

Editorial

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New insights into non-invasive screening of esophageal squamous cell carcinoma

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Esophageal cancer remains a leading cause of cancer-related death worldwide. Its incidence and mortality rank seventh and sixth among all cancers in 2020 [1], respectively. The geographical regions with most esophageal cancer cases and deaths include central and eastern Asia (known as the Asian esophageal cancer belt), parts of Africa, and some South American countries, with esophageal squamous cell carcinoma (ESCC) being the major histological subtype (comprising nearly 90 % of cases). Due to the asymptomatic nature of early-stage lesions, more than 80 % of ESCC were diagnosed at an advanced stage. This has led to an extremely poor prognosis of ESCC, with 5-year survival rates less than 20 % in most countries.

Cancer screening could facilitate earlier detection and longer patient survival. Upper gastrointestinal endoscopy with biopsy is currently the only well-established method for ESCC screening and diagnosis. Population-based studies in ESCC endemic regions in China have confirmed that Lugol chromoendoscopic screening increases the early detection rate and decreases mortality. However, endoscopy is an invasive procedure, with relatively low patient acceptability and risks of procedure-related complications. Furthermore, endoscopy is not sufficiently available in many high-risk areas, and the cost-effectiveness of implementing endoscopic screening at a population level remains suboptimal. Therefore, to improve the coverage and efficiency of population-based ESCC screening, there is an urgent need to develop less invasive screening methods with acceptable cost, accessibility, and diagnostic accuracy. Recently, many emerging non-invasive screening modalities have been developed, which holds promise for improving ESCC early detection rate.

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Esophageal cytology

Esophageal balloon cytology was invented by Prof. Shen Qiong from China in 1960s, and was widely used in ESCC screening in Linxian which is a well-known ESCC high-risk area. Cell specimen from the esophageal mucosa could be collected by the inflated balloon sampler during device retrieval, and smear cytology slides were subsequently made for cytologists to find squamous dysplasia. Patients with abnormal cytological findings would be subsequently referred to endoscopy or treatment. Groundbreaking as this technique was, the sensitivity for detecting histology-confirmed squamous dysplasia was less than 50 % in asymptomatic participants [2]. This low sensitivity may be due to sampling error (missing cells during collection & slides preparation) and difficulties in interpretation of esophageal cell morphology. Furthermore, the balloon retrieval process could cause considerable patient discomfort. Due to these reasons, balloon cytology was not widely used in other high-risk regions. Recently, a sponge cell sampler (sponge within a soluble gelatin capsule) has been reported to provide increased cell yields ($>10^6$ cells) and improved patient acceptability. A research group in China combined sponge cytology and deep learning cell recognition and classification. After cell collection, slides preparation and digitalization, the artificial intelligence (AI) scanner automatically indicates potentially abnormal cells for cytologists to confirm, which could decrease the possibility of missed diagnosis while reducing cytologists' working load. During validation in community-based screening of 1,844 participants, the AI-assisted sponge cytology achieved a sensitivity of 90.0 % and specificity of 93.7 % [3]. This group also developed and validated a machine learning model for ESCC and esophagogastric junctional adenocarcinoma screening based on 17,498 screening participants in China, which could assess the disease risk based on computational cytology features and risk factors in a fully-automated manner, achieving an area under the curve (AUC) of 0.960 [4].

In addition to cytologist- or AI-based morphological analysis, biomarker test based on cytology specimen also showed promising results in recent years. Sponge cytology

combined with p53 immunohistochemical staining showed a sensitivity of 100 % and specificity of 97 % for detecting 4 cases of high-grade squamous dysplasia from Golestan, Iran. Sponge cytology combined with a DNA methylation panel including cg20655070, SLC35F1, and ZNF132 also showed acceptable results for detecting ESCC, though the sample size was relatively small and participants were not recruited from a screening setting [5]. Considering current research evidences, esophageal cytology tests based on AI morphology and novel biomarkers, if further validated in screening population, has the potential to be implemented in mass screening in high-risk areas and reduce ESCC mortality.

Biomarker-based liquid biopsy

Liquid biopsy refers to the technique that captures materials originate from cancer tissues (DNA, RNA, proteins, cells) through body fluid specimen (blood, saliva, urine, etc.). Blood-based molecular targets for ESCC screening mainly includes auto-antibodies (anti-p53 antibody, etc.), circulating tumor DNA (ctDNA), and cell-free non-coding RNAs (including miRNA, lncRNA, etc.). Most of previous studies have shown only modest results in screening population, and no biomarkers were currently approved for clinical application. Recently, with the substantial development in high-throughput sequencing and quantitative detection technology, some studies on blood-based ESCC screening have shown promising results. Methylation assay of ctDNA KCNA3 and OTOP2 has produced an AUC, sensitivity, and specificity of 0.88, 81.5 %, and 92.9 % for detecting ESCC [6]. An international team has discovered and independently validated a serum 8-miRNA panel (including miR-103, miR-106b, miR-151, miR-17, miR-181a, miR-21, miR-25, and miR-93), and the AUCs were 0.80–0.93 in large international validation cohorts [7]. Importantly, they also found the superiority of the 8-miRNA signature to current clinical serological markers for early ESCC patients.

Saliva has the advantage of complete non-invasive sampling, and salivary biomarkers were found to be potentially useful in ESCC screening. Li et al. proposed a salivary exosome 6-miRNA signature (miR-1268a, miR-4505, miR-1972, miR-4274, miR-4701-3p, and miR-6126) and showed a high accuracy for distinguish patients with all-stage ESCC [8]. A bi-signature of tRNA-derived small RNAs (tRNA-GlyGCC-5 and sRESE) in saliva exosome was also found to be highly effective to discriminate ESCC and healthy ones with high sensitivity (90.50 %) and specificity (94.20 %) [9]. Besides values in

screening and diagnosis, those signatures were also found to have prognostic significance.

Breath volatile organic compounds

Human breath contains hundreds of volatile organic compounds (VOCs), which is a potential approach for completely non-invasive disease screening. A systematic review and meta-analysis showed that the pooled AUC of VOCs diagnosing gastroesophageal cancer was 0.95 [10]. For ESCC, the research evidence is still far from sufficient. A pilot study from China reported an AUC of 0.943 for detecting ESCC. However, these preliminary results should be interpreted with caution due to the relatively small sample size (29 ESCC and 57 healthy relatives), insufficient numbers of early-stage cancers, and lack of blinding.

Future perspectives

The non-invasive screening of ESCC has taken a substantial step forward in recent years. The acceptability and accuracy of esophageal cytology test have been significantly improved due to the combination with AI or novel biomarkers. Both blood-based and saliva-based biomarkers have shown promising results in ESCC screening. Breath test is an attractive screening approach though more research evidences are warranted. For all the screening methods, it is crucial to validate their performances in a screening setting and carefully evaluate their cost-effectiveness before recommended in population-based programs. Besides developing new screening modalities, how to better make use of existing methods (alone or in combination) at a population level is also a pivotal research question. Better combining multiple non-invasive screening tests with the current system of endoscopic confirmation and subsequent treatment and follow-up could transform screening strategy and reduce ESCC mortality.

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