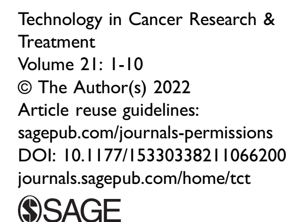
Original Article


Technology in Cancer Research & Treatment
Volume 21: 1-10
© The Author(s) 2022
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/15330338211066200
journals.sagepub.com/home/tct
SAGE

A Rapid Cytological Screening as pre-Endoscopy Screening for Early Esophageal Squamous Cell Lesions: A Prospective Pilot Study from a Chinese Academic Center

Yadong Feng[1*], Yan Liang[1,2*], Bin Yao[3*], Jiajia Xu[4], Juncai Zang[3], Youyu Zhang[1], Jiong Zhang[1], Guangpeng Xu[3], Bo Wei[3], Xiangyi Yao[5], Peilin Huang[6], and Ruihua Shi[1,2]

Abstract

Background: Cytological detection of early esophageal squamous cell carcinoma (ESCC) remains challenging. Therefore, we introduced a rapid cytological screening method and evaluated its efficacy as a pre-endoscopy screening for early ESCC and precursor lesions. **Methods:** This method consisted of a sponge sample retrieval, automatic liquid-based cytological treatment and slides preparation, computer-assisted screening and manual diagnosis. Efficacy for detection of early ESCC and precursor lesions was evaluated. Also, diagnostic efficiency was compared with manual diagnosis. **Results:** Eighty-three patients with early ESCC and precursor lesions and 2,090 asymptomatic participants with high risks of ESCC were enrolled. Whole procedure was accomplished within two working days. Abnormal cells were detected in all 83 patients, and in 272 (13.01%) subjects among 2,090 asymptomatic participants. Early ESCC, high-grade intraepithelial neoplasia, low-grade intraepithelial neoplasia and reflux esophagitis and normal endoscopic findings were detected in 8, 13, 11, 187 and 53 participants with abnormal cells, respectively. The calculated sensitivity, specificity, positive predictive value and negative predictive value for detection of early ESCC and precursor lesions were 100%, 88.34%, 11.76%, and 100%, respectively. Compared with manual diagnosis, this method was accomplished in a shorter time duration (5.4 ± 0.45 min vs 320.2 ± 132.4 min, $p < 0.001$), a higher diagnostic accuracy (96.7% vs74.4%, $p = 0.015$) and a better inter-observer agreement (93.3% vs66.7%, $K = 0.286$, $p < 0.001$). **Conclusions:** Our study provides a promising methodology as pre-endoscopy screening for early ESCC and precursor lesions.

Keywords

pre-endoscopy screening, cytological detection, early esophageal squmaous cell carcinoma, precursor lesions, rapid diagnosis

Received: March 15, 2021; Revised: October 24, 2021; Accepted: November 24, 2021.

[1] Department of Gastroenterology, Zhongda Hospital, School of Medicine, Southeast University, 87 Dingjiaqiao Road, 210009, Nanjing, China
[2] Nanjing Medical University, 101 Longmian Road, 211166, Nanjing, China
[3] Nanjing Froeasy Technology Development CO., LTD, C1 Building, Red Maple Park of Technological Industry, 210046, Nanjing, China
[4] Department of Pathology, Zhongda Hospital, School of Medicine, Southeast University, 87 Dingjiaqiao Road, 210009, Nanjing, China
[5] Faculty of Art Economic, University of Manitoba, 60 Shore Street, Winnipeg, Canada, r3T 2C8
[6] Research Institution of Southeast University, 87 Dingjiaqiao Road, 210009, Nanjing, China

*Yadong Feng, Yan Liang and Bin Yao contributed equally to this paper.

Corresponding Authors:
Yadong Feng, Department of Gastroenterology, Zhongda Hospital, School of Medicine, Southeast University, 87 Dingjiaqiao Road, 210009, Nanjing, China.
Email: drfengyd@126.com

Peilin Huang, Research Institution of Southeast University, 87 Dingjiaqiao Road, 210009, Nanjing, China.
Email: 13951940316@163.com

Ruihua Shi, Department of Gastroenterology, Zhongda Hospital, School of Medicine, Southeast University, 87 Dingjiaqiao Road, 210009, Nanjing, China; Nanjing Medical University, 101 Longmian Road, 211166, Nanjing, China.
Email: ruihuashi@126.com

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access page (https://us.sagepub.com/en-us/nam/open-access-at-sage).

Introduction

Esophageal cancer (EC) is the eighth most common cancer, and the sixth most frequent cause of cancer mortality worldwide.[1] In China, esophageal squamous cell carcinoma (ESCC) is the predominant histological subtype of esophageal cancer, which also accounts for about 70% of worldwide cases.[2,3] Endoscopic Lugol's iodine staining[4] is widely applied for screening of early ESCC[5] and its precursor lesions in high-risk areas.[6] However, massive endoscopy screening for early ESCC is medical-source demanding. Furthermore, due to the truth that ESCC is a rare disease even in high-risk areas, which has an incidence of approximately 30/100,000 per year,[7,8] endoscopic screening for early ESCC in the general population can be regarded as high-cost. In addition, acceptance of endoscopic examination by patients is also relatively low. Subsequently, limiting the sample size of endoscopic screening precisely by identifying individuals who really need endoscopy examinations is more desirable.

Cytological detection can be used for a preliminary screening before endoscopy in areas prone to a high incidence of ESCC.[9,10] However, due to low accuracy, sensitivity and specificity, cytological screening for early ESCC remains challenging. Some methods, such as sponge and balloon cytology, have been reported to be not suitable for massive screening of ESCC.[10,11] In recent years, some improvements have been achieved in terms of sampling device. A capsule device (Cytosponge, Medtronic, Minneapolis, MN) was designed to collect esophageal specimens. According to previous studies, this newly designed device has high acceptability among most participants.[11-14] This capsule sampler has been used to detect early ESCC.[11] Retrieved specimen can be well processed by a liquid-based method, thus, will facilitate further cytological diagnosis.[11-16] Manually, cytological screening for abnormal cells among a large amount of cells is not practical. Thus, Cytosponge-derived samples are always evaluated by an alternative method, such as immunochemical analysis or fluorescent in-situ hybridization targeting p53.[11-14] In case of large scale screening for early esophageal lesions, application of a simple and efficient cytological method as a primary screening is desirable. Furthermore, how to improve the efficiency of diagnosis is a crucial question that needs to be resolved.

A barrier that hampers cytology as a preliminary screening is the diagnostic efficiency and reproducibility. Application of artificial intelligence (AI) provides a novel method for solution of this dilemma of analyzing large amounts of bio-images.[17,18] By using a deep-learning approach, a computer-assisted screening system that has robust and fast cell recognition can be constructed.[18] Thus, great progress has been achieved in improving the diagnostic accuracy and effectiveness of preliminary assessments of a large number of cells.[17,18] Although a computer-assisted analysis may promote the efficiency of cytological diagnosis, no such method for screening of early esophageal squamous lesions is available.

In this study, for the interest of establishing a novel pre-endoscopy screening method, we designed a rapid cytological screening scheme, which consists of fast specimen retrieval, automated sample processing, computer-assisted fast cytological screening and manual confirmation. The aim of this study is to investigate the efficacy of this method as a primary screening in detection of early ESCC and precursor lesions.

Methods

This study was registered on chineseclinicaltrials.gov (ChiCTR1900028524). The protocol is in accordance with the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in a prior approval by the review board of a Chinese tertiary academic hospital, Zhongda Hospital, which is affiliated to Southeast University (Ref No.2019ZDSYLL092-P01). Written informed consent was obtained from each participant before capsule ingestion. This study was performed between July seventh, 2019 and August 30th, 2020.

All main devices, including the cytosponge capsule (Shikang I®; application for a Chinese invention patent is currently underway, 201911049863.7), cell preservation solution (Chinese invention patent, ZL201810314036.5), Feulgen-Eosin staining (Chinese invention patent, ZL 201710732464.5), the double charge-coupled device camera (Chinese invention patent, ZL201110071786.2 and ZL201110071790.9) and the computer-assisted screening system (Chinese software copyright, 0791278, 0977924 and 3031912) are products of Nanjing Froeasy Technology Development CO., Ltd (Foreasy Tech Co., Nanjing, China). As, Cytosponge is not available in China, Shikang I® is a newly designed analog for retrieval of esophageal epithelial samples and is recommended by a newly issued Chinese guideline for early screening of early ESCC and precursor lesions.[19]

Capsule Device and Cytological Sample Collection

The capsule device was showed in Figure 1. The main components are an ingestible gelatin capsule, a polyurethane made sponge mesh and an attached string. The capsule was designed to restrain the sponge mesh. The capsule sponge was introduced into the stomach by ingestion with water, with the end of the string held outside the mouth without any tension. The device was kept in the proximal stomach for 5 min for capsule dissolution and release of the sponge mesh. When released from the capsule, the sponge mesh was used for collection of epithelial cytological samples by gently pulling the attached string out of the mouth. Then the string was cut and the sponge derived specimen was kept in cell preservation solution. Epithelial samples from the upper gastrointestinal tract, including the gastric fundus, the cardia and the esophagus, were available. For the aim of screening of esophageal lesions, squamous cells were of interest and chosen for evaluation.

Brief Description of the Computer-Assisted Screening System

A strategy of screening abnormal nuclei was applied by this fast cytological screening system. Esophageal squmaous cells were

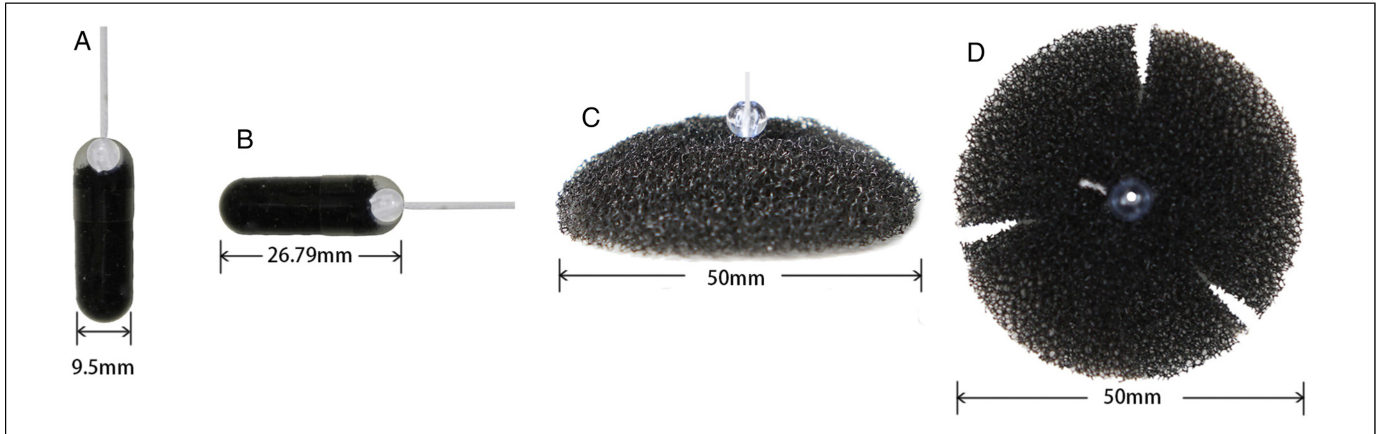


Figure 1. Details of Shikang I® capsule sponge. As shown in A and B, the capsule sponge device consists of an ingestible gelatin capsule (26.79 mm in length and was 9.5 mm in diameter), a compressed sponge mesh made of polyurethane, and a 60-cm long string. The string is fixed to the sponge mesh via a small glass bead. When released from the capsule, the sponge mesh is contained within a 50-mm elliptical doom structure (C), and it is assembled by three independent, identically sized components (D).

retrieved from 71 patients with early ESCC and precursor lesions by endoscopic brushing, and were divided into training and validation materials. In the training phase, 55,600 Feulgen-Eosin stained cells from 21 patients were set as training set. Digital images of cells and nuclei were acquired by automated filming and scanning by the double charge-coupled device equipped camera (Foreasy Tech Co., Nanjing, China). The pixel value of single cell and relevant nucleus was 89 × 89 dpi. Abnormal cells were manually annotated by expert pathologists and were classified as atypical squamous cells (ASC), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL) and squamous cell carcinoma (SCC).[20-22]

Convolutional neural networks (CNNs) were applied for establishment of this system. Seg Net, which is a fully convolutional network, was used for automated orientation and segmentation of digital images of nuclei and whole cells. A deep residual network (Res Net) was applied for identifying and classifying different morphometric features. Hence, squmaous cells could be automatically identified and classified. The training procedure was developed by iterations through the entire training set. After 300 epochs, the training procedure was terminated due to no improvement in diagnostic accuracy. DNA image cytometry was set as the main parameter for identifying abnormal cells. DNA stemline aneuploidy was assumed if the mean value of the nuclear DNA content of a stemline was >/<10% of 2c or 4c. Single-cell DNA aneuploidy was assumed if ≥5c occurred (DNA index≥2.5). To increase the diagnostic sensitivity of suspicious malignant nuclei, a ratio between digitally abnormal and diploid nuclei of ≥2.5 was set as an auxiliary threshold. By using 86,200 cells from 50 patients with early ESCC and precursor lesions with as validation set, the sensitivity and specificity in identifying abnormal cells were 95.82% and 92.05%, respectively.

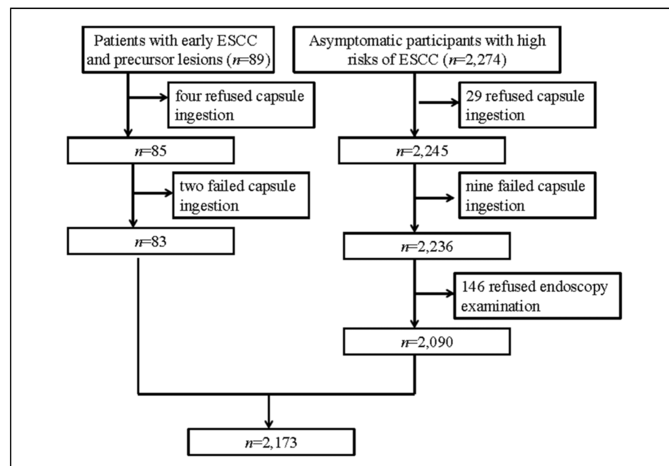


Figure 2. Flowchart of this study. ESCC: esophageal squmaous cell carcinoma

Participants

The flowchart is listed in Figure 2. There were two groups of participants: (1) patients with early ESCC and precursor lesions[23] confirmed by prior endoscopy and biopsy (Group A); (2) asymptomatic participants, who were with high risk factors of ESCC[19] and tended to undergo endoscopy examination (Group B). Participants with capsule and endoscopic results were enrolled. Endoscopic Lugol's iodine staining and biopsy were set as gold standard.

Specimen Processing, Slides Preparation and Staining

All specimens were processed by a liquid-based thin layer cell preparation technique. A two-stage cell enrichment protocol was adopted. In each round, the sponge was washed with 60 ml of preservative fluid, and oscillated for 15 min. We actually performed a third round of cell enrichment in several

specimens; however, no cell was detected in the third round. All cells were transferred to the slides using a sedimentation technique and treated by Feulgen-Eosin staining. One hundred and twenty slides were prepared for each case.

Computer -Assisted Cytological Diagnosis and Histopathological Diagnosis

Slides were subjected to a double charge-coupled device camera for automated scanning (Figure 3), followed by a computer-assisted screening. Typical images of ASC, LGIN, HGIN and SCC are shown as Figure 4. Details of the working display of this system are shown in Figure 5. Some main parameters, including DNA content, ratio between digitally abnormal and all diploid nuclei, total cell count and abnormal cell count, parameters of abnormal cells in detail, were annotated automatically. Therefore, abnormal cells could be identified and annotated. Also, clumped abnormal cells could be differed from those normal cells (supplementary Figure 1). Annotated cells were manually reviewed by two expert pathologists (Jiajia Xu and Peilin Huang). And, abnormal cells were confirmed according to previous diagnostic criteria.[20-22]

For histopathological diagnosis, early ESCC and precursor lesions, including early ESCC, high-grade intraepithelial neoplasia (HGIN) and low-grade intraepithelial neoplasia (LGIN), were defined according to 2019 WHO classification of tumors of the digestive system.[23]

Comparison of Computer-Assisted Diagnosis and Manual Diagnosis

Thirty Feulgen-Eosin stained slides from 15 patients with early ESCC and HGIN were randomly selected. According to previous cytological diagnosis, ASC, LSIL, HSIL and SCC were present in 7, 7, 9 and 7 slides, respectively. Three expert cytopathologists were masked to the cytological results and were asked to make a manual diagnosis and a computer-assisted diagnosis.

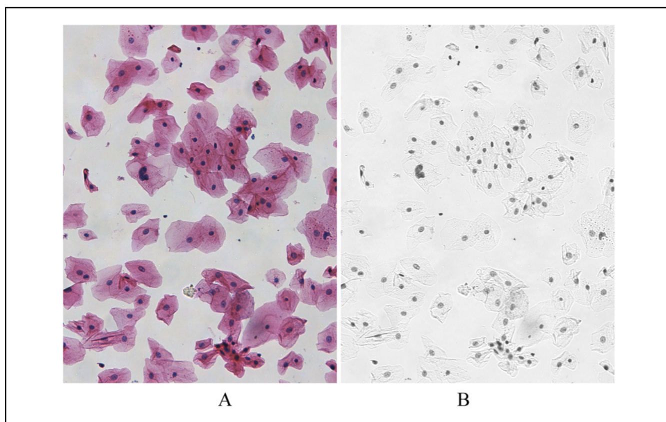


Figure 3. Digital images of cells by a double charge-coupled device camera (×20). Cells were processed in Feulgen-Eosin staining. A. whole cells in color images; B. nuclei in black images.

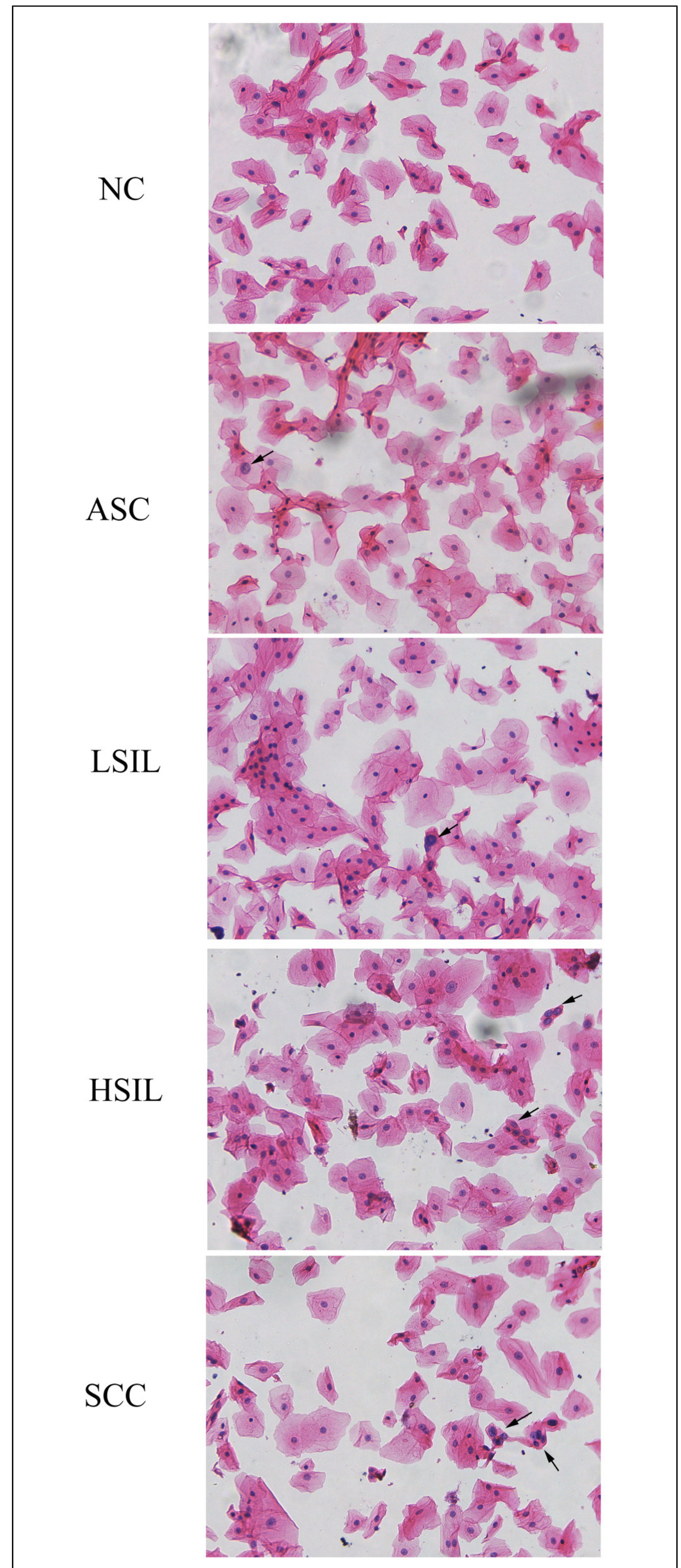


Figure 4. Esophageal squmaous cells in Feulgen-Eosin staining. Cells were present in ×20. NC: normal squmaous cells; ASC: atypical squamous cells; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; SCC: squamous cell carcinoma. Typical cells of ASC, LSIL, HSIL and SCC were labeled with black arrows.

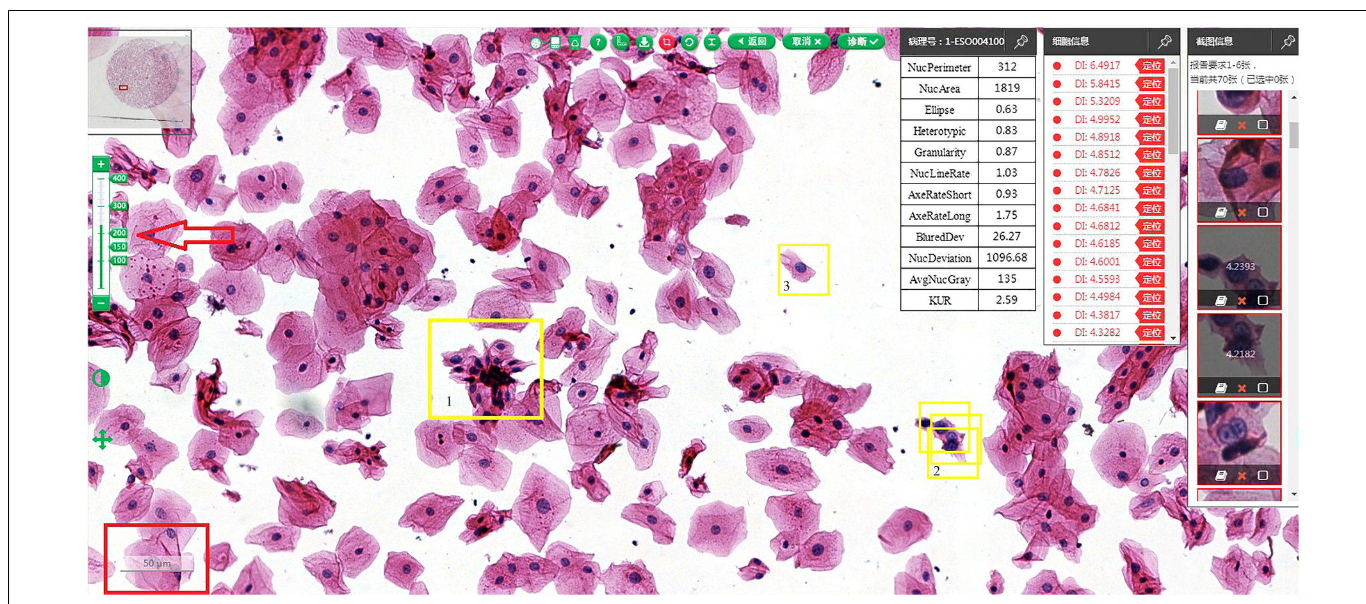


Figure 5. Working display of this computer-assisted screening system (in Chinese). Cells were treated in Feulgen-Eosin staining. Abnormal cells were annotated automatically, and the visual field can be enlarged and contracted. Cell images, DNA index along with some main parameters, including nuclear perimeter, nuclear area, ellipse, nuclear heterotypic, granularity of chromatin, nuclear line rate, rate in short axe, rate in long axe, nuclear blured deviation, nuclear deviation, average nuclear gray and kurtosis were listed. The whole view of scanned image of a prepared slide was shown in the black box. And, location of interested cells in the slide was shown in the small red box. Abnormal epithelial squmaous cells, as presented in the yellow boxes, were automatically identified. In detail, 1 and 2 were clumped SCCs, 3 was single LSIL. Typical normal esophageal epithelial squmaous cell was shown in the red box. Red arrow was a control bar for zooming in and zooming out.

A manual system from Foreasy Tech Co., which consists of a microscope with a $\times$20 objective, a condenser numerical aperture (NA) of 0.75 and a camera adapter of factor 0.87, a double charge-coupled device camera, and a computer with a high-resolution monitor, was used for manual diagnosis. Each slide was subjected to this system, and DNA content and cytological features were evaluated manually. Nuclei from lymphocytes, with coefficient of variation <5%,[24] were chosen as internal reference cells for evaluation of DNA content. Smears that were suspicious for abnormal nuclei were chosen for further diagnosis. Manual diagnosis was made according to criteria for squamous epithelial cells lesions.[20-22]

For computer-assisted diagnosis, potential abnormal cells were annotated by the computer-assisted screening system, and were subjected for further manual diagnosis. In this procedure, normal cells confirmed by automated recognition were remitted from manual review. Outcomes of diagnosis and inter-observer agreements were compared between two modalities of diagnosis.

Statistical Analysis

Continuous variables are expressed as mean $\pm$ standard deviation (SD), while categorical variables are expressed as frequencies and percentages. Setting the pathological diagnosis as the gold standard, the diagnostic accuracy, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of computer-assisted cytological diagnosis were calculated. The Chi-square test, one-way analysis of variance (ANOVA), the Mann–Whitney *U* test were performed using SPSS statistical software for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered to be statistically significant.

Results

Participants

Eighty-nine patients from Group A and 2,274 participants from Group B were initially recruited. Among them, four patients from Group A and 29 participants from Group B denied capsule ingestion. Two patients from Group A and nine participants from Group B failed to ingest a capsule. Subsequently, 2,319 (83 from Group A and 2,236 from Group B) participants successfully ingested the capsule. In Group B, 272 participants were with abnormal cells, and they all underwent endoscopy examination. Among those who were with negative cytological results, 146 participants denied a followed-up endoscopy. Finally, 2,173 participants were included (Figure 2). Basal characteristics of enrolled patients and participants were listed in Table1 and 2.

Overview of Capsule Ingestion, Sample Processing and Laboratory Evaluation

Finally, 2,269 participants (80 from Group A and 2,189 from Group B) swallowed the capsule at the first attempt, while the remaining 50 participants (3 from Group A, 47 from Group

B) completed the ingestion at the second attempt. The mean duration of capsule ingestion was 3.2 ± 1.36 (2-8) minutes. Transient gagging was presented in 45 participants. No detachment of sponge or other adverse events occurred. Cell enrichment, slides preparation and Feulgen-Eosin staining were completed in 50 and 40 min, respectively. The duration of scanning was one minute per slide; hence, scanning for all slides was accomplished within 120 min. Manual confirmation for automatically identified abnormal cells from 120 slides was completed in 50.45 ± 10.75 (8-142) minutes. The whole procedure, from specimen retrieval to finally manual diagnosis, was completed within two working days.

Cytological Diagnosis and Relationship with Endoscopic Findings

Results of Group A are listed in Table 3. Mean total cell counts and average cell counts per slide were 7,676,803 ± 119,609 (5,201,081-10,092,737) and 63,973 ± 9967 (43,342-84,106), respectively. Abnormal cells were detected for all 83 patients, with a mean number of 159 ± 242 (11-1201). ASC, LSIL, HSIL and SCC were present in 17, 31, 24 and 11 patients, respectively. Among 17 patients with ASC, 16 and 1 patient(s) were with histologically confirmed LGIN and HGIN, respectively. Therefore, setting ASC as threshold, the positive rate was about 98.5% in detecting histologically confirmed ESCC and HGIN, which are with urgent clinical significances.

Results of Group B are listed in Table 4. Mean total cell counts and average cell counts per slide were 7,438,869 ± 1,175,753 (4,894,116-9,353,476) and 61,990 ± 9797 (40,784-77,945), respectively. Among these, 272 participants were with abnormal cells. There were 143, 99, 24 and 6 participants with ASC, LSIL, HSIL and SCC, respectively. Mean number of abnormal cells was 59 ± 16 (9-204). Cytological diagnosis was compared with endoscopy examination and biopsy (Table 5). There were 8, 13, 11, 187 and 53 participants with early ESCC, HGIN, LGIN and reflux esophagitis and normal endoscopic findings in those who with abnormal cells, respectively. And, 1,746 and 72 participants were with normal findings and reflux esophagitis in those who with normal cytological results, respectively. According to clinically significance, 21 patients with histologically confirmed ESCC and HGIN were re-visited for consideration of high risk factors. Their mean age was 65.3 ± 13.2(55-72) years. Among them, 6 were from areas with high incidence of ESCC, 7, 11, 8, 9 and 3 were with smoking, heavy drinking, a history of SCC, family history of ESCC and with multiple (≥2) risk factors, respectively. The sensitivity, specificity, PPV and NPV of detection of early ESCC and the precursor lesions were 100%, 88.34%, 11.76% and 100%, respectively.

Evaluation of Diagnostic Efficacy, Accuracy and inter-Observer Agreement

Manual confirmation by expert cytopathologists for abnormal cells identified from thirty slides by computer-assisted diagnosis presented with a shorter time duration for diagnosis (5.4 ± 0.45 min vs 320.2 ± 132.4 min, $p < 0.001$), a significant higher diagnostic accuracy (96.7% vs74.4%, $p = 0.015$) and a better inter-observer agreement (28/30, 93.3% vs20/30, 66.7%, K = 0.286, $p < 0.001$) than those of manual diagnosis only.

Discussion

Since the 1960s, some cytological methods, including endoscopic brushing, sponge and balloon cytology, have been introduced into screening for early ESCC.[10,25] These methods are performed without endoscopy or sedation, and can be applied at scale in limited resource settings. However, these detections are mainly limited by unsatisfactory results.[25] Recently, Cytosponge has been applied for sampling for esophageal specimens[11-14,26] and proved to be promising in detection of early esophageal lesions.[11-14] Since manual diagnosis is not practical for population-based large scale screening, we have

Table 1. Charactristics of 83 patients with early ESCC and precursor lesions.

Total patients	n = 83
Age, years(range)	61.27 ± 9.23(55-72)
Gender,(n)	
male	52
female	31
Lesions,(n)	
Early ESCC	25
HGIN	31
LGIN	27

ESCC: esophageal squmasous cell carcinoma; HGIN: high-grade intraepithelial neoplasia; LGIN: low-grade intraepithelial neoplasia

Table 2. Charactersitics of asymptomatic participants with high risk factors.

Total participants	n = 2,090
Age, years(range)	55.45 ± 10.53(43-75)
Gender(n)	
male	1,274
female	816
Risk factors,(n)	
family history of ESCC	256
smoking	1,437
heavy drinking	1,279
history of SCC	109
Numbers of high risk	
factors,(n)	1,329
1	371
2	224
3	166
4	

ESCC: esophageal squmasous cell carcinoma; SCC: squmasous cell carcinoma

Table 3. Results from patients with early ESCC and precursor lesions.

Patients	n = 83
Mean total cell counts, mean ± SD (range)	7,676,803 ± 119,609 (5,201,081-10,092,737)
Mean cell counts per slide, mean ± SD (range)	63,973 ± 9967 (43,342-84,106)
Mean numbers of abnormal cells	159 ± 242 (11-1201)
Cytological diagnosis, (n)	
ASC	17
LSIL	31
HSIL	24
SCC	11

ESCC: esophageal squmaous cell carcinoma; SCC: squmaous cell carcinoma; ASC: atypical squamous cells; LSIL: low-grade squamous intraepithelial lesion; HSIL:high-grade squamous intraepithelial lesion

developed a rapid screening procedure and evaluated its efficiency as a pre-endoscopy screening. Specimens were retrieved by a capsule ingestion, and sequentially subjected for cytological processing, computer-assisted fast screening and manual diagnosis.

In this study, a rapid sample collection, processing and diagnosis procedure was present. Esophageal sampling was achieved by using a newly designed sponge device. Although this newly designed sponge is with a larger size than that of Cytosponge, it showed a novel acceptability and a satisfactory result in sample collecting. An automated procedure, including automated sample processing, slides preparation and staining, was used to process all specimens. Based on our data, the proportion of abnormal cells was very small, and manual screening for few cells of interest among all available cells is very challenging. Thus, some other examinations, such as immunochemical staining of trefoil factor3[12] and p53,[11] as well as fluorescent in-situ hybridization of p53,[14] were applied as alternative methods for evaluation of Cytosponge derived specimens. Although a batch strategy can be applied for laboratory treatment, it is time-consuming to evaluate all prepared slides by manual diagnosis. Furthermore, the cost is also increased by performing such adjunctive detections. Hence, an AI-assisted diagnosis is appreciated for such diagnosis. According to a recent study by Gehrung M et al.,[27] a deep learning based well-trained framework to analyze samples of the Cytosponge-TFF3 test is feasible for diagnosis of Barrett's esophagus for early detection of esophageal adenocarcinoma. In this study, we have developed a computer-assisted screening platform for a primary screening. Abnormal DNA content, that 5cEE (5c exceeding events, DNA index score >2.5) ≥1[28] was set as the first cut-off value for identifying potential abnormal nuclei. In previous studies, 9cEE ≥1 is always used for diagnosis of cancerous cells.[18,29] However, 9cEE ≥1 is not sufficient for screening of precursor lesions,[30] and a lower threshold was adopted. Due to the same reason, a ratio between digitally abnormal and diploid nuclei of ≥2.5[20-22] was set as the second criterion. Subsequently, few potential cells with abnormal nuclei were identified and subjected

Table 4. Results from asymptomatic participants.

Total participants	n = 2,090
Mean total cell counts, mean ± SD (range)	7,438,869 ± 1,175,753 (4,894,116-9,353,476)
Mean cell counts per slide, mean ± SD (range)	61,990 ± 9797 (40,784-77,945)
Cytological diagnosis	
Normal results	1,818
Abnormal results,(n)	272
ASC	143
LSIL	99
HSIL	24
SCC	6
Mean numbers of abnormal cells	59 ± 16 (9-204)
Endoscopic diagnosis, (n)	
Normal cytological results	1,818
normal findings	1,746
reflux esophagitis	72
Abnormal cytological results	272
normal findings	53
reflux esophagitis	187
LGIN	11
HGIN	13
early ESCC	8

ASC: atypical squamous cells; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; SCC: squmaous cell carcinoma; LGIN: low-grade intraepithelial neoplasia; HGIN: high-grade intraepithelial neoplasia; ESCC: esophageal squmaous cell carcinoma;

for manual diagnosis. Taking advantages of Feulgen-Eosin staining, morphological characteristics of whole cells and relevant nuclei are clearly presented, which makes followed manual diagnosis convenient, high efficient and time saving. In this study, a 100% diagnostic accuracy was achieved in patients with early ESCC and precursor lesions. The proportion of subjects with abnormal cells was 13.01% (272/2090), and was similar with previous studies.[11,14] In addition, all participants with

Table 5. Comparsion between cytological results and endoscopic/histopathological outcomes in asymptomatic participants

	Cytology				
Endoscopy	ASC ($n = 143$)	LSIL ($n = 99$)	HSIL ($n = 24$)	SCC ($n = 6$)	Normal findings ($n = 1,818$)
ESCC, $n = 8$	0	0	2	6	0
HGIN, $n = 13$	0	0	13	0	0
LGIN, $n = 11$	0	2	9	0	0
RE, $n = 259$	98	89	0	0	72
Normal findings, $n = 1799$	45	8	0	0	1746

ASC: atypical squamous cells; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; SCC: squmaous cell carcinoma; LGIN: low-grade intraepithelial neoplasia; HGIN: high-grade intraepithelial neoplasia; ESCC: esophageal squmaous cell carcinoma; RE: reflux esophagitis

endoscopically and histopathologically confirmed early ESCC and precursor lesions were with abnormal cytological results. Thus, this method is clinically significant as a potential method as pre-endoscopy screening for early ESCC and precursor lesions. Participants who really need endoscopy examination can be identified, and follow-up endoscopy for confirmation can be performed in a limited population. Also, unnecessary endoscopy can be avoided in those who with negative cytological findings. Hence, this detection may make endoscopic screening medical-source saving.

To achieve a high diagnostic efficiency is one crucial aim of cytological detection. AI-assisted diagnosis provides a novel solution for boring pathological diagnosis, and also demonstrates a high efficiency.[18,27] In this study, a final cytological diagnosis for batch specimen can be completed within two working days by using a semi-automated detection assisted analysis. This present semi-automated diagnosis was superior to manual diagnosis by presenting a higher diagnostic efficiency, reproducibility and inter-observer agreement.[18,27] Therefore, this method meets the requirements of widespread screening. Batch processing and rapid confirmation for multiple specimens are technically feasible in a single procedure, and the following-up endoscopy can be performed timely.

The proportions of endoscopically and histopathologically confirmed early ESCC, HGIN and LGIN were 0.038%, 0.062% and 0.057%, respectively, and were significantly lower than those from extremely high-prevalence regions,[11,31,32] which were about 0.19%, 0.36% and 2.57%,[32] respectively. This difference may be due to our study was conducted in a region with a relatively lower incidence of disease. Although ASC is a low diagnostic threshold, we adopted it for primary screening for two main reasons. Firstly, the diagnostic threshold in cytological screening for early ESCC is not fully established, and ASC is commonly used for cytological detection of histopathologically confirmed ESCC.[11] Secondly, there were some worries about increased cases of false negative diagnosis or misdiagnosis if a higher threshold was applied. As a result, many individuals with ASC were identified in this patient cohort. Because of presence of ASC as atypical changes in moderate to severe esophagitis,[11] many cases of reflux esophagitis were confirmed by endoscopy examinations. Subsequently, the specificity and PPV were not high. The present specificity and PPV are similar with those from previous studies by performing immunochemical staining and FISH targeting p53.[11,14] By using this threshold, the numbers of participants who need endoscopy was significantly limited. Therefore, this method can make large-scale endoscopy screening much cost-saving. This method is superior to previous studies by presenting a satisfactory sensitivity and NPV.[11,14-16] Subsequently, this cytological detection as a primary screening can be warranted by identifying subjects who need endoscopy examination from general population. A higher diagnostic threshold will be evaluated in the next stage by comparing outcomes of cytological detections and endoscopic findings in a large scale of population.

In recent years, wide-area transepithelial sampling of the esophagus with computer-assisted three-dimensional analysis (WATS[3D], CDx Diagnostics, Suffern, NY) has been applied for early detection of Barrett's esophagus.[33-35] WATS[3D] and our method are also a deep learning based detection, and these two methods are similar by providing digital images for manual diagnosis. These two detections facilitate manual diagnosis, and improve inter-observer agreement among pathologists.[33] Differences between WSTA[3D] and our analysis are listed as follows. Firstly, these two methods are initially designed for detection of different diseases. Secondly, sample collections are different. Endoscopic brushing is needed in WSTA[3D] sample collection. Third, WSTA[3D] provides a simulated image of an in vivo, en face view of a gland for further evaluation. And multiple two-dimensional images are needed to synthesize a single three-dimensional image.

There are some limitations of this study. Firstly, since this is a preliminary pilot study, the sample size is relatively small. In the next stage, a multicenter research study will be conducted for further evaluation. Secondly, this is a one-time study, whereas in future work, additional data will be acquired, and a cost-effectiveness analysis will be performed in the next stage.

Conclusion

We have demonstrated a new strategy for the rapid screening for early esophageal lesions. Due to the high accuracy and efficiency, this working scheme may provide a novelly efficient, accurate, and medical-resource-saving screening method for screening early ESCC and precursor lesions. This method is competent for pre-endoscopy screening for detecting early esophageal lesions. Since this the first-stage research, the diagnostic threshold for this detection should be further evaluated. For this aim, more patients and asymptomatic participants with high risks of ESCC are needed for further evaluation. And, the correlation between cytology and endoscopic findings should be explored.

Acknowledgements

We thank Mrs. Xiaomei Sun and Dr Lihua Zhang for their help in sample collection and treatment.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. Mr. Bin Yao, Ms. Juncai Zang, Mr. Bo Wei and Mr. Guangpeng Xu are employees of Foreasy Tech Co. They are the main members of two projects. They do not have any financial arrangement for this research and the preparation of this manuscript.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by Jiangsu Provincial Special Progarm of Medical Science (BE2019710 and BE2018620) and Nanjing Technological Development Program (201803056).

ORCID iD

Yadong Feng https://orcid.org/0000-0001-9259-3840

Supplemental Material

Supplemental material for this article is available online.

References

1. Ferlay J, Colombet M, Soerjomataram I, et al. Estimating the global cancer incidence and mortality in 2018: gLOBOCAN sources and methods. *Int J Cancer*. 2019;144(8):1941-1953. doi: 10.1002/ijc.31937
2. National Health Commission of the People's Republic of China. Chinese Guidelines for diagnosis and treatment of esophageal carcinoma 2018 (English version). *Chin J Cancer Res.* 2019;31-(2):223-258. doi: 10.21147/j.issn.1000-9604.2019.02.01
3. Zeng H, Chen W, Zheng R, et al. Changing cancer survival in China during 2003-15: a pooled analysis of 17 population-based cancer registries. *Lancet Glob Health*. 2018;6(5):e555-e567. doi: 10.1016/S2214-109X(18)30127-X
4. Dawsey SM, Fleischer DE, Wang GQ, et al. Mucosal iodine staining improves endoscopic visualization of squamous dysplasia and squamous cell carcinoma of the esophagus in linxian, China. *Cancer*. 1998;83(2):220-231.
5. Kuwano H, Nishimura Y, Oyama T, et al. Guidelines for diagnosis and treatment of carcinoma of the esophagus April 2012 edited by the Japan esophageal society. *Esophagus*. 2015;12(1):1-30. doi: 10.1007/s10388-014-0465-1
6. Yang S, Wu S, Huang Y, et al. Screening for oesophageal cancer. *Cochrane Database Syst Rev*. 2012;12, Cd007883:1-27. doi: 10.1002/14651858.CD007883.pub2.
7. Chen Q, Yu L, Hao C, et al. Effectiveness evaluation of organized screening for esophageal cancer: a case-control study in linzhou city, China. *Sci Rep*. 2016;6:35707. doi: 10.1038/srep35707
8. Li YY, Du LB, Hu XQ, et al. A suggested framework for conducting esophageal cancer screening in China. *J Dig Dis*. 2018;19-(12):722-729. doi: 10.1111/1751-2980.12675
9. Pan QJ, Roth MJ, Guo HQ, et al. Cytologic detection of esophageal squamous cell carcinoma and its precursor lesions using balloon samplers and liquid-based cytology in asymptomatic adults in llinxian, China. *Acta Cytol*. 2008;52(1):14-23. doi: 10. 1159/000325430
10. Gao QY, Fang JY. Early esophageal cancer screening in China. *Best Pract Res Clin Gastroenterol*. 2015;29(6):885-893. doi: 10. 1016/j.bpg.2015.09.018
11. Roshandel G, Merat S, Sotoudeh M, et al. Pilot study of cytological testing for oesophageal squamous cell dysplasia in a high-risk area in northern Iran. *Br J Cancer*. 2014;111(12):2235-2241. doi: 10.1038/bjc.2014.506
12. Kadri SR, Lao-Sirieix P, O'Donovan M, et al. Acceptability and accuracy of a non-endoscopic screening test for Barrett's Oesophagus in primary care: cohort study. *Br Med J.* 2010;341, c4372:1-8. doi: 10.1136/bmj.c4372.
13. Katzka DA, Geno DM, Ravi A, et al. Accuracy, safety, and tolerability of tissue collection by cytosponge versus endoscopy for evaluation of eosinophilic esophagitis. *Clin Gastroenterol Hepatol*. 2015;13(1):77-83. e2.doi: 10. 1016 /j. cgh. 2014.06.026.
14. Haisley KR, Dolan JP, Olson SB, et al. Sponge sampling with fluorescent in situ hybridization as a screening tool for the early detection of esophageal cancer. *J Gastrointest Surg*. 2017;21-(2):215-221. doi: 10.1007/s11605-016-3239-3
15. Vidyavathi K, Harendrakumar ML, Lakshmana Kumar YC. Correlation of endoscopic brush cytology with biopsy in diagnosis of upper gastrointestinal neoplasms. *Indian J Pathol Microbiol*. 2008;51(4):489-492. doi: 10.4103/0377-4929.43737
16. Mariano VS, Pastrez PRA, Mafra Costa A, et al. Impact of brush cytology analysis for the diagnosis of esophageal squamous cell carcinoma: the quality of liquid-based preparation of cytological slides. *Acta Cytol*. 2019;63(3):240-246. doi: 10.1159/000496567
17. Sarwar S, Dent A, Faust K, et al. Physician perspectives on integration of artificial intelligence into diagnostic pathology. *NPJ Digit Med*. 2019;2(28):1-7. doi: 10.1038/s41746-019-0106-0
18. Böcking AH, Friedrich D, Meyer-Ebrecht D, et al. Automated detection of cancer cells in effusion specimens by DNA karyometry. *Cancer Cytopathol*. 2019;127(1):18-25. doi: 10.1002/cncy. 22072
19. National quality control center of digestive endoscopy, national clinical research center for digestive disease (Shanghai), national early gastrointestinal-cancer prevention & treatment alliance, Chinese endoscopist association, Chinese society of digestive endoscopy, Chinese society of health management, cancer endoscopy professional committee of China anti-cancer association. Chinese Experts consensus on the protocol of early esophageal cancer and pre-cancerous lesion screening (2019, Xinxiang). *Chin J Dig Endosc.* 2019;36(11):793-801. doi: 10.3760/cma.j.issn.1007-5232.2019.11.001. [in Chinese].
20. Shufan Liu, Xiu Kan. *Cellular Pathology*. China Union Medical University Press, 2011. 270-296.
21. Koss LG. Cytologic diagnosis of oral, esophageal, and peripheral lung cancer. *J Cell Biochem Suppl*. 1993;17F:66-81. doi: 10.1002/jcb.240531010
22. Lin Y, Totsuka Y, He Y, et al. Epidemiology of esophageal cancer in Japan and China. *J Epidemiol*. 2013;23(4):233-242. doi: 10. 2188/jea.je 20120162
23. WHO classification of tumors editorial board. *WHO classification of Tumors: Digestive System Tumors*. 5th ed. IARC Press; 2019:23-58.
24. Giroud F, Haroske G, Reith A, et al. 1997 ESACP consensus report on diagnostic DNA image cytometry. Part I: basic considerations and recommendations for preparation, measurement and interpretation. European society for analytical cellular pathology. *Anal Cell Pathol*. 1998;17(4):189-200. doi: 10.1155/1998/390837
25. Codipilly DC, Qin Y, Dawsey SM, et al. Screening for esophageal squamous cell carcinoma: recent advances. *Gastrointest Endosc*. 2018;88(3):413-426. doi: 10.1016/j.gie.2018.04.2352
26. Freeman M, Offman J, Walter FM, et al. Acceptability of the cytosponge procedure for detecting Barrett's Oesophagus: a qualitative study. *BMJ Open*. 2017;7(3):e013901. doi: 10.1136/bmjopen-2016-013901
27. Gehrung M, Crispin-Ortuzar M, Berman AG, et al. Triage-driven diagnosis of Barrett's Esophagus for early detection of esophageal adenocarcinoma using deep learning. *Nat Med*. 2021;27(5):833-841. doi: 10.1038/s41591-021-01287-9
28. Bradley G, Odell EW, Raphael S, et al. Abnormal DNA content in oral epithelial dysplasia is associated with increased risk of progression to carcinoma. *Br J Cancer*. 2010;103(9):1432-1442. doi: 10.1038/sj.bjc.6605905

29. Kayser K, Blum S, Beyer M, et al. Routine DNA cytometry of benign and malignant pleural effusions by means of the remote quantitation server euroquant: a prospective study. *J Clin Pathol*. 2000;53(10):760-764. doi: 10.1136/jcp.53.10.760
30. Yang X, Xiao X, Wu W, et al. Cytological study of DNA content and nuclear morphometric analysis for aid in the diagnosis of high-grade dysplasia within oral leukoplakia. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2017;124(3):280-285. doi: 10.1016/j.oooo.2017.05.509
31. He Z, Liu Z, Liu M, et al. Efficacy of endoscopic screening for esophageal cancer in China (ESECC):design and preliminary results of a populationa-based randomized controlled trial. *Gut*. 2019;68(2):198-206. doi: 10.1136/gutjnl-2017-315520
32. He Z, Zhao Y, Guo C, et al. Prevalence and risk factors for esophageal squamous cell cancer and precursor lesions in anyang, China: a population-based endoscopic survey. *Br J Cancer*. 2010;103(7):1085-1088. doi: 10.1038/sj.bjc.6605843
33. Vennalaganti PR, Naag Kanakadandi V, Gross SA, et al. Inter-Observer agreement among pathologists using wide-area transepithelial sampling With computer-assisted analysis in patients With Barrett's Esophagus. *Am J Gastroenterol*. 2015;110(9):1257-1260. doi: 10.1038/ajg.2015.116
34. Docimo SJr, Al-Mansour M, Tsuda S. SAGES TAVAC safety and efficacy analysis WATS3D (CDx diagnostics, sufern, *NY).Surg Endosc*. 2020; 34 (9): 3743-3747. doi: 10. 1007/s00464-020-07503-w.
35. Singer ME, Smith MS. Wide area transepithelial sampling with computer-assisted analysis (WATS^{3D}) Is cost-efective in Barrett's Esophagus screening. *Dig Dis Sci*. 2021;66(5):1572-1579. doi: 10.1007/s10620-020-06412-1