immunophenotypic patterns will aid in patient management.

Keywords: Immunophenotype; Aberrant; Acute leukemia; Flow cytometry; Children

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1. Introduction

Childhood acute leukemia (AL), specifically acute lymphoblastic leukemia (ALL), is the most common malignancy observed in children.[1] Various classifications of AL have been introduced over time by literature and the World Health Organization

(WHO).[2] Hematopoietic neoplasm, or AL, exhibits a heterogeneous molecular profile, requiring a more precise diagnostic categorization and subcategorization.[2] Recent classifications of AL are more prognostic-oriented compared to older classification systems, as they are based on immunophenotype, cytogenetics, and molecular typing.[3] Proper classification is instrumental in enhancing patient management.[4]

Immunophenotype-based classifications are more lineage-specific (B-cell ALL [B-ALL], T-cell ALL [T-ALL], and acute myeloid leukemia [AML]) and carry prognostic significance. Therefore, this study has been conducted in specific regions of India. Recently, AL has been confirmed with immunophenotyping using flow cytometry. Occasionally, the asynchronous expression of antigens or antibodies on the cell surface is referred to as an aberrant phenotype, such as the expression of T-cell lineage or B-cell lineage markers in AML or the expression of myeloid lineage markers in T/B ALL.[4] Certain cases cannot be classified as ALL and AML based solely on morphology, cytochemistry, and immunohistochemistry.[5] This phenomenon is due to the co-expression of lymphoid and myeloid immunophenotype markers on the cell surface, or the presence of two distinct cell populations.[6] These cases are diagnosed with the availability of flow cytometry and are labeled as biphenotypic, hybrid, and mixed leukemia.[7]

The aim and objective of the present study are as follows: (i) to determine the immunophenotypic pattern of childhood AL in Delhi-National Capital Region (NCR), (ii) to provide the clinical and hematological profile of the immunophenotype of AL in children, and (iii) to correlate with prognostic outcomes.

2. Methods

2.1. Study design and data acquisition

This retrospective cross-sectional study was performed at a tertiary care institute in Delhi, India. Informed consent was not applicable as the study was retrospective and involved no direct contact with patients. Data were retrieved from the departmental archive.

2.2. Bone marrow aspirate (BMA) and trephine biopsy analysis

Clinical data, BMA, and trephine biopsy slides of pediatric patients diagnosed with AL through bone marrow aspiration, biopsy, and flow cytometry between January 2019 and June 2021 were analyzed. A total of 209 samples of BMA and biopsies were collected from 95 pediatric patients with AL. Of these, 141 remission samples were excluded, and a total of 68 patient samples were analyzed for clinical profile, hematological profile, and immunophenotype. Children with inadequate/suboptimal BMAs and unavailable flow cytometry findings were excluded from the study. All children confirmed with AL through flow cytometry were included in the study.

2.3. Immunophenotyping

Immunotyping through flow cytometry was conducted using the Cytomics FC 500 (Beckman Coulter, USA). Peripheral blood and BMA samples were collected fresh in EDTA (ethylenediaminetetraacetic acid-anticoagulant) vials. The samples were analyzed within 24 h of collection. For most of the cases, a pre-fixed panel of antibodies (CD34, HLA-DR, CD45, CD19, CD20, CD79a, CD10, TdT, CD3, c-CD3, CD4, CD2, CD7, CD8, CD23, CD103, CD38, CD200, CD117, CD13, CD33, MPO, CD64, CD11b, and CD15) was used in conjunction with fluorescein isothiocyanate (FITC [FL1), phycoerythrin (PE [FL2), ECD (FL3), and PC5 (FL5) dyes. The samples were processed as per standard protocols for surface and cytoplasmic antibodies. Results were obtained by gating the blast cells with side scatter (SSC) versus forward scatter followed by SSC versus CD45 gating.

AL, based on immunophenotype, was initially divided into four groups and subsequently into subgroups. Group 1 comprised B-ALL expressing CD19, c-CD79a, and c-CD22, along with variable CD34/HLA-DR, CD10, CD24, and PAX5. Group 2 consisted of T-ALL expressing TdT and variably expressing c-CD3, CD3, CD2, CD5, and CD7. Group 3 included AML, predominantly expressing MPO and variably expressing CD117, CD33, CD13, CD64, and CD15. Group 4 covered mixed phenotypic AL/aberrant immunophenotypic AL antigens. All four groups were correlated with their clinical details, hematological profiles, and prognostic outcomes.

The aberrant immunophenotype was defined as the expression of surface antigen on a leukemic cell that differs from the normal maturation process of the cell lineage.[4] While recent classifications of AL are based on molecular typing, the immunophenotype-based French–America–British (FAB) classification remains robust for patient management. It was feasible for routine practice and cost-effective. The FAB classification includes eight subtypes of AML (M0 – M7) and three subtypes of ALL (L1 – L3) (Table 1).[8]

The 2016 WHO revised classification of leukemia is based on immunophenotypes and molecular characteristics (Table 2).[9]

In addition, demographic profiles, clinical details, hemograms, and flow cytometry analyses of the patients were noted.

Table 1: The French–America–British classification of acute leukemia[8]

AML	ALL
M0 – AML with no Romanowsky or cytochemical evidence of differentiation	L1
M1 – Myeloblastic leukemia with little maturation	L2
M2 – Myeloblastic leukemia with maturation	L3
M3 – APL	
M3h – APL, hypergranular variant	
M3v – APL, microgranular variant	
M4 – AMML	
M4eo – AMML with dysplastic marrow eosinophils	
M5 – AMoL	
M5a – AMoL, poorly differentiated	
M5b – AMoL, differentiated	
M6 – "Erythroleukemia"	
M6a – AML with erythroid dysplasia	
M6b – Erythroleukemia	
M7 – Acute megakaryoblastic leukemia (AMkL)	

Abbreviations: AML: Acute myeloid leukemia; APL: Acute promyelocytic leukemia; AMML: Acute myelomonocytic leukemia; AMol: Acute monoblastic leukemia; ALL: Acute lymphoblastic leukemia.

2.4. Statistical analysis

Continuous data were reported as mean ± standard deviation (*SD*) for normally distributed variables and as median with an interquartile range (IQR) for skewed variables. Categorical data were reported as percentages. $P < 0.05$ was considered statistically significant. We used STATA 14 software for statistical analysis. All continuous variables were assessed for normal or skewed distribution (*SD* > 40% of mean). For variables with a skewed distribution, the median (IQR) was reported. Comparisons of more than two unpaired groups were performed using the Kruskal–Wallis test, with Dunn's test applied if the Kruskal–Wallis test was significant. All categorical variables (>2 unpaired groups) were assessed using the Pearson Chi-square test to assess significant variability. The contingency coefficient (*C*) was calculated to measure the strength of the relationship between variables, with values ranging from 0 (no association) to 1 (very strong association), where 0 means no association and 1 is a very strong association, which shows the strength of the relationship between variables. SATA 14 software was used for data analysis.

3. Results

Data from a total of 68 patients obtained during the study period were analyzed for clinical profile, hematological

Table 2: 2016 WHO classification of acute leukemia[9]

S. No.	Types of leukemias
1.	AML and related neoplasms
	AML with recurrent genetic abnormalities
	AML with t (8;21) (q22;q22.1); *RUNX1-RUNX1T1*
	AML with in v (16) (p13.1q22) or t (16;16) (p13.1;q22); *CBFB-MYH11*
	APL with *PML-RARA*
	AML with t (9;11) (p21.3;q23.3); *MLLT3-KMT2A*
	AML with t (6;9) (p23;q34.1); *DEK-NUP214*
	AML with inv (3) (q21.3q26.2) or t (3;3) (q21.3;q26.2); *GATA2, MECOM*
	AML (megakaryoblastic) with t (1;22) (p13.3;q13.3); *RBM15-MKL1*
	Provisional entity: AML with BCR-ABL1
	AML with mutated *NPM1*
	AML with biallelic mutations of *CEBPA*
	Provisional entity: AML with mutated RUNX1
	AML with myelodysplasia-related changes
	Therapy-related myeloid neoplasms
	AML NOS
	AML with minimal differentiation
	AML without maturation
	AML with maturation
	Acute myelomonocytic leukemia
	Acute monoblastic/monocytic leukemia
	Pure erythroid leukemia
	Acute megakaryoblastic leukemia
	Acute basophilic leukemia
	Acute panmyelosis with myelofibrosis
	Myeloid sarcoma
	Myeloid proliferations related to Down syndrome
	TAM
	Myeloid leukemia associated with Down syndrome
	Blastic plasmacytoid dendritic cell neoplasm
	Acute leukemias of ambiguous lineage
	Acute undifferentiated leukemia
	MPAL with t (9;22)(q34.1;q11.2); *BCR-ABL1*
	MPAL with t (v; 11q23.3); *KMT2A* rearranged
	MPAL, B/myeloid, NOS
	MPAL, T/myeloid, NOS
2.	B-lymphoblastic leukemia/lymphoma
	B-lymphoblastic leukemia/lymphoma, NOS B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
	B-lymphoblastic leukemia/lymphoma with t (9;22) (q34.1;q11.2); *BCR-ABL1*

(Cont'd...)

Table 2: ***(Continued)***

S. No.	Types of leukemias
	B-lymphoblastic leukemia/lymphoma with t (v; 11q23.3); *KMT2A* rearranged
	B-lymphoblastic leukemia/lymphoma with t (12;21) (p13.2;q22.1); *ETV6-RUNX1*
	B-lymphoblastic leukemia/lymphoma with hyperdiploidy
	B-lymphoblastic leukemia/lymphoma with hypodiploidy
	B-lymphoblastic leukemia/lymphoma with t (5;14) (q31.1;q32.3) *IL3-IGH*
	B-lymphoblastic leukemia/lymphoma with t (1;19) (q23;p13.3); *TCF3-PBX1*
	Provisional entity: B-lymphoblastic leukemia/lymphoma, BCR-ABL1-like
	Provisional entity: B-lymphoblastic leukemia/lymphoma with iAMP21
3.	T-lymphoblastic leukemia/lymphoma
	Provisional entity: Early T-cell precursor lymphoblastic leukemia
	Provisional entity: Natural killer cell lymphoblastic leukemia/lymphoma

Abbreviations: WHO: World Health Organization; AML: Acute myeloid leukemia; APL: Acute promyelocytic leukemia; NOS: Not otherwise specified; TAM: Transient abnormal myelopoiesis; MPAL: Mixed phenotype acute leukemia.

profile, and immunophenotype. The male-to-female patient ratio for AL was 4.6:1. The mean ± *SD* age of affected children was 6.2 ± 2.9 years. Most commonly, patients were referred to the laboratory for a workup of suspected AL (45.6%). The rest of the patients were incidentally diagnosed during the workup with pancytopenia or pyrexia of unknown origin. The clinical presentations of patients ($n = 68$) included anemia (66%), fatigue/weakness (63%), loss of appetite (60%), loss of weight (58%), fever (52%), failure to thrive (42%), hepatosplenomegaly (36%), bone pain (30%), lymphadenopathy (25%), and bleeding from gums/skin rashes (23%). The hematological profiles of these patients revealed mean ± *SD* values of red blood cell count of 2.6 ± 0.9 × 10^9/μL and hemoglobin (Hb) of 7.6 ± 2.3 g/dL. The median value of total leukocyte count (TLC × 10^3/μL) was 8,450 (4100 – 27,950), hematocrit (L/L) 0.22 (0.19 – 0.29), blast in peripheral smear 59 (24 – 80), blasts in BMA 95 (75 – 98), and platelets 31,000 (18,500 – 60,000).

Based on immunophenotype, the frequencies of the various groups of AL were as follows: B-ALL (51.5%; 35/68), T-ALL (10.3%; 07/68), AML (22%; 15/68), and mixed phenotype AL (MPAL) (16.2%; 11/68). These four groups were compared with clinical features and hematological profiles. The median (IQR) age (years) for different AL groups were as follows: B-ALL (5 [4 – 9]), T-ALL (8 [6 – 12]), AML (5 [4.5 – 6]), and MPAL (8 [5 – 10]); the difference was not statistically significant.

Table 3 describes clinical features in different groups of AL. Several clinical definitions in children include:

(i) Hepatomegaly: Defined as liver edge papable 2 – 3.5 cm below the right costal margins in children and newborns
(ii) Splenomegaly: Defined as a palpable splenic edge >2 cm below the left costal margins
(iii) Lymphadenopathy: Defined as any palpable lymph nodes >1 cm in diameter.

No statistically significant difference was observed between the different groups of AL for clinical features. On the other hand, Table 4 illustrates the hematological profile in different groups of AL.

TLC exhibited a significant difference among the different groups of AL. After applying the Dunn test, t*P*-value of TLC showed significant differences ($P = 0.003$) between T-ALL and B-ALL, AML versus T-ALL ($P = 0.038$), and MPAL versus B-ALL ($P = 0.041$).

Furthermore, peripheral blood smear (PS), BMA, and trephine biopsy diagnoses were correlated with four groups of AL determined through flow cytometry. These samples (PS, BMA, and biopsy) diagnosed 60.3% of cases as AL, classified as B-ALL (26 cases), T-ALL (5 cases), AML (1 case), and MPAL (9 cases). In addition, 22.1% of cases were diagnosed as AML, further identified as AML (14 cases) and MPAL (1 case), and 17.6% of cases were diagnosed as ALL, further identified as B-ALL (9 cases), T-ALL (2 cases), and MPAL (1 case). The overall concordance was significant ($P = 0.0001$) between BMA (morphology) and flow cytometry with χ^2 (6) = 58.79, and the contingency coefficient ($C = 0.9110$) indicated a strong association.

The prognostic outcomes (dead and surviving) for the four groups of immunophenotypes were compared in Table 5.

T-ALL (57.2%) and AML (86.7%) were significantly higher in the deceased patient group in comparison to the surviving patient group ($P = 0.023$).

Based on immunophenotype, the highest mortality rate was significantly higher in AML patients, followed by T-ALL and MPAL. Patients with B-ALL (Common-B-ALL) demonstrated the most favorable prognosis among all immunophenotypic groups.

The immunophenotypic groups were, further, subdivided into eight subgroups based on the expression of antigens. The frequency of immunophenotypes of AL in children in Delhi-NCR is shown in Table 6.

Table 3: Clinical manifestations in different groups of AL patients based on immunophenotype between January 2019 and June 2021

Clinical features	B-ALL (%)	T-ALL (%)	AML (%)	MPAL (%)	*p*-value
Fever	80	100	53.3	81.8	0.071
Bone pain	42.9	71.3	40	36.4	0.473
Weight loss	85.7	100	80	81.8	0.647
Loss of appetite	85.7	100	86.6	90.9	0.739
Fatigue/weakness	94.3	100	86.7	90.9	0.675
Paleness/anemia	97.1	100	100	90.9	0.547
Hepatomegaly	57.5	71.4	33.3	54.5	0.316
Splenomegaly	51.4	85.7	33.3	54.5	0.151
Lymphadenopathy	37.1	57.1	13.3	54.5	0.098

Abbreviations: AL: Acute leukemia; ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; B-ALL: B-cell acute lymphoblastic leukemia; MPAL: Mixed phenotypic acute leukemia; T-ALL: T-cell acute lymphoblastic leukemia.

Table 4: Hematological profiles in different groups of AL patients based on immunophenotype between January 2019 and June 2021

Laboratory parameters	B-ALL	T-ALL	AML	MPAL	*P*-value
TLC (10^3/μL)	5,100 (3,300 – 19,400)	62,000 (9,140 – 94,100)	8,500 (4,200 – 27,500)	17,200 (7,700 – 30,000)	0.031*
RBC (10^9/μL)	2.7 (2.2 – 3.1)	2.7 (2.5 – 3.0)	2.4 (2.2 – 3.1)	2.4 (1.9 – 3.4)	0.725
Hb (g/dL)	7.5 (6.5 – 9.3	7.2 (6.2 – 8.8)	7.2 (6.4 – 9.4)	6.8 (5.8 – 9.2)	0.971
Hematocrit (L/L)	0.22 (0.19 – 0.28)	0.22 (0.21 – 0.29)	0.24 (0.2 – 0.28)	0.22 (0.18 – 0.32)	0.868
Platelets (10^6/μL)	31,000 (20 000 – 60,000)	27,000 (17,000 – 68,000)	38,000 (12,000 – 66,000)	31,000 (16,000 – 49,000)	0.876
PS blast	52 (21 – 80)	82 (37 – 97)	59 (28 – 76)	60 (38 – 80)	0.362
BMA blast	95 (90 – 98)	98 (98 – 98)	93.5 (70 – 98)	72.5 (0 – 98)	0.282

Note: *P<0.05.
Abbreviations: AL: Acute leukemia; ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; B-ALL: B-cell acute lymphoblastic leukemia; BMA: Bone marrow aspirate; Hb: Hemoglobin; MPAL: Mixed phenotypic acute leukemia; PS: Peripheral blood smears; T-ALL: T-cell acute lymphoblastic leukemia; TLC: Total leukocyte count; TRBC: Total red blood cell count.

Table 5: An immunophenotypic pattern of AL in deceased and surviving patient groups

Immunophenotypic groups	Deceased patient group (%)	Surviving patient group (%)	*P*-value
B-ALL (n=35)	40	60	0.023*
T-ALL (n=7)	57.2	42.8	
AML (n=15)	86.7	13.3	
MPAL (n=11)	45.5	54.5	

Note: *P<0.05.
Abbreviations: AL: Acute leukemia; ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; B-ALL: B-cell acute lymphoblastic leukemia; MPAL: Mixed phenotypic acute leukemia; T-ALL: T-cell acute lymphoblastic leukemia.

In Group I, B-ALL subgroups were Common-B-ALL in 88.6% and Pre-B-ALL in 11.4%. In Group 2, T-ALL subgroups were Cortical-T-ALL in 71.4% and Pre-T-ALL in 28.6%. In Group 3, AML was predominantly of the M2 subtype in 73.3% and AML-M4 in 26.7%. Group 4 comprised MPAL, with 90.9% showing aberrant expression of myeloid markers with B-ALL and 9.1% showing aberrant expression of lymphoid markers with AML. These eight subgroups of immunophenotypes were also compared with clinical features and hematological profiles, but no significant differences were found.

4. Discussion

Immunophenotypic patterns of childhood AL have been extensively reported in the literature.[10,11] In Indonesia, for instance, 62.8% of cases were classified as ALL (83% of ALL being B-ALL and 17% T-ALL), while 23% were classified as AML, and 7.9% were of unknown origin, with only 0.2% biphenotypic pediatric patients.[10] In this study, we found that T-ALL (57.2%) and AML (86.7%) were significantly higher (P = 0.023) in the deceased patient group compared to the surviving patient group, whereas B-ALL and MPAL were significantly higher in the surviving patient groups. A study from North India reported 81.0% ALL, 15.8% AML, and 3.2% MPAL in pediatric patients.[12] Conversely, a study from South India reported an excess of T-ALL and a paucity of common ALL in children over the past

Table 6: The frequency of immunophenotypes in groups and subgroups of acute leukemia of children in Delhi-NCR, India

S. No.	Groups	Subgroups	Immunophenotype	Type of leukemia	Frequency % (*n*)
1.	B-ALL	Ia	CD34, HLA-DR, CD19, CD20, CD79a, CD10	Common-B-ALL	88.6 (31/35)
2.		Ib	CD19, CD20, CD79a, HLA-DR, CD34	Pre-BALL	11.4 (4/35)
3.	T-ALL	IIa	TdT, c-CD3, CD3, CD2, CD1a, CD5, CD7, CD4, CD10, CD8	Cortical- T-ALL	71.4 (5/7)
4.		IIb	c-CD3, CD2, CD3, CD5, CD7, *TdT	Pre-T-ALL	28.6 (2/7)
5.	AML	IIIa	CD34, HLA-DR, CD13, CD33, MPO, CD117	AML-M2	73.3 (11/15)
6.		IIIb	CD13, CD33, C-MPO, CD117, CD15, CD64, CD11b	AML-M4	26.7 (4/15)
7.	MPAL	Iva	CD34, CD19, CD10, CD79a, HLADR, CD20, CD13, CD33	My+BALL	90.9 (10/11)
8.		IVb	Strong+ (CD33, CD64, cMPO); Weak+ (TdT, CD79a, CD10, CD3)	Ly+AML	9.1 (1/11)

Notes: ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; B-ALL: B-cell acute lymphoblastic leukemia; c: cytoplasmic; Ly+AML: Aberrant expression of lymphoid markers with AML; MPAL: Mixed phenotypic acute leukemia; My+BALL: Aberrant expression of myeloid markers with B-ALL; T-ALL: T-cell acute lymphoblastic leukemia; *TdT (not done); +: Positivity; NCR: National Capital Region.

20 years.[13] These differences might be related to different geographical factors and ethnicities.

Our current study presents an overall epidemiological pattern of immunophenotype observed in Delhi-NCR, the capital region of India, where people from across the country reside. We observed the following overall frequencies of AL: B-ALL (51.5%), T-ALL (10.3%), AML (22%), and MPAL (16.2%). While the overall immunophenotypic pattern is similar to that reported in the literature from around the world and studies from North India, the lower numbers observed in our study might be related to sample size. Notably, this pattern differs from South India, where T-ALL is predominantly observed.

There is no significant difference between all leukemic groups (B-ALL, T-ALL, AML, and MPAL) for age in our study, corroborating the findings from the study by Sharma *et al.*[12]

The most common clinical manifestations of AL patients were anemia, weight loss, loss of appetite, and fever, which were also corroborated by other studies.[14,15] Sharma *et al.*[12] reported physical findings in AL patients: hepatomegaly in 69% of the cases, splenomegaly in 56.5%, and lymphadenopathy in 62.3%. Sharma *et al.*[14] found significant differences between ALL and AML for fever and between MPAL and AML for lymphadenopathy. However, we did not find significant differences in clinical features between different groups of AL, potentially attributed to the small sample size.

The hematology profile of AL evaluated in our study is comparable to other studies.[13-15] We found a significant difference ($P = 0.031$) for TLC between various groups of leukemia. After applying the Dunn test, *P*-value of TLC exhibited a significant difference between T-ALL and B-ALL, between AML and T-ALL, and between MPAL and B-ALL. However, a few studies did not find a significant difference in Hb and TLC between different groups of AL (MPAL vs. ALL/AML).[12,14] Sharma *et al.*[12] noted a significant difference in mean platelets count between MPAL and ALL/AML. Apart from TLC, we did not observe a significant difference in the hematological profile among different AL groups. This variation is represented by the diversity in hematological profiles across different patients.

Supriyadi *et al.*[10] found a very good concordance ($\kappa = 0.82$) between morphology and immunophenotype using a three-color method with a panel of 15 monoclonal antibodies ($n = 387$). Similarly, we also observed a significant association ($P = 0.0001$) between BMA morphology and flow cytometry with $\chi 2$ value of (6) = 58.79 and a C (0.9110) indicating a strong strength of association between both methodologies.

Few studies from Northern India have reported the immunophenotype in 85% of patients as Blineage ALL (PproB- ALL 8%, ccommonB -ALL 74%, and PpreB -ALL 18%), and in 15% of patients as Tlineage ALL (PproT -ALL 29%, PpreT -ALL 11%, cortical- T -ALL 44%, and MmatureT -ALL 16%).[16] The immunophenotypic pattern is similar to the results of our study results (Table 4), but the overall proportion is low in the present study, which might be related attributed to the small sample size.

Rajalekshmy *et al.*[17] from Chennai, India, reported the immunophenotypic pattern of ALL from Chennai, India, indicating T-ALL in 53.6%, B-ALL in 46.4% (precursor B 6.4%, pre-B 5.6%, Common-B-ALL 20.8%,and B- 04%) and unclassified in 5.6%.[17] This study reported a high incidence of T-ALL high in children, which is notably very unusual, and may be it related to different immunophenotypic patterns in across different geographic and ethnic groups. Gupta *et al.* reported immunophenotypic patterns from Kolkata, demonstrating 81.7% B-ALL (Common B-ALL 95.2% and Pro-B-ALL 4.8%), T-ALL comprising

18.3% (Cortical-T -ALL 27.9%, Pro-T- ALL 8.2%, early thymic -T- ALL 9.8%, and medullary-T- ALL 24.6%), AML comprising 32.1% with recurrent cytogenetic abnormalities (11.9% t [8;21], 12.3% t [15;17] that is acute promylocytic leukemia, 2.9% with inversion 16/t [16;16], 3.9% MLL gene rearrangement, and 1.1% with 3q abnormalities), and 2.3% MPAL.[18]

Different studies from North India have reported. The aberrant phenotypes with myeloid antigens at 42.5% and 11% were reported by in ALL cases different studies from North India in ALL cases.[14,16] These findings were different from our study results; we found an overall aberrant antigen expression of 16.2%, and with myeloid antigen expressed most predominantly in 90.8% in of B-ALL (My+B-ALL, 10/11 cases). We found one case (9.1%) expressing aberrant lymphoid antigen in AML (Ly+AML). Other studies have found, which reported the most common aberrant expressing antigen CD13 as the most commonly aberrantly expressed antigen (at 32.2% and 25.6%, respectively).[14,18] Whereas in our study, we found observed mostly a predominance of 14.7% of aberrant expression of myeloid antigens CD13 (70%), CD33 (50%), and both (20% common) aberrant expressing the myeloid antigen. Sharma *et al*.[14] found 2.99% MPAL, out of them, which only seven pediatric patients showed aberrant lymphoid antigen expression in AML.[14] The overall configuration of the immunophenotypic pattern of childhood AL has changed from the maximum proportion that majority of AL cases were being B-ALL to a significant portion of non-B-ALL (T-ALL, AML, and MPAL) (constituting approximately 40 – 45%) of cases in the worldwide and in India. In our study, we found that 48% of AL cases were non-B -ALL proportions of AL, which affects the prognosis of AL.

The present study evaluated the prognostic significance of different immunophenotypes of in AL. We found the maximum highest mortality rate of 86.7% in AML, followed by 57.2% in T-ALL 57.2% and 45.5% in MPAL. B-ALL (subtype-: Common -B -ALL) showed the best most favorable prognosis (with a minimum mortality rate of 40%). Similarly, the world literature worldwide reported a favourable prognosis associated with B-ALL and a poor prognosis related to AML and T-ALL.[19] A study by Santos *et al*.[20] provided the relavance of immunophenotypic markers as independent prognostic factors that could be included integrated into clinical protocols, for risk stratification and therapeutic guidance.[20]

The limitations of our study were included: (i) the sample size was a small, (ii) The lack of further confirmation by molecular techniques and (iii) we were unable to correlate with cytogenetic findings of AL.

5. Conclusion

The immunophenotypic pattern in Delhi-NCR shows B-ALL (51.5%) as the most prevalent, followed by AML (22%), MPAL (16.3%), and T-ALL (10.2%). Based on immunophenotype, the most common childhood AL is B-ALL (Common-B-ALL), in contrast to South India, where T-ALL predominates. However, the mortality rate is highest in AML in comparison to other AL subtypes. Among B-ALL cases, Common-B-ALL demonstrates the most favorable prognosis, consistent with findings in global literature.

The different immunophenotypic patterns observed between Delhi-DCR and South India suggest the presence of underlying factors, warranting further investigation. Additional studies are required to elucidate the reasons behind these differences. Enhanced understanding of immunophenotypic patterns and their prognostic value within specific geographical areas will not only facilitate improved patient management but also aid in the formulation of health-care policies.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization: Anju Khairwa
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Ethics approval and consent to participate

The University College of Medical Sciences ethics committee approved the study protocol with the number (IECHR-2021-51-15R1). The study was conducted in accordance with the ethical guidelines of the college. This retrospective cross-sectional study was performed at a tertiary care institute in Delhi, India. Informed consent was not applicable as the study was retrospective, and there was no direct contact with patients. Data were retrieved from the departmental archive.

Consent for publication

This retrospective cross-sectional study was performed at

a tertiary care institute in Delhi, India. Informed consent for publication was not applicable as the study was retrospective, and there was no direct contact with patients. Data were retrieved from the departmental archive.

Availability of data

Data used in this work are available from the corresponding author on reasonable request.

Further disclosure

The findings have been presented in a conference DAPCON July 31, 2022, at AIIMS, New Delhi, India as a poster.

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ACCSCIENCE PUBLISHING

Tumor Discovery

CASE REPORT

Malignant proliferating trichilemmal tumor post-chemotherapy: A case report

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Abstract

The malignant proliferating trichilemmal tumor (MPTT) is an exceptional adnexal neoplasm of the skin, primarily observed in the scalp of elderly women, often precipitated by local trauma or chronic inflammation. Notably, MPTT exhibits a propensity for local aggressiveness, frequently leading to diagnostic challenges reminiscent of squamous cell carcinoma. The primary treatment modality entails surgical excision with adequate margins, yielding a relatively low local recurrence rate. Herein, we present the case of a 50-year-old woman diagnosed with MPTT of the scalp several years subsequent to undergoing chemotherapy for breast cancer, initially misdiagnosed as squamous cell carcinoma upon biopsy. This case marks only the third reported instance associating MPTT with chemotherapy following breast cancer treatment. We aim to draw attention to this particularly aggressive tumor and its potential origination from benign trichilemmal cysts due to chemotherapy.

Keywords: Malignant proliferating trichilemmal tumor; Trichilemmal cyst; Breast cancer; Chemotherapy

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1. Background

Proliferating trichilemmal tumors (PTTs) are a rare entity of adnexal tumors arising from the isthmic portion of the outer root sheet of the hair follicle.[1,2] They represent only 0.1% of skin tumors.[1,3] Malignant proliferating trichilemmal tumors (MPTTs), on the other hand, are even rarer. Their exact incidence is undetermined due to the rarity of reported cases in the literature, the absence of case series, and the lack of clinical and histological features differentiating them from squamous cell carcinoma. MPTTs appear to occur mostly in the scalp region of elderly women. They are locally aggressive tumors associated with recurrence and even nodal or distant metastatic spread. Herein, we report a case of an MPTT of the scalp in a female patient. It marks only the third reported case of MPTT associated with a history of breast cancer treated by chemotherapy.[4,5] Through this case report, we aim to attract the attention of specialists to investigate a potential causal link between chemotherapy and MPTT.

2. Case presentation

A 50-year-old woman was referred to the Maxillofacial Department of our hospital by her oncologist due to a scalp mass. The patient reported that the lesion had been present

for 20 years but started growing rapidly over the past 10 months, becoming painful, itchy, and spontaneously bleeding. Thirteen years ago, the patient was diagnosed with breast carcinoma, for which she underwent radical mastectomy with lymph node dissection, followed by radiotherapy, chemotherapy, and hormonotherapy. Unfortunately, access to the patient's medical records was not possible. The patient also reported a family history of multiple pilar cysts and disclosed having four of them removed 3 years ago. There was no history of trauma or chronic irritation. During the clinical examination, a firm, nodular mass was identified on the parietal region of the scalp, measuring 4 × 3 cm, with irregular borders, a hairless and ulcerated surface, and telangiectasia (Figure 1). In addition, seven subcutaneous cysts were discovered in the different areas of the scalp, ranging in size between 0.5 cm and 2 cm (Figure 2). Notably, no palpable neck lymph nodes were detected.

A biopsy of the mass revealed histological features compatible with squamous cell carcinoma. Subsequent craniofacial and cervical-thoracic computed tomography scans revealed no evidence of bone invasion, lymph node involvement, or distant metastasis. Following these findings, the patient underwent surgical excision of the parietal lesion with macroscopical margins of 1 cm, along with the removal of the largest cyst. The final histological examination depicted a malignant tumor proliferation with lobulated architecture, coupled with focal stromal invasion demonstrating increasing nuclear and cytoplasmic pleomorphism, accompanied by numerous and often atypical mitotic figures (Figure 3A, B and C). In addition, lymphovascular invasion was observed. The definitive diagnosis rendered was a parietal MPTT associated with multiple trichilemmal cysts. Remarkably, no recurrence or metastasis was detected during the 18-month follow-up period.

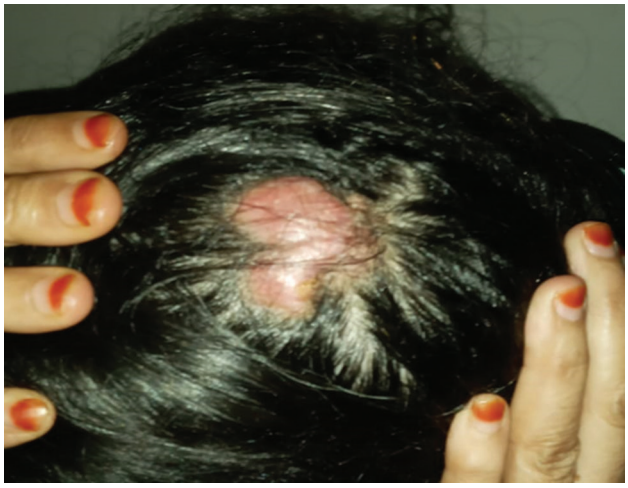

Figure 1. Nodular mass of the parietal scalp with irregular ulcerated borders, hairless surface, and telangiectasia.

3. Discussion

The first case of trichilemmal cysts was reported by Wilson Jones in 1966. Trichilemmal cysts occur in 5% to 10% of the population, with barely 2% progressing into PTTs.[3,6] The term MPTT was first proposed by Headington[7] in 1976 and later adopted by Saida *et al.*[8] in 1983 to define a PTT exhibiting malignant features such as infiltrative growth pattern, cytological atypia, atypical mitosis, and lymph node metastasis. Despite being rare, MPTTs predominantly arise in the scalp region (90%), with 84% of cases reported in mainly women aged between 40 and 80 years.[9,10] Other implicated locations include the face, breast, back, chest, abdomen, buttocks, pubis, vulva, back of hand, and wrist.[9,11]

MPTTs are usually characterized as slowly enlarging, firm, painless nodular lesions ranging in size from 1 to 30 cm, often with unremarkable cutaneous covering. These nodules persist for a significant duration before undergoing abrupt and rapid tumor evolution, marked by pain, ulceration, bleeding, or purulent discharge, indicative of malignant metamorphosis.[1,9,10,12-14] Cases of regional lymph node metastasis have also been reported.[8,13-15]

Although malignant degeneration is rare, MPTTs commonly arise from preexisting trichilemmal cysts. Alternatively, several authors have reported cases of MPTTs originating *de novo*, from organoid nevi[16] or seborrheic keratosis.[9] Many researchers have suggested a hypothetical malignant transformation pathway between trichilemmal cysts and MPTTs progressing from the adenomatous stage of trichilemmal cysts to the epitheliomatous stage of PTTs and eventually to the carcinomatous stage of MPTTs.[3,4,9,10,12,17] As in our case, the commonly found accompaniment of MPTTs with simple trichilemmal cysts may support this theory, as does the coexistence of benign and malignant areas within the same neoplasm.[9] Trauma and chronic inflammation are the most incriminated factors in this degeneration. Only two cases of MPTT associated with chemotherapy have been reported in the literature;[4,5] further research and reports are necessary to establish a solid causality link between each of these factors and MPTTs.

MPTTs may be easily confused with squamous cell carcinoma since they share many clinical similitudes and are both rare cases. Similarly, MPTTs must be distinguished from PTTs or trichilemmal carcinoma because they affect prognosis and treatment approach. Histologically, MPTT is described as a tumor that invades neighboring tissues accompanied by anaplasia,

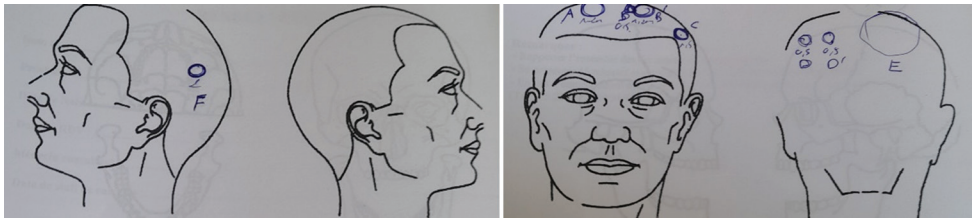

Figure 2. Mapping of the patient's nodular scalp lesions.

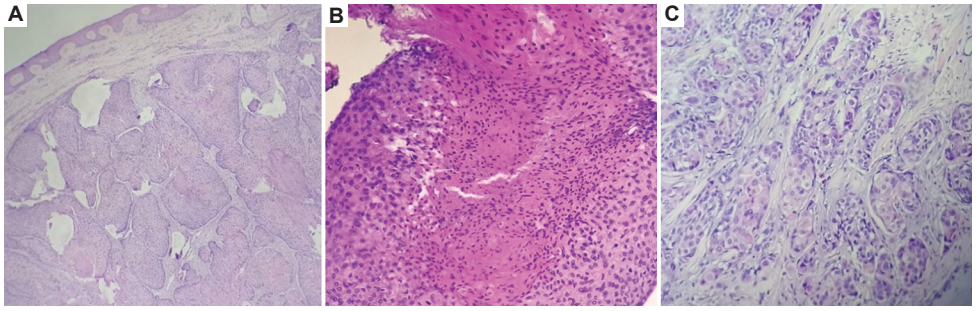

Figure 3. Malignant proliferating trichilemmal tumor. (A) A mid-dermal lobulated proliferation with (B) trichilemmal keratinization, (C) marked cytonuclear atypia and numerous mitosis.

aneuploidy, necrosis, and high mitotic activity, especially atypical mitosis. In addition, vascular and neural invasion may also be observed.[2,5,7,18] These histological features, as well as the positivity of proliferation markers such as Ki67 and p53, and the presence of lymph node or distant metastasis, are essential in distinguishing MPTT from PTT.[1,2,4,19] Trichilemmal keratinization is also an important indicator in differentiating PTTs from squamous cell carcinomas;[2] it is characterized by abrupt, compact, amorphous keratinization of epithelial cells enveloping the cyst wall in the absence of a granular cell layer.[1,2,4,9,13] Immunohistochemical studies are also valuable in distinguishing MPTT from squamous cell carcinoma. CD34, an important immune determinant indicating trichilemmal differentiation, is known to be expressed in MPTTs but not in squamous cell carcinomas.[1,2,4,19] Nevertheless, negative staining with CD34 has been detected in several case reports of MPTTs.[2,4,17] Based on clinicopathological findings, Ye *et al.*[20] classified MPTTs into three groups: Group I comprises benign lesions with regular histological contours, modest nuclear atypia, and absence of pathologic mitoses, necrosis, or nerve/vessel invasion, with no reported recurrence. Group II encompasses low-grade malignant tumors with irregular histological contours and local invasion into the deep dermis and subcutis, with potential for local recurrence. Group III consists of high-grade malignant tumors exhibiting invasive growth patterns, remarkable nuclear atypia, atypical mitoses, geographic necrosis, and involvement of nerves or vascular structures, with a high recurrence rate, lymph node involvement, and a tendency for distant metastasis. Our case aligns with the classification of high-grade MPTT. Additional differential diagnoses of MPTT include dermoid cyst, basal cell carcinoma, keratoacanthoma, invasive Bowen's disease, trichoblastoma, cylindroma, spiradenoma, sebaceous carcinoma, clear cell hidradenocarcinoma, and pilomatrix carcinoma.[3,11,14,16]

MPTTs are characterized by aggressiveness, with estimated rates of local recurrence and lymph node metastasis ranging from 3.7 to 6.6% and 1.2 to 2.6%, respectively.[10,13,21] The true metastatic rate of MPTT remains unknown, yet reports indicate figures as high as 25% for grade III lesions.[3,20,21] Metastases have been reported at various stages, ranging from initial presentation to as late as 10 years thereafter.[6] Consequently, a radical approach to treatment is warranted for MPTTs.

Primary treatment typically entails surgical excision with a 1 cm margin of uninvolved tissue.[3,9,11,16] For current lesions, certain authors advocate for a 2 cm margin. Mohs surgery has been proposed as a potentially less invasive and more efficient technique.[22,23] However, the literature lacks conclusive evidence regarding the contiguity of MPTTs. Non-contiguous lesions negate the benefit of Mohs surgery, as skip lesions lead to inaccurate clearance margins.[3] The role of adjuvant chemotherapy or radiotherapy remains debatable, particularly in cases of localized disease, due to the exceptional nature of the condition, requiring further evaluation. Certain authors consider it unnecessary,[3,9] while others have used adjuvant radiotherapy to the treated area and neck to prevent recurrence[7] or to reduce

tumor volume.[3,11,20,21] In one case, it has been used with curative intent.[21] For MPTTs with distant metastasis, palliative chemotherapy with administration of cisplatin, adriamycin, and vindesine or 5-fluorouracil, followed by palliative radiotherapy, is recommended by some authors.[3,8,9,11,14,16] However, standardized recommendations are currently lacking due to the scarcity of literature and the absence of randomized trials.

4. Conclusion

The MPTT is now widely recognized in the literature, yet it still eludes a definitive nosological diagnostic and, therefore, a therapeutic consensus. Indeed, the literature expresses a certain ambiguity, and no epidemiological or physiological data are 100% certain, for several reasons: the scarcity of reported cases and literature reviews, the absence of large case series, the existence of various terminologies, and the clinical and histological similarities between MPTT and other tumors, especially squamous cell carcinoma. Regarding the physio-pathological link between chemotherapy and the transformation of PTT into MPTT, our case marks only the third reported instance in the literature. However, these reports are limited to case studies, offering no conclusive explanations for the potential link between the two. Further specialized research and publications are warranted to establish such a link.

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Conflict of interest

The authors declare no conflict of interest.

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Ethics approval and consent to participate

Our study is exempted from ethical approval. Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Consent for publication

Written informed consent was obtained from the patient for publication of this case report and accompanying images.

Availability of data

Data can be obtained from the corresponding author upon reasonable request.

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ACCSCIENCE PUBLISHING

Tumor Discovery

CASE REPORT

Pyogenic granuloma of maxillary median gingiva in a pediatric patient: A case report and literature review

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Abstract

Pyogenic granuloma (PG) is a painless pedunculated granulomatous lesion that develops on the skin and mucous membranes. PG in children is relatively rare. We herein report a case of PG of the maxillary median gingiva in a pediatric patient. The patient was a 6-year-old Japanese boy who presented with a painless mass with bleeding after exfoliation of the left upper deciduous central incisor. The lesion had undergone continuous, rapid growth for approximately 10 days. Intraoral findings revealed a painless, elastic, soft, and non-pedunculated mass, measuring 15 × 10 mm, in the left upper deciduous central incisor area. To differentiate it from malignancy, an excisional biopsy was performed on the patient under general anesthesia. Histological findings confirmed that the patient suffered from PG. At the time of writing this paper, the patient had been clinically followed up for the 18th month after the surgery, showing no signs of tumor recurrence.

Keywords: Pyogenic granuloma; Child; Gingiva

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1. Background

Pyogenic granuloma (PG) is a painless pedunculated granulomatous lesion that develops on the skin and mucous membranes. PG of the oral cavity commonly occurs in the lips, gingiva, and tongue, but its occurrence in children is relatively rare.[1] The etiology of PG has not yet been clearly explained in the literature. Differentiating PG from malignant tumors is necessary as they share a similar trait – rapid mass growth.

We herein report a case of PG of the maxillary median gingiva in a pediatric patient.

2. Case presentation

The patient was a 6-year-old Japanese boy who presented with a painless mass with bleeding after exfoliation of the left upper deciduous central incisor. The patient received anti-inflammatory treatment at a nearby dental clinic. Since the lesion exhibited continuous, rapid growth for approximately 10 days, the patient

was eventually referred to our department for further examination and treatment.

Regarding the intraoral findings, a painless, elastic, soft, and non-pedunculated mass, measuring 15 × 10 mm, was found in the left upper deciduous central incisor area (Figure 1). There was no remarkable personal or family medical history. Panoramic and dental X-ray showed no significant abnormalities. Computed tomography (CT) revealed no obvious bone resorption or destruction (Figure 2). Magnetic resonance imaging revealed a high signal intensity on T2-weighted short tau inversion recovery imaging and a well-defined mass measuring 13 × 10 mm with a contrast effect on T1-weighted imaging (Figure 3). Considering the above findings, the patient underwent an excisional biopsy under general anesthesia in the same month. The excision range was designed with a safety margin of approximately 1 mm around the tumor (Figure 4A), and the lesion was resected as a single mass with the right upper deciduous central incisor (Figure 4B and C). After resection, the healthy gingiva and periosteum at the margins of the resection area were sutured with an absorbable thread. Histologically, a granulomatous lesion with telangiectasia was observed, showing no signs of malignancy (Figure 5). The lesion was a highly vascularized fibrous connective tissue stroma exhibiting numerous dilated blood capillaries, which were of varying sizes and shapes and lined with a single layer of endothelial cells. Based on these findings, the final diagnosis was PG. At the time of writing this paper, the patient had been clinically followed up for the 18th month after the surgery, showing no signs of tumor recurrence. The gingival morphology was also restored to a healthy state. Normal eruption of the successional maxillary anterior teeth was observed (Figure 6).

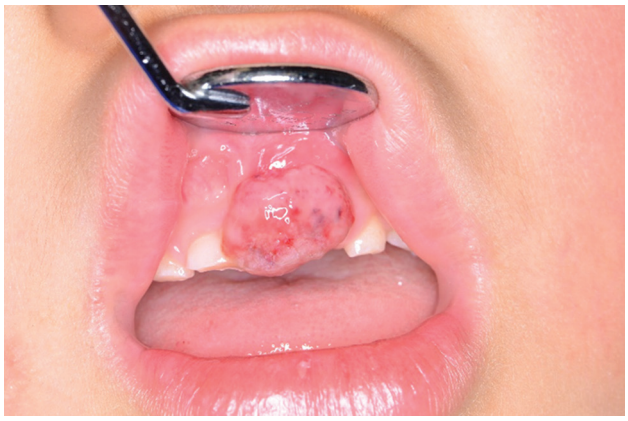

Figure 1. Pre-operative intraoral findings. A mass measuring 15 × 10 mm was found in the left upper deciduous central incisor area.

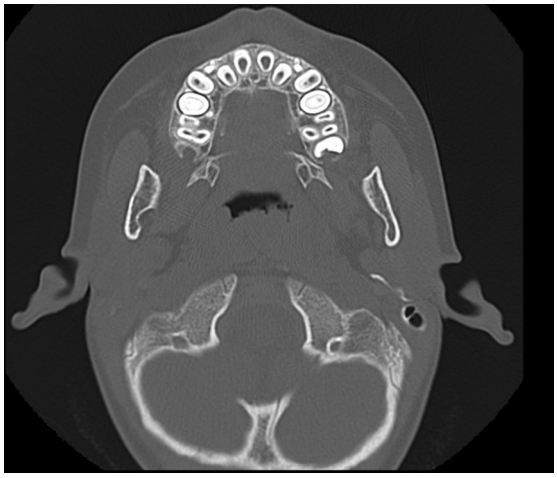

Figure 2. Computed tomography image. No bone resorption and destruction were observed in the upper deciduous central incisor area.

3. Discussion

PG is a granulomatous exophytic lesion that develops on skin and mucous membranes. PG of the oral cavity commonly occurs on the lips, gingiva, and tongue. It occurs more frequently in females than in males, with a ratio of 2:1.[2] The occurrence of PG is most common before the fifth decade of life.[3] The incidence of PG has risen tremendously in the last two decades.[2]

The occurrence of PG is relatively rare in children. To the best of our knowledge, only 26 cases of PG in children below 10 years old, including the current case, have been reported in the English literature. Most of the PG lesions develop on the gingiva, but two cases occurred on the upper lip and one case on the lower lip. The mean maximum diameter of the PG was 16.25 mm. The occurrence of PG was slightly more common in boys. The youngest documented patient who had been diagnosed with PG was 8 weeks old, with the lesion developing on the perigingiva of the congenital tooth. Table 1 presents

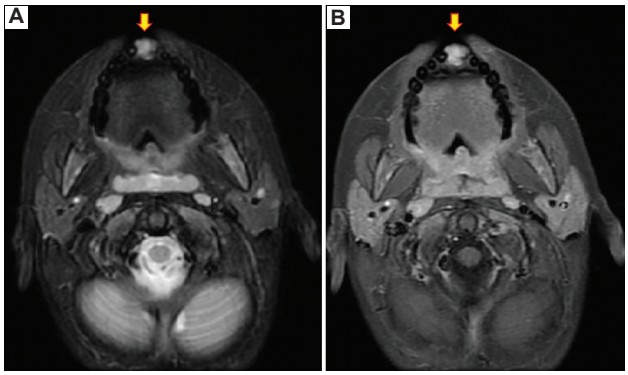

Figure 3. Magnetic resonance imaging images. (A) T2-weighted STIR imaging showed a high signal intensity (arrow). (B) Gadolinium contrast T1-weighted imaging showed a well-defined mass, measuring 13 × 10 mm, with a contrast effect (indicated by arrow).

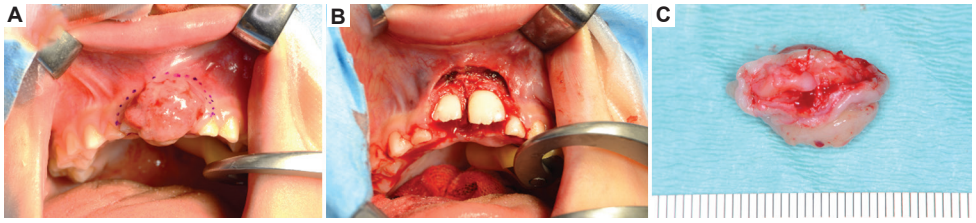

Figure 4. Operative findings. (A) The resection range was designed with a safety margin of approximately 1 mm around the tumor. (B) The lesion was resected in one lump together with the right upper deciduous central incisor. (C) The resected specimen.

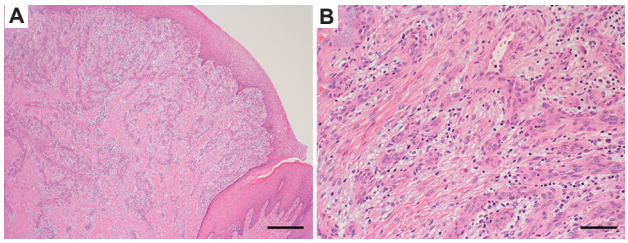

Figure 5. Histopathological findings of a granulomatous lesion with telangiectasia. (A) Low-magnification field (MM ×4); scale bar: 250 μm. (B) High-magnification field (MM ×20); scale bar: 50 μm.

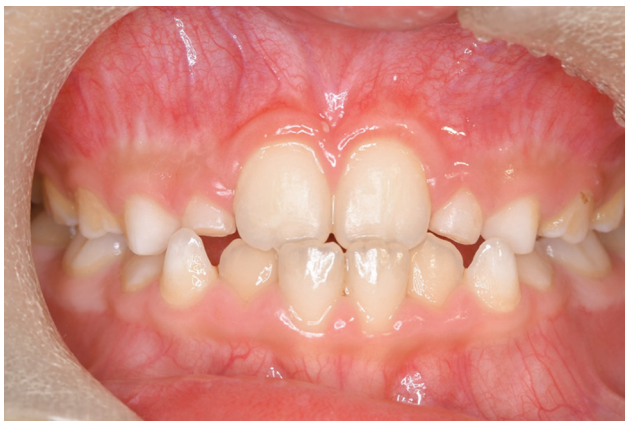

Figure 6. Examination of the patient's oral cavity during follow-up at the 18th month after the surgery. There were no signs of local recurrence.

the baseline characteristics and clinical outcomes of 26 pediatric PG patients under 10 years old, of which 25 of them have been reported in the literature.[4-27]

PG is considered an inflammatory hyperplasia that is unrelated to infection and is caused by various stimuli, including low-grade local irritation, traumatic injury, hormonal factors, drugs, and bone marrow transplant.[28] Of the 26 cases mentioned above, Cheney-Peters and Lund reported the development of PG after bone marrow transplantation for pediatric hematological cancers.[17] Although the mechanism by which PG develops after bone marrow transplantation is unclear, previous reports suggest that inflammation, graft-versus-host disease, and calcineurin inhibitors, such as cyclosporine A and tacrolimus, probably play a role in its development.[29-34] Lindsay and Srivaths reported two cases of PG associated with hemophilia A, in which one case exhibited involuted presentation, with the lesion slowly resolving over the subsequent weeks, and the other demonstrating a lesion which was completely resolved following infusion of recombinant factor 8.[13] However, there is no confirmation on whether the diagnosis of PG was accurate because histopathological examinations were not performed. In addition, Cheney-Peters and Lund concluded that the occurrence of PG in hemophilia patients is grounded in tissue inflammation from vascular trauma and prolonged bleeding, and the resultant increase in circulating systemic inflammatory markers likely promotes the growth of these vascular lesions, which are prone to bleeding, thereby initiating a vicious cycle.[17] Thus, when PG occurs in the oral region, it is important to search for hidden systemic and local factors. In our case, there were few possibilities of PG originating from a systemic disease because there were no abnormalities in the pre-operative examination, and the patient has no relevant medical or family history. Further systemic examination, which was however not conducted, could have identified the etiology in this case.

A definitive diagnosis of PG can be made only with a histopathological examination. Histologically, it is a granulomatous lesion with inflammatory cell infiltration and vascular endothelial cell proliferation. The occurrence of PG is accompanied by the formation of numerous large and small endothelium-lined vessels, which sometimes form lobular aggregates. The arrangement of these lobular aggregates provides invaluable hints for pathologists to make an accurate diagnosis. In some cases, the vascular epithelium may be decimated due to ulceration or trauma or covered by a stratified squamous epithelium.

According to the International Society for the Study of Vascular Anomalies classification, PG lesions exhibiting lobular proliferation of capillaries and endothelial cells are classified as benign vascular tumors.[35] Epivatianos *et al.* reported that PG lesions can be categorized into lobular capillary hemangioma (LCH) and non-LCH types, depending on whether the capillaries proliferated

Table 1. Cases of PG in pediatric patients under 10 years old

Case no.	Year	Authors	Sex	Age	Location	Number of tumors	Treatment	Pathological diagnosis	Size (mm)	Number of recurrences	Final outcome
1	2000	Willies-Jacobo *et al.*[4]	M	Infant	Gingiva	1	Resection	PG	10×8×5	0	—
2	2001	Akyol *et al.*[5]	M	4 months	Tongue	1	Resection	PG	10×8×8	0	Good
3	2001	Milano *et al.*[6]	M	3 years	Gingiva	1	Excisional biopsy/Extraction	PG	—	0	Good
4	2005	Jurkiewicz *et al.*[7]	M	8 weeks	Tongue	1	Resection	PG	30×25	0	Good
5	2006	Shenoy and Dinkar[8]	F	8 years	Gingiva	1	Excisional biopsy	PG	20×10×10	0	Drop out
6	2008	de Souza *et al.*[9]	F	8 years	Lower lip	1	Excisional biopsy	PG	9×8×5	0	Good
7	2009	das Chagas *et al.*[10]	F	2 years	Upper lip	1	Excisional biopsy	PG	16	0	Good
8	2012	An *et al.*[11]	M	8 years	Gingiva	1	Excision	PG	25×20×15	0	Good
9	2013	Ximenes *et al.*[12]	M	4 year	Tongue	1	Excision	PG	8×6×4	0	Good
10	2014	Lindsay and Srivaths[13]	M	4 years	Tongue	1	Spontaneously involuted	None	—	0	Good
11	2014	Lindsay and Srivaths[13]	—	1.5 years	—	1	Recombinant factor 8 infusion	None	—	0	Good
12	2015	Jain *et al.*[14]	M	8 years	Gingiva	1	Excision (advised)	PG	30×30	0	—
13	2016	Agarwal *et al.*[15]	M	8 days	Gingiva	1	Excision	PG	8×5	0	Good
14	2016	Nirmala *et al.*[16]	M	8 years	Gingiva	1	Excision	PG	28×28×9	0	Good
15	2016	Cheney-Peters and Lund[17]	M	1.5 year	Tongue	2	Excision	PG	10×5,10×10	0	Good
16	2018	Peters *et al.*[18]	F	5 years	Tongue	1	Excisional biopsy	PG	6×12	0	Good
17	2019	Lim *et al.*[19]	F	3 years	Tongue	1	Resection	PG	40×30	1	Good
18	2019	Alaoui *et al.*[20]	M	10 years	Tongue	1	Excision	PG	9×6×5	0	Good
19	2020	Agel and Ahluwalia[21]	F	6 years	Gingiva	1	Excisional biopsy/extraction	PG	15×10	0	Good
20	2020	Ahsan Razi *et al.*[22]	M	10 years	Gingiva	1	Excisional biopsy	PG	17×8	0	Good
21	2020	Tazegül and Bodrumlu[23]	M	8 years	Gingiva	1	Excisional biopsy	PG	15	0	Good
22	2020	Tatar *et al.*[24]	M	10 years	Lower lip	1	Excision	PG	3	0	Drop out
23	2021	Kavitha *et al.*[25]	F	8 years	Gingiva	2	Excision	PG	15×10, 20×15	0	—
24	2022	Nassar and Besar[26]	—	10 years	Tongue	1	Excisional biopsy	PG	20×20	0	Good
25	2023	Gera *et al.*[27]	M	6 years	Gingiva	1	Excision	PG	5×10×8	0	Good
26	2023	The current case	M	6 years	Gingiva	1	Excisional biopsy/extraction	PG	8×6	0	Good

Abbreviations: F: Female; M: Male; PG: Pyogenic granuloma.

in a lobular shape based on clinicopathological and immunohistochemical characteristics, and that these two lesion types differ histologically per their pathogenesis.[36] Our case was categorized as non-LCH. However, Kawachi found that capillary hemangiomas and PG differ from each other because of their distinct expression of vascular endothelial growth factors.[37] They also concluded that capillary hemangiomas showed proliferating activity of capillaries, such as endothelial cells and pericyte-like perivascular cells, with the appearance of mast cells and lobules, whereas PG exhibited remarkable proliferative activity mainly of endothelial cells and inflammatory changes. However, it is currently unclear whether capillary hemangiomas and PG are of the same pathological classification.

The development of PG can be categorized according to their respective macroscopic findings into three distinct stages, namely (i) cellular phase, (ii) capillary phase/vascular phase, and (iii) involution phase.[38] Differential diagnoses in the oral region include benign reactive lesions, such as fibromas, hemangiomas, and peripheral giant cell granulomas, as well as malignant tumors, such as metastatic tumors of the oral soft tissue and Kaposi's sarcoma.[2,39] A histopathological examination is essential to distinguish rapidly growing PG from malignant tumors, as in the present case. In our case, an excisional biopsy was performed to avoid having to perform a second procedure if the tumor was not malignant.

Treatment options for PG include electrocautery, electrocoagulation, radiation therapy, and cryotherapy,[28,40] but resection of the lesion together with the surrounding healthy tissue has become a prevailing treatment approach in recent years. However, inadequate resection may result in rapid recurrence. The recurrence rate of PG has been reported to be 6% – 16%.[3,41] Accordingly, a radical cure of the initial resection is important. Furthermore, Asnaashari *et al.* concluded that using lasers for PG lesion removal could reduce stress and fear among pediatric patients and minimize discomfort both during and after surgery.[42] In the aforementioned 26 cases of pediatric PG, resection or excision was performed in most cases, with only one case reporting recurrence within the follow-up period.

For a PG lesion manifesting rapid growth, as in our case, a biopsy is required to rule out malignancy. However, physicians should anticipate tougher restrictions in terms of facilities, examinations, and treatments if the patient is a child. In addition, it is important for health-care personnel to cater to the mental health needs of the affected children and their families. In the present case, the patient was admitted to our hospital because pediatric hospitalization was not feasible at the previous facility which tended to his treatment. We collaborated with the pediatrician who was responsible for the examination and treatment of the patient. We strived to provide prompt yet comprehensive care to the patient while reducing procedures as much as possible. Our tailored treatment plan proved to be effective as a result of the smooth handling of the perioperative treatment and management for the patient.

4. Conclusion

We presented a case of PG in the anterior maxillary region of a child, contributing significantly to the relevant literature. To rule out malignancy, it is important for physicians to collaborate closely with pediatricians to promptly examine and treat the rapidly growing tumors in children, which manifest possible signs of malignancy, as in the present case.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

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Writing – review & editing: Takeshi Karube, Kaori Yago, Hajime Okita, Seiji Asoda

Ethics approval and consent to participate

Not applicable.

Consent for publication

Informed consent has been obtained from the patient for publication of this case report.

Availability of data

The data can be requested from the corresponding author following reasonable request.

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ACCSCIENCE PUBLISHING

Tumor Discovery

CASE REPORT

Calcified peripheral schwannoma mimicking a cervical lymph node: A case report and literature review

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Abstract

Peripheral schwannomas are typically benign tumors originating from Schwann cells of peripheral nerves. Although often asymptomatic, such tumors may present diagnostic challenges since they resemble other known structures, leading to potential misinterpretations. Here, we present the case of a 17-year-old patient with a cervical mass that initially posed a diagnostic dilemma, resembling a cervical lymph node on clinical examination. The calcified nature of the tumor added complexity to its identification, and our case underscores the significance of a good diagnostic evaluation. The literature on calcified peripheral schwannomas is scarce, but the current case contributes to the understanding of atypical presentations. Diagnostic modalities, including imaging studies and histopathological examination, play a pivotal role in confirming the schwannoma diagnosis and differentiating it from other entities. Surgical excision remains the primary treatment, but awareness of varied presentations is crucial for accurate management. This case report serves as a valuable addition to the literature, highlighting calcified peripheral schwannomas mimicking cervical lymph nodes. Medical practitioners should maintain a high index of suspicion for schwannomas in unusual clinical cases, ensuring accurate diagnosis and appropriate management.

Keywords: Cervical mass; Schwannoma; Calcification

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1. Introduction

Cervical schwannomas are benign tumors of the peripheral nerves developed exclusively from Schwann cells. Initially described by Verocay in 1908,[1] they are estimated to be present in the neck region in 25 % of all cases.[2] The nerve of origin remains unidentified in 50% of the cases,[3] but they are found to be predominantly formed within the vagus nerve.[2] Other structures such as the sympathetic cervical chain can be, although rarely, the origin of this benign entity.[2] Here, we report the case of a chronic cervical schwannoma, which was initially misinterpreted as a cervical lymph node.

2. Case presentation

The present case revolves around a 17-year-old girl with no significant medical history, who reported a 2-year history of progressive swelling in the upper right laterocervical

region behind the mandibular angle. The patient exhibited no signs of numbness, compression (such as dysphagia or dyspnea), or discomfort. On examination, a firm painless mass, approximately 2 cm × 2 cm, was palpable at level 2 on the right side. The mass was mobile in both superficial and deep planes, with no skin changes overlying. Oto-rhino-laryngoscopic examination revealed normal findings. Ultrasound examination identified a well-defined solid cystic mass, measuring 2.5 cm in diameter, hypoechoic with no internal flow. There was no continuity between the proximal and the distal ends. Infracentimetric lymph node structures were identified alongside the mass. Given their proximity, the original diagnosis of pathological lymph node was maintained, and thus, no further radiological exploration was pursued. A fine-needle aspiration cytology (FNAC) of the mass showed an atypia of undetermined significance. Surgical exploration under general anesthesia revealed a white nodular formation located medially to the anterior border of the sternocleidomastoid muscle. The mass was distant from the neurovascular bundle and easily dissected from the surrounding structures. A complete excision was carried out (Figures 1 and 2).

Histologically, the nodular mass was calcified, exhibiting necrotic rearrangement. Histopathologic examination revealed spindle-shaped and elongated tumor cells, which showed no mitotic activity, pointing to a plausible diagnosis of schwannoma (Figure 3). The post-operative course was not marked with any remarkable clinical changes, and after a one-year follow-up, the patient showed no signs of recurrence.

3. Discussion

Schwannomas are benign tumors that originate in any peripheral nerves, with the exception of the olfactory and optic nerves.[4] They affect the head-and-neck region in 20 – 45% of cases, with the vestibular nerve being the primary origin of development.[5] The occurrence of schwannomas is common in patients between 20 and 50 years old, regardless of gender.[4] The clinical signs of cervical schwannomas depend on various factors including the location, size, and the nerve of origin. The most typical clinical presentation is isolated laterocervical mass, which is asymptomatic and characterized by slow mass growth.[5] Additional symptoms may be present in some cases, which can be explained by an external mass compression of the oro-pharyngolaryngeal axes or the adjacent nerves.[4] In our case, the patient did not exhibit any symptoms other than progressive swelling.

Identifying the nerve of origin in cervical schwannomas poses a significant challenge. This is evidenced by a comprehensive case study involving patients with non-vestibular schwannomas of the head and neck, where specific nerve of origin could only be determined in 16 out of 26 patients (62%), with the origin of the remaining cases, presumably from unnamed small nerve plexus branches, left unidentified.[4] Similarly, an article from India highlights the difficulty of determining the nerve of origin for this tumor entity, suggesting a successful chance of identification as low as 50%.[3]

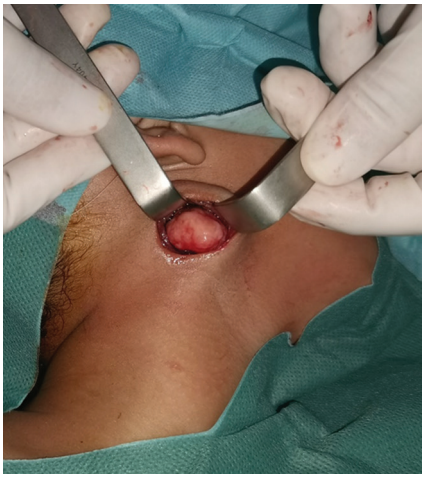

Figure 1. Peroperative image showing the nodular mass.

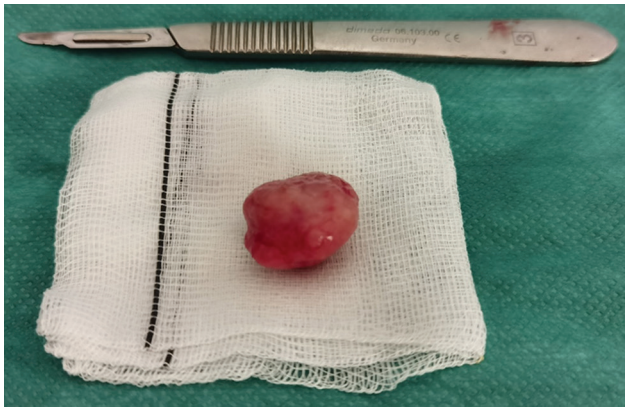

Figure 2. Excised mass before histological examination.

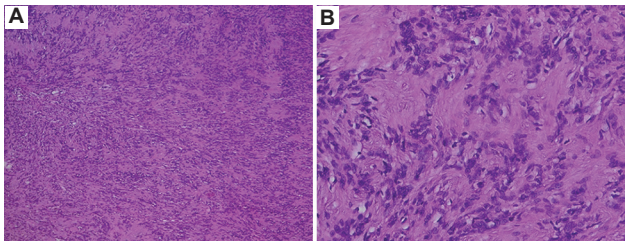

Figure 3. Microscopic examination of hematoxylin-eosin-stained sections of surgically resected tumor under ×20 (A) and ×40 (B) magnification. The sections show cytologically bland spindle cells against a vague nuclear palisading and fibrillary background.

Whether surgical approach is employed to resect cervical schwannomas is determined by the location and size of the mass. For instance, accurately identifying location of the mass on vital structures such as neurovascular bundle and spinal nerve is crucial for decision-making on whether a horizontal or Paul André incision should be employed.[3,6] In our case, the nerve of origin was not identified. However, considering the location of the mass and the ease with which it was dissected from the surrounding structures during the surgical exploration, we also believe that the origin was likely a small nerve of the plexus branches.

Although the definitive diagnosis relies strictly on histopathologic means, pre-operative imaging proves to be an essential tool for diagnosing cervical schwannomas. Imaging can yield crucial information regarding the size and location of the mass, as well as the involvement of surrounding structures, such as the neurovascular bundle, and provide guidance in performing a fine need aspiration cytology or biopsy (FNAC or FNAB, respectively) through ultrasonography.[4,7] Both computed tomography (CT) and magnetic resonance imaging (MRI) findings assist with ruling out differential diagnoses such as paraganglioma or congenital cysts. These radiological modalities can also help identify the nerve of origin by analyzing the mass effect on the vascular axis.[6] In the case of the involvement of vagus nerve, the tumor tends to widen the space between the internal carotid or common carotid artery and the internal jugular vein, whereas the involvement of sympathetic nerve displaces the jugulocarotid axis forward.[8] Ultrasonographic findings alone can be insufficient to differentiate between schwannomas and pathologic lymph nodes, especially since the exploration is operator dependent.[9] In a comparative study, Ahn *et al.* reported that core needle biopsy (CNB) delivered a high-accuracy performance in diagnosing extracranial schwannomas of the head and neck (96.5% specificity and 100% sensitivity), as compared to FNAC.[7] However, despite its relatively low sensitivity in this respect,[10] FNAC can be used to rule out other potential malignant lesions.[9] A FNAC was performed in our case guided by ultrasonography showing an atypia of undetermined significance.

While the tumor progression is typically slow, and malignant transformation is exceptionally rare, complete excision of the lesion remains the optimal treatment for symptomatic cervical schwannomas.[5,6] Claude-Bernard-Horner syndrome (miosis, ptosis, anhidrosis, and enophthalmos) is the most common post-operative complication in schwannomas of the cervical sympathetic chain.[6] This complication can be confirmed in an anatomopathological examination by identifying spindle cells with Verocay bodies or without tissue cellularity.[11] In addition to their morphological characteristics, schwannomas can be differentiated from leiomyomas and palisaded myofibroblastoma by virtue of their distinctive immunoreactivity profile.[9] Schwannomas typically demonstrate diffuse expression of S100 protein, while showing no reactivity for antibody against muscle markers such as desmin and smooth muscle actin.[12]

Calcification is a rare occurrence in schwannomas. This is supported by a study by Din *et al.* who identified only 27 cases of calcifications out of 2116 cases of schwannoma, of which only three cases were detected in the cervical region.[13] Recurrence of schwannoma is exceedingly uncommon, even in cases where the tumor is incompletely resected.[14]

4. Conclusion

Schwannoma should be considered a differential diagnosis in patients with a neck mass due to the potential misinterpretation as lymphadenopathy, even if ultrasonography screening is performed. Radiological examinations including CT scan and MRI play an important role in accurate diagnosis-making as well as pre-surgical analysis of anatomical structures.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

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Ethics approval and consent to participate

Written consent was obtained from the patient and his family to participate in this study.

Consent for publication

Written consent was obtained from the patient and his family for the publication of this case report and accompanying images.

Availability of data

Not applicable.

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ACCSCIENCE PUBLISHING

Tumor Discovery

CASE REPORT

High-grade sinonasal adenocarcinoma as an unusual presentation: A case report

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Abstract

The occurrence of malignant tumors, such as adenocarcinoma, is commonplace in the nasal region, characterized by a notably low incidence. This rarity often contributes to delayed diagnosis, rendering the condition challenging to manage. Here, we present the case of a 70-year-old man who presented with chronic left nasal obstruction, accompanied by headaches and recurrent epistaxis. The diagnostic investigation revealed an extensive and aggressive nasal adenocarcinoma, necessitating a hemi-maxillectomy for tumor excision. Subsequent reconstruction of the ipsilateral cheek was accomplished using a pectoralis major flap. Despite the initial intervention, the patient experienced a recurrence 1 year later. In sinonasal adenocarcinoma, nasal obstruction stands out as the predominant symptom, complemented by potential manifestations of swelling and facial deformation. The inherently aggressive nature of this tumor underscores the imperative for early diagnosis and meticulous management to achieve optimal therapeutic outcomes. The comprehensive approach involving surgical intervention and reconstruction reflects the complexity in treating advanced nasal adenocarcinomas.

Keywords: Adenocarcinoma; Malignancy; Nose; Paranasal sinuses

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1. Introduction

Primary sinonasal adenocarcinomas are rare tumors exhibiting a broad morphological spectrum. They are generally divided into two groups: intestinal-type sinonasal adenocarcinoma (ITAC) and non-ITAC subtypes.[1] These neoplasms are estimated to constitute merely 13% of all documented cases of sinonasal carcinoma.[2,3]

The classification of adenocarcinoma holds paramount significance as it not only dictates the tumor's behavior but also predicts the patient's prognosis. Observational data indicate that individuals afflicted with low-grade adenocarcinomas typically endure prolonged symptoms, experience reduced pain, and are less prone to deformities. Conversely, high-grade lesions entail more extensive involvement of the paranasal sinuses, signifying heightened invasiveness.[4]

The primary objective of implementing surgery is to completely remove the lesion to ensure a favorable outcome. While various external approaches can be employed, endoscopic surgery has gained prominence in recent years. This shift is attributed to its comparable clinical outcomes, lower incidence of complications, and diminished

mortality rates in contrast to alternative surgical procedures.[5,6] Radiotherapy pre-dominantly serves as a palliative or complementary measure in the post-operative phase.[5,7] Despite its positive effects, chemotherapy has waned in popularity due to its suboptimal reproducibility of therapeutic effects. Evidently, surgery remains the most advantageous treatment modality, whether utilized independently or in conjunction with radiotherapy.[5] Adjuvant radiotherapy is recommended for high-grade tumors and those classified as T3 or T4 stage.[6]

The case under consideration involves a 70-year-old patient diagnosed with high-grade adenocarcinoma of the nasal cavity, presenting initially with the left nasal obstruction. After the initial intervention, the patient became subject to a recurrence of the tumor with extension to the left cheek, highlighting the aggressive nature and metastatic potential of this subtype of adenocarcinoma.

2. Case report

A 70-year-old man, with a history of chronic smoking (30 packs/year) and occasional alcohol consumption, presented at the clinic due to persistent, unilateral left nasal obstruction, and recurrent episodes of epistaxis that had been ongoing for 6 months. The patient did not report any associated symptoms such as facial pain, anosmia, or rhinorrhea.

On anterior rhinoscopy, a notable deviation of the right nasal septum was identified. Subsequent endoscopic examination revealed a sizable, lobulated mass within the left nasal cavity, characterized by a tan, fleshy, and firm consistency, fully occupying the cavity. Despite its considerable size and where it was located, the mass did not display hypervascularization. A biopsy was conducted, confirming the presence of a glandular tumor. However, the specific origin of the tumor could not be determined through endoscopic examination due to its extensive dimensions.

Further, examination, which included the evaluation of the nasopharynx, eyes, neck, and cranial nerves, revealed normal findings. A comprehensive magnetic resonance imaging (MRI) assessment of the paranasal sinuses disclosed a massive and expansive lesion occupying the entire left nasal cavity up to the level of the choana (Figure 1). The lesion was measured at 9.0 × 4.8 × 2.1 cm in dimensions, with evident contrast entrapment, and the absence of associated lymphadenopathy. Given the increased risk of bleeding and the intent to perform an *en bloc* resection, the initial biopsy was deferred, and a decision was made to proceed directly with tumor resection.

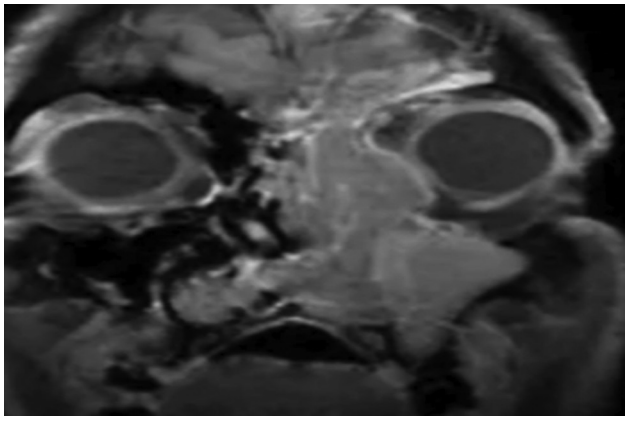

Figure 1. Pre-operative magnetic resonance imaging of the patient's nasal cavity

The patient underwent surgical intervention, which included tumor resection with hemi-maxillectomy. Preservation was undertaken for the medial orbital wall and the anterior skull base. The lesion was successfully removed and pathologically identified as a glandular tumor. Subsequent immunohistochemical analysis revealed a high-grade non-intestinal adenocarcinoma classified as pT3N0M0 (Figure 2). Postoperatively, the patient was referred to the oncology department for comprehensive oncological management due to the aggressive nature of the identified adenocarcinoma.

The patient was lost to follow-up for a year. On returning to the clinic, he suffered from a left jugal ulcerating and infiltrating mass, which was fistulized to the skin. Notably, no discernible signs of local recurrence were observed during the rhinoscopy. Figure 3 depicts the recurrent lesion, which was unfortunately neglected by the patient. The ulceration and infiltration of the jugal mass, along with the fistulization of the skin, reflect the progression of the disease due to a lack of care during the time when the patient was lost to follow-up.

A computed tomography (CT) scan revealed a well-defined left jugal tissue process measuring 69 × 62 mm, extending over 60 mm. The extension encompasses the medial aspect of the left nasal fossa, the left ramus, the left horizontal branch of the mandible, and the left zygomaticus. Posteriorly, it extended to the left masseter muscle, displaying a loss of the separation line (Figure 4). Surgical excision (exeresis) of the tumor was executed, followed by the reconstruction of the resulting defect. The reconstruction involved employing a temporalis muscle flap, complemented by a skin graft to address the loss of skin substance. The procedure also included a dissection of the homolateral lymph nodes (Figure 5).

On completion of the surgery, a definitive anatomopathological study was conducted, revealing the

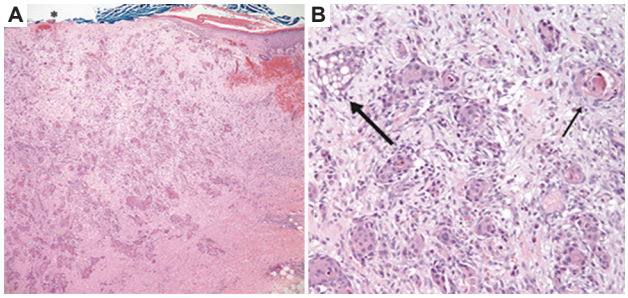

Figure 2. Characteristic histopathologic features of adenosquamous carcinoma. (A) Infiltrative pattern of neoplastic nests, dermal fibrosis/sclerosis, elastosis, and focal ulceration (denoted by asterisk) (hematoxylin-eosin staining; magnification ×40. (B) Keratinizing cysts (denoted by small arrow) and glandular elements (denoted by large arrow) (hematoxylin-eosin staining; magnification ×200.

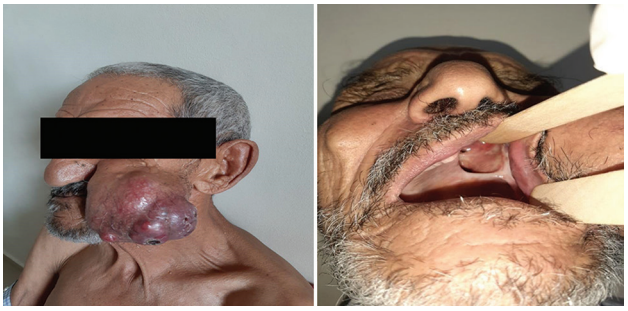

Figure 3. Image showing tumor recurrence at the left jugal level

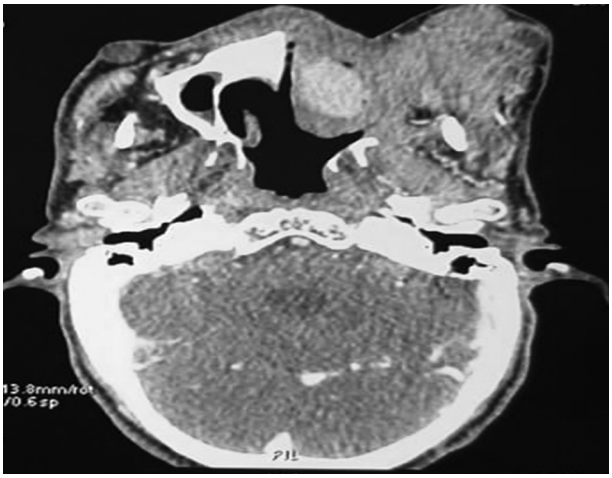

Figure 4. Pre-operative compared tomography scan showing well-bounded, round, and heterodense jugal tissue process measuring 69 × 62 mm

characteristic appearance of cutaneous adenosquamous carcinoma. Notably, the findings included the presence of vascular emboli and peri-nervous infiltration. Importantly, no associated lymph node metastases were identified.

Subsequent to the anatomopathological assessment, the patient was referred to the oncology department for further

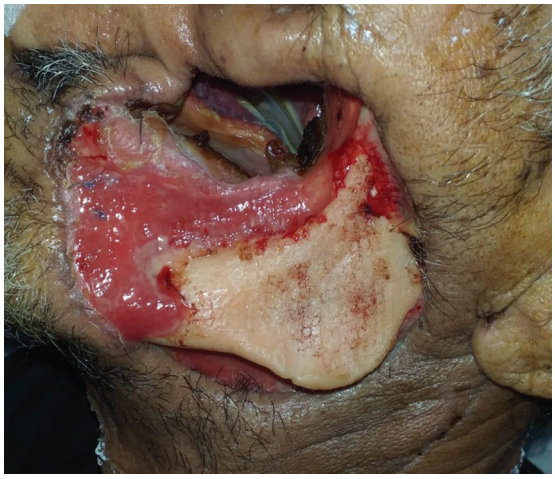

Figure 5. Image showing the loss of substance after excision of the cheek mass

treatment. This referral underscores the necessity for a comprehensive oncological management plan, considering the aggressive nature of adenosquamous carcinoma and the distinctive morphological characteristics observed in the anatomopathological analysis.

3. Discussion

Glandular neoplasms account for 4 – 8% of all primary malignancies within the nasal cavity.[1,2] Reports on high-grade sinonasal adenocarcinomas of the non-intestinal type are scant.[2,9] This type of tumor is more common among male individuals and affects people across a broad age spectrum, spanning from adolescents to the elderly.[3,4] Nasal obstruction, often accompanied by swelling or facial deformities, emerges as the predominant clinical symptom. The nasal cavity and maxillary sinuses are the most frequently implicated sites, although in some cases, such as the one presented here, extension to other sinuses is possible. Unlike adenocarcinomas of the intestinal type, no discernible risk factors have been identified for high-grade non-intestinal adenocarcinomas.[5]

The prognosis for high-grade tumors is typically unfavorable, even if aggressive therapeutic interventions are implemented, associated with a meager 3-year survival rate of 20%.[6,7] Historically, the average 5-year survival rate for sinus cancer has shown a notable increase from 28% in the 1960s to 51% in the 1990s.[8] Recent studies, including an analysis by Turner and Reh, highlighted an incremental clinical improvement following sinonasal cancer treatment, with a 5-year relative survival rate estimated to have risen from approximately 49.7% in 1973 to 56.4% in 2001.[4] Choussy *et al.*'s analysis of 418 patients reports a 5-year overall survival rate of 64%.[10]

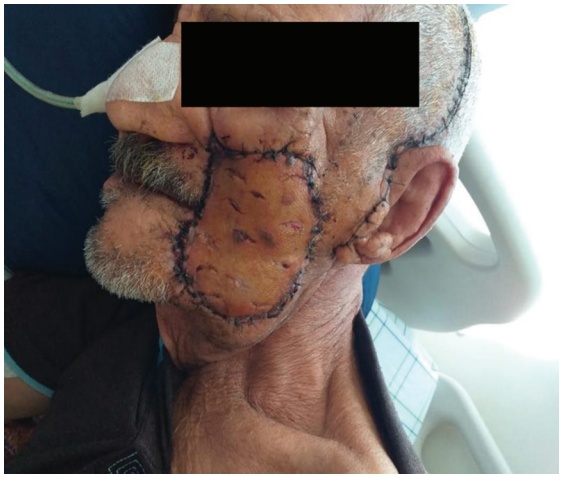

Figure 6. Image showing reconstruction with a flap of the temporalis muscle

Distinguishing between low-grade and high-grade adenocarcinomas is pivotal for treatment planning and prognostic assessments. Histologic features indicative of high-grade adenocarcinomas encompass solid growth patterns with sheets of cells, poorly defined irregular glandular patterns, hyperchromatism, moderate to prominent nuclear pleomorphism, and a heightened mitotic rate.[9,10]

Diagnostic evaluation of sinonasal masses may involve radiologic modalities such as CT and MRI. Either modality can reveal tumor involvement in various anatomical regions, including the nasopharynx, intracranial cavity, paranasal sinuses, orbits, infratemporal fossa, and pterygopalatine fossa. Determining the tumor origin on CT may pose challenges, especially in cases involving a common wall, such as the medial wall of the maxillary sinus, and the detection effort could be further compounded by obstructive sinusitis. In such instances, MRI proves to be a valuable adjunct for detection.

The primary therapeutic modality for sinonasal adenocarcinoma is complete surgical excision. In a study by Alessi *et al.* involving 13 patients, the adequacy of surgical margins emerged as the single most crucial factor in treatment success, although no specific margin size was recommended. In anatomical regions such as the superior nasal vault and skull base, achieving clear margins may necessitate multiple excisional biopsies due to the potential presence of microscopic disease, even in apparently normal-appearing mucosa. Surgical approaches for low-grade adenocarcinoma should be less radical compared to the more aggressive strategies warranted for high-grade lesions.[11,12] Adjuvant radiation therapy is recommended for high-grade lesions and recurrent low-grade lesions.[13]

Adenocarcinomas detected in their early stage can be managed with only surgical treatment, which results in an impressive 5-year survival rate of 83.4%. For patients with advanced-stage disease, the combined approach of surgery and radiotherapy, which yields a 5-year survival rate of 66.6%, is recommended. Notably, radiotherapy alone does not confer a significant benefit in terms of 5-year survival when compared with no-treatment.[14]

This case provides valuable insights, emphasizing the rarity and aggressiveness of high-grade non-intestinal sinus adenocarcinomas. Nasal congestion emerges as a prominent and non-specific symptom, often contributing to delayed diagnoses. Thus, multidisciplinary assessments play a pivotal role in effective patient management. Complete tumor resection through meticulous surgical approaches proves superior to endoscopic methods. This case report underscores the necessity to perform surgical resection to ensure optimal clinical outcomes (Figure 6).

4. Conclusion

Adenosquamous carcinoma is aptly characterized as a locally aggressive, high-risk subtype within the spectrum of cutaneous squamous cell carcinoma. Histopathological attributes of this tumor subtype, such as tumor thickness and perineural invasion, confer a heightened risk, and the incidence of locoregional recurrence is notably common. Distinguishing adenosquamous carcinoma from other sinonasal tumors is imperative due to its markedly distinct prognosis. Achieving a definitive diagnosis necessitates a comprehensive anamnesis, encompassing a detailed patient history and clinical evaluation. The diagnostic process is further refined through radical endoscopic resection, aimed at achieving a total excision of the lesion. This surgical approach is crucial for both diagnostic accuracy and therapeutic efficacy. In addition to the surgical intervention, satisfactory immunohistochemistry results play a pivotal role in confirming the diagnosis of adenosquamous carcinoma. Immunohistochemical analyses provide valuable insights into the specific molecular markers and protein expressions associated with this subtype, aiding in its conclusive identification. In summary, recognizing adenosquamous carcinoma as a distinct and aggressive subtype underscores the importance of implementing a thorough diagnostic investigation. A comprehensive patient history, radical endoscopic resection for total excision, and reliable immunohistochemistry results collectively contribute to achieving a definitive diagnosis and informing the subsequent course of therapeutic interventions.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Sara Moujrid, Fadoua El Mourabit
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Methodology: Sara Moujrid, Fadoua El Mourabit
Writing – original draft: Sara Moujrid, Fadoua El Mourabit
Writing - review & editing: All authors

Ethics approval and consent to participate

Verbal consent had been obtained from the patient before participation.

Consent for publication

Verbal consent had been obtained from the patient for publishing this case report while maintaining his anonymity.

Availability of data

Not applicable.

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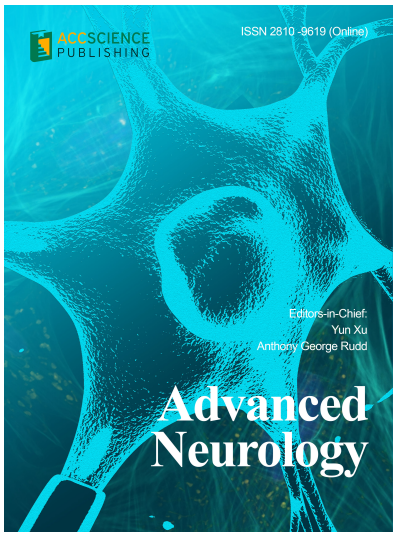

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