Clin. Lab. 2020;66:XXX-XXX

ORIGINAL ARTICLE

Technical and Clinical Performance of Two Methods to Detect Squamous Cell Carcinoma Antigen Levels for Comparing Pathological Diagnosis Coincidence Rates in Lung, Cervical, and Head and Neck Cancers

Yanming Liu [1, #], Yue Cao [2, #], Shan He [3], Wencan Cai [1]

[#] *The authors contributed equally*
[1] *Department of Clinical Laboratory, Yuebei People's Hospital, Shaoguan, Guangdong Province, China*
[2] *Department of Medical Technology, Shaoguan Medical College, Shaoguan City, Guangdong Province, China*
[3] *Department of Medical Examination, Southern Medical University, Guangzhou Guangdong Province, China*

SUMMARY

Background: **Detection of serum squamous cell carcinoma (SCC) antigen (SCCA) contributes to the diagnosis and therapeutic monitoring of SCC. However, the results obtained from different detection systems are not always consistent.**

Methods: **In this study, we compared the performance of electrochemiluminescence assays (ECLIAs) and flow fluorescence immunoassays (FFIAs) (e.g. using a Luminex 200/xMap) in the detection of SCCA for the diagnosis of SCC of the lung (LSCC), cervix (CSCC), and head and neck (HANSCC) in serum samples from 154 healthy individuals and 236 patients with SCC. We also evaluated the consistency of the SCCA results with the pathological diagnosis for both methods.**

Results: **SCCA levels obtained from ECLIAs were significantly higher than those obtained from FFIAs for all groups. However, the results from the two methods were well correlated (r = 0.918). The diagnostic coincidence rates (FFIA versus ECLIA) for SCCA results in patients with LSCC, CSCC, and HANSCC were 40.82% versus 52.04%, 36.14% versus 57.14%, and 16.36% versus 23.64%, respectively, and the negative coincidence rate (FFIA versus ECLIA) in healthy individuals was 98.05% versus 98.70%. The cutoff value, sensitivity, specificity, and area under the receiver operating characteristic curve of SCCA diagnosis (FFIA versus ECLIA) in LSCC, CSCC, and HANSCC were 1.12, 77.55%, 85.34%, and 0.87 versus 3.07, 85.71%, 91.52%, and 0.91, respectively; 1.21, 81.93%, 90.2%, and 0.90 versus 3.84, 89.16%, 95.24%, and 0.95, respectively; and 1.01, 62.27%, 82%, and 0.81 versus 3.35, 58.18%, 89.65%, and 0.85, respectively.**

Conclusions: **Serum SCCA levels detected by ECLIAs were significantly higher than those detected by FFIAs, with higher detection performance and pathological diagnosis coincidence rate in the patients with LSCC and CSCC simultaneously.**

(Clin. Lab. 2020;66:xx-xx. DOI: 10.7754/Clin.Lab.2019.190912)

Correspondence:
Yue Cao, MD
Department of Medical Technology
Shaoguan Medical College
Xinhua South Road No. 108
Wujiang District
Shaoguan City
Guangdong Province
China
Email: 65395008@qq.com

Yanming Liu, MD
Department of Clinical Laboratory
Yuebei People's Hospital
Huimin South Road No.133
Wujiang District
Shaoguan City
Guangdong Province
China
Email: ybyylym@gdmu.edu.cn

Manuscript accepted December 11, 2019

KEY WORDS

squamous cell carcinoma antigen, electrochemiluminescence assay, flow fluorescence immunoassay, squamous cell carcinoma

LIST OF ABBREVIATIONS

ECLIA - electrochemiluminescence
FFIA - flow fluorescence immunoassay
SCCA - squamous cell carcinoma antigen
LSCC - lung squamous cell carcinoma
CSCC - cervical squamous cell carcinoma
HANSCC - head and neck squamous cell carcinoma
IQR - interquartile range
CV - coefficient of variation
LoQ - limit of quantitation
LoD - limit of detection
HAMA - human anti-mouse antibodies
RF - rheumatoid factor
HC - healthy controls
DCR - Diagnostic compliance rate
CI - confidence interval
AUC - area under curve

INTRODUCTION

Squamous cell carcinoma (SCC) is an epithelial malignancy that can be derived from different types of tissues, including the lungs, cervix, esophagus, oral cavity, and liver [1,2]. SCC antigen (SCCA) is a glycoprotein with a molecular weight ranging from 45 to 55 kDa, produced in normal squamous epithelial skin cells; this antigen acts as a serine protease inhibitor [3]. Because epithelial cells are widely distributed in the respiratory tract and the female reproductive tract, the incidences of SCC in these sites are high [4]. SCCA has more than 10 subtypes, which can be classified as acidic (isoelectric point [pI] < 6.25) or neutral (pI ≥ 6.25) [5]. Acidic SCCA proteins are easily released from the cytosol and are the main cause of elevated SCCA levels in serum [6]. In addition, SCCA seems to be associated with the invasion and metastasis of SCC; thus, serum SCCA can be used to monitor patients with SCC [7]. Non-small cell lung cancer accounts for approximately 85% of all lung cancers, of which about 35% are SCC [8]. In addition, approximately 80% of cervical cancer cases and 90% of head and neck cancer cases are SCC [9].

Detection of serum SCCA contributes to the diagnosis and therapeutic monitoring of SCCs, including lung SCC (LSCC), cervical SCC (CSCC), and head and neck SCC (HANSCC; e.g., laryngeal cancer, nasopharyngeal carcinoma, and esophageal cancer). However, significant differences in SCCA measurement results and reference intervals have been observed for healthy individuals when using different detection systems, which may be related to the different detection principles and antigenic epitopes used [10].

Accordingly, in this study, we compared the diagnostic performances of flow fluorescence immunoassays (FFIAs) and electrochemiluminescence assays (ECLIAs) for detection of SCCA in a clinical setting.

MATERIALS AND METHODS

Study design and serum samples

A retrospective case-control study, involving 390 screened subjects, was performed in 2018 by measuring the serum SCCA level at Yuebei Peoples' Hospital. PASS 15.0 was used to calculate the sample size in line with literature [10]. Samples were obtained from healthy controls (154 cases) and patients with LSCC (98 cases), CSCC (83 cases), or HANSCC (55 cases) diagnosed by postoperative pathology. The main inclusion criteria for healthy controls were as follows: apparently healthy with normal routine results (including leukocyte counts and levels of C-reactive protein, alanine aminotransferase, bilirubin, aspartate aminotransferase, urea, and creatinine to exclude inflammation, liver disease, or kidney disease); no known history of tumor disease, benign disorders, and chronic diseases; no smoking habit; and no psoriasis, allergic dermatitis, or other severe skin diseases [11]. Patients with LSCC, CSCC, and HANSCC were required to have primary tumors diagnosed pathologically but to have not yet received therapy or surgery. General exclusion criteria for all the groups were as follows: age less than 18 years, current pregnancy, and history of other malignant diseases. Because SCCA levels tend to be higher in patients with advanced chronic kidney disease with a glomerular filtration rate of less than 30 mL/min/1.73 m^2 [9] and in patients with psoriasis or other serious skin lesions [12], these patients were excluded. Serum samples were collected prior to treatment and after obtaining consent from patients with SCC. This study was approved by the Medical Ethics Committee of Yuebei Peoples' Hospital. For each serum sample, information regarding age and gender was also collected.

Sample storage and assays

Serum samples were stored at approximately -80°C prior to analysis. SCCA was measured twice using a Luminex 200 immunoassay analyzer (Tellgen, China Shanghai) and a Roche E602 Electrochemiluminescence immunoassay analyzer (Roche, Germany). The FFIA was a double-antibody sandwich flow fluorescence immunoassay. Capture antibodies against SCCA were covalently crosslinked on fluorescently encoded microspheres, made into suspensions of a certain concentration, and then incubated with serum at 37°C for 30 minutes. The phycoerythrin-labeled detection antibody solution against SCCA was added to form a complex of (microsphere-capture antibody)-SCCA-(detection antibody-phycoerythrin) at 37°C. Detection was performed on a Luminex multi-function flow dot matrix

Table 1. FFIA and ECLIA assay performance characteristics.

Parameter	FFIA SCCA assay	ECLIA SCCA assay
20-day precision	Total within-laboratory % CV ≤ 6.25%	Total within-laboratory % CV ≤ 6.25%
LoQ	0.5 ng/mL	0.6 ng/mL
LoD	0.5 ng/mL	0.2 ng/mL
Range	0.5 - 50 ng/mL	0.1 - 70 ng/mL
Extended range with auto dilution	1:10 auto-dilution to 500 ng/mL	1:20 auto-dilution to 1,400 ng/mL
Cutoff value	0 - 1.5 ng/mL	0 - 2.7 ng/mL
Hook effect	no high dose hook effect when SCCA ≤ 500 ng/mL	no high dose hook effect when SCCA ≤ 1,000 ng/mL
HAMA/RF and interferences	within ± 10% for HAMA/RF and potential interferents, no notable endogenous interferences observed	within ± 10% for HAMA/RF and potential interferents, no notable endogenous interferences observed

CV - coefficient of variation, LoQ - limit of quantitation, LoD - limit of detection, HAMA - human anti-mouse antibodies, RF - rheumatoid factor.

instrument, and the fluorescence signal of the microspheres was positively correlated with the SCCA concentration in the serum. The ECLIA used SCCA-specific monoclonal antibodies that recognized both human SCCA1 and SCCA2 subtypes in an equimolar model, which utilized the biotin streptomycin double-antibody sandwich principle. Values were then read on Elecsys and Cobas e602 analyzers. A 15-µL sample of peripheral blood was required for the test, and the total detection time was 18 minutes. The two detection systems were calibrated and verified by the manufacturer's engineers routinely, according to the user manual for each instrument. Masks and gloves were worn throughout the testing to prevent false-positive increases in SCCA caused by exogenous contamination, such as saliva and sweat [9]. The performance characteristics for the FFIAs and ECLIAs are described in Table 1.

Statistical analysis

Each variable was presented as the median and interquartile range (IQR). Statistical analysis was performed using IBM SPSS ver. 21.0 (IBM Corp., Armonk, NY, USA). Statistical comparisons were carried out using Student's *t*-tests or Pearson's chi-square tests. According to the SCCA reference ranges for healthy individuals determined by FFIAs and ECLIAs, patients were divided into positive and negative groups (Table 2). SCCA-negative percentages in healthy controls and SCCA-positive percentages in the other three groups were calculated and compared between the two methods by paired chi-square tests. To determine the sensitivity, specificity, and optimal cutoff values of the parameters, receiver operating characteristic (ROC) curve analysis was performed to compare the SCCA diagnostic performances for FFIAs and ECLIAs in patients with LSCC, CSCC, and HANSCC. All reported p-values were two-sided, and results with p-values of less than 0.05 were considered statistically significant.

RESULTS

Patient demographics

The developmental cohort consisted of serum samples from 98 patients with LSCC, 83 patients with CSCC, 55 patients with HANSCC (14 of whom had laryngeal cancer, 20 of whom had nasopharyngeal carcinoma, and 21 of whom had esophageal cancer), and 154 healthy controls (Table 2). The mean ages of the patients in the LSCC, CSCC, HANSCC, and healthy control groups were 57.2, 50.6, 46.2, and 52.3 years, respectively. There was a greater proportion of men in the LSCC and HANSCC groups.

Distribution and comparison of FFIAs and ECLIAs for SCCA

The SCCA levels determined by FFIAs and ECLIAs in each group are shown in Table 2 and Figure 1A - D. Data showed a non-normal distribution. Significantly higher median levels of SCCA were observed by FFIAs in patients with CSCC patients (3.84 ng/mL; IQR: 1.5 - 6.34 ng/mL) compared with that in apparently healthy women in the control group (0.70 ng/mL; IQR: 0.45 - 0.89 ng/mL), patients with LSCC (1.68 ng/mL; IQR: 1.12 - 2.29 ng/mL), and patients with HANSCC (1.22 ng/mL; IQR: 0.83 - 1.92 ng/mL). Median levels of SCCA measured by ECLIAs in patients with CSCC (12.15 ng/mL; IQR: 6.42 - 16.83 ng/mL) were also significantly higher than those in apparently healthy women in the control group (1.98 ng/mL (IQR: 1.39 - 2.66 ng/mL), patients with LSCC (5.87 ng/mL; IQR: 3.42 - 7.02 ng/mL), and patients with HANSCC

Table 2. Baseline characteristics of the study population.

Group	HC		LSCC		CSCC		HANSCC		p-value
n	154		98		83		55		
Gender (male/female)	102/52		68/30		0/83		38/17		
Age (year) [M (IQR)]	52.3 (39.9 - 64.7)		57.2 (46.7 - 67.7)		50.6 (37.7 - 63.5)		46.2 (16.4 - 66.8)		0.35
WBC (10^9/L) [M (IQR)]	6.56 (4.68 - 9.34)		5.88 (3.80 - 8.49)		7.02 (4.40 - 10.54)		6.35 (5.02 - 8.22)		0.08
CRP (mg/dL) [M (IQR)]	0.32 (0.14 - 0.83)		0.40 (0.03 - 0.92)		0.42 (0.12 - 0.77)		0.38 (0.09 - 0.80)		0.12
TBIL (µmol/L) [M (IQR)]	8.50 (5.23 - 18.53)		9.90 (4.55 - 20.30)		10.92 (5.12 - 22.35)		8.93 (6.02 - 1.33)		0.06
ALT (U/L) [M (IQR)]	20.50 (8.50 - 39.02)		20.30 (5.50 - 38.47)		18.46 (4.30 - 35.80)		22.52 (3.8 - 38.42)		0.32
AST (U/L) [M (IQR)]	14.50 (3.50 - 29.36)		15.30 (5.50 - 29.04)		13.46 (4.30 - 29.09)		19.52 (3.8 - 32.35)		0.13
Creatinine (µmol/L) [M (IQR)]	74.50 (45.00 - 129.45)		85.30 (55.60 - 138.64)		83.46 (44.70 - 140.19)		79.52 (63.8 - 132.64)		0.09
Urea (mmol/L) [M (IQR)]	4.50 (3.50 - 9.17)		5.30 (2.50 - 9.94)		4.46 (1.30 - 10.95)		6.52 (2.8 - 11.04)		0.27
SCCA (ng/mL) [M (IQR)]									
FFIA	T: 0.75 (0.52 - 0.92) F: 0.70 (0.45 - 0.89)		1.68 (1.12 - 2.29)		3.84 (1.50 - 6.34)		1.22 (0.83 - 1.92)		< 0.001 [a]
ECLIA	T: 2.05 (1.46 - 2.58) F: 1.98 (1.39 - 2.66)		5.87 (3.42 - 7.02)		12.15 (6.42 - 16.83)		4.03 (2.55 - 5.06)		< 0.001 [a]
p-value	< 0.001 [b]		< 0.001 [b]		0.002 [b]		0.043 [b]		
Pos/Neg (n)	+	-	+	-	+	-	+	-	
FFIA (cutoff: 0 - 1.5 ng/mL)	3	151	40	58	30	53	9	46	
ECLIA (cutoff: 0 - 2.7 ng/mL)	2	152	51	47	48	35	13	42	
DCR (%)									
FFIA		98.05	40.82		36.14		16.36		
ECLIA		98.70	52.04		57.14		23.64		
McNemar test p-value		1.00 [c]	0.043 [c]		0.001 [c]		0.388 [c]		

HC - healthy controls, LSCC - lung squamous cell carcinoma, CSCC - cervical squamous cell carcinoma, HANSCC - head and neck squamous cell carcinoma, SCCA - squamous cell carcinoma antigen, M (IQR) - median with inter quartile range, WBC - white cell count, CRP - C-reactive protein, TBIL - total bilirubin, ALT - alanine aminotransferase, AST - aspartate aminotransferase, Pos - Positive, Neg - negative, DCR - diagnosis coincidence rate. [a] - compared between each group of the same method, [b] - compared between two methods of the same group, [c] - compared between two methods' DCR (%) of the same group.

Table 3. Diagnostic value of FFIA and ECLIA SCCA in discriminating SCC from HC.

Methods	SCC vs. HC	Cutoff	Sensitivity% (95% CI)	Specificity% (95% CI)	AUC (95% CI)
FFIA	LSCC vs. HC	1.12	77.55 (68.01 - 85.36)	85.34 (88.48 - 95.38)	0.87 (0.82 - 0.92)
	CSCC vs. HC-F	1.21	81.93 (71.95 - 89.52)	90.20 (90.68 - 96.88)	0.90 (0.85 - 0.95)
	HANSCC vs. HC	1.01	67.27 (53.29 - 79.32)	82.00 (75.32 - 89.75)	0.81 (0.74 - 0.89)
ECLIA	LSCC vs. HC	3.07	85.71 (77.19 - 91.96)	91.52 (88.52 - 96.43)	0.91 (0.87 - 0.95)
	CSCC vs. HC-F	3.84	89.16 (80.41 - 94.92)	95.24 (90.38 - 98.48)	0.95 (0.92 - 0.99)
	HANSCC vs. HC	3.35	58.18 (44.11 - 71.35)	89.65 (86.38 - 93.06)	0.85 (0.78 - 0.92)

SCC - squamous cell carcinoma, HC - healthy controls, HC-F - females of HC group, LSCC - lung squamous cell carcinoma, CSCC - cervical squamous cell carcinoma, HANSCC - head and neck squamous cell carcinoma, CI - confidence interval, AUC - area under curve.

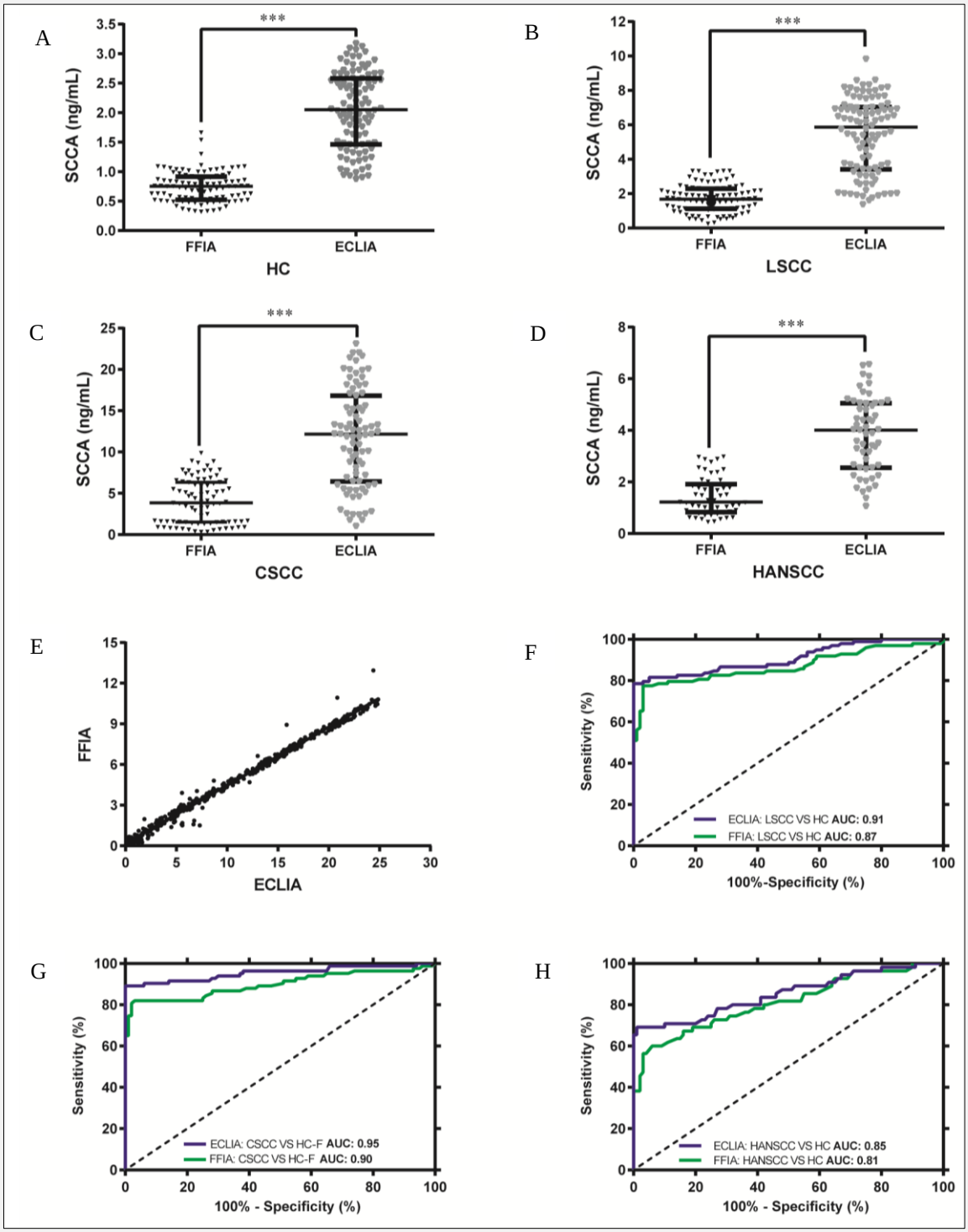

Figure 1. SCCA level comparison and ROC analysis of two methods in each group.

(A) FFIA SCCA levels versus ECLIA SCCA levels in HC group (n = 154). (b) FFIA SCCA levels versus ECLIA SCCA levels in LSCC group (n = 98). (C) FFIA SCCA levels versus ECLIA SCCA levels in CSCC group (n = 83). (D) FFIA SCCA levels versus ECLIA SCCA levels in HANSCC group (n = 55). (E) Correlation analysis of two methods' SCCA levels in all the groups (Y = 0.429 X +0.1738, R^2 = 0.843, p < 0.01, n = 390). (F) ROC analysis of SCCA levels as a diagnostic biomarker for differentiating LSCC from healthy peoples with two methods. (G) ROC analysis of SCCA levels as a diagnostic biomarker for differentiating CSCC from healthy peoples with two methods. (H) ROC analysis of SCCA levels as a diagnostic biomarker for differentiating HANSCC from healthy peoples with two methods.

(4.03 ng/mL; IQR: 2.55 - 5.06 ng/mL). SCCA concentrations were found to be higher when measured by ECLIAs than when measured by FFIAs for each group ($p < 0.01$); however, the results from the two methods were well correlated ($r = 0.918$; $p < 0.01$; Figure 1E).

Comparison of SCCA diagnostic performances of FFIAs and ECLIAs

The 99% confidence intervals for healthy individuals for FFIAs (0 - 1.5 ng/mL) and ECLIAs (0 - 2.7 ng/mL) were verified by our laboratory before the experiment [13]. For FFIAs, the numbers of SCCA-negative versus -positive cases for the healthy control, LSCC, CSCC, and HANSCC groups were 151 versus 3, 58 versus 40, 53 versus 30, and 46 versus 9, respectively. For ECLIAs, the numbers of SCCA-negative versus -positive cases in the healthy control, LSCC, CSCC, and HANSCC groups were 152 versus 2, 47 versus 51, 35 versus 48, and 42 versus 13, respectively (Table 3). ECLIAs showed higher AUCs than FFIAs for all groups (Figure 1F - H). The diagnostic performance (sensitivity/specificity) of SCCA was higher in CSCC than in LSCC and HANSCC (Table 3).

DISCUSSION

Due to the central role in staging and monitoring patients with various forms of SCC, the routine measurement of SCCA is rapidly increasing in clinical laboratories. SCCA is a glycoprotein originally isolated from SCC tissue by Kato et al. [14] and is encoded by two highly homologous genes with different biological characteristics (neutral SCCA1 and acidic SCCA2). SCCA1 inhibits the activity of papain, e.g., cysteine protease activity, whereas SCAA2 inhibits the activity of chymotrypsin, e.g., serine protease, cathepsin, and chymase activities [15]. The expression levels of SCCA1 and SCCA2 vary significantly among different tumor types [3,8,16,17], as well as in patients with several nonmalignant skin disorders and renal failure [13]. Moreover, SCCA1 and SCCA2 levels differ in patients with different types of SCC, and SCCA1 and SCCA2 typing detection is more effective than total SCCA detection. When we initially began using FFIAs for SCCA detection, our laboratory received complaints of lower positive rates for SCCA in patients with SCC from clinicians. For example, the FFIA-positive coincidence rate of SCCA in patients with CSCC at our hospital was approximately 10%, which was much lower than that reported in previous studies [9]. It should be noted that previous studies paid more attention to the comparison of intra- and inter-assay imprecision of two SCCA methods, but ignored the importance of the relationship between serum SCCA level and pathological diagnosis on which this study focused [18].

FFIAs and ECLIAs, which target the common epitopes of SCCA1 and SCCA2, were used in this study to detect total serum SCCA levels. By comparing differences in serum SCCA levels, diagnosis coincidence rates, and clinical performances of FFIAs and ECLIAs, we found that SCCA levels determined by ECLIAs were significantly higher than those determined by FFIAs, with a larger reference range in the normal population. Additionally, we showed that the diagnosis coincidence rate for ECLIAs with pathological diagnosis in patients with LSCC and CSCC was significantly higher than that of FFIAs. Additionally, there were no significant differences in negative coincidence rates for ECLIA and FFIA in healthy controls. Beside SCCA levels determined by ECLIAs in some patients with SCC in this study were close to the upper limit of the ECLIA reference range, although SCCA levels determined by FFIAs were at the lower level of the FFIA reference range. Finally, we found that diagnostic performance, including sensitivity, specificity, and AUCs, were the highest for patients with CSCC in our study.

Owing to differences compared with the standard reference materials traced by the two methods and the different antigenic epitopes, the correlation coefficient ($r = 0.918$) of the two methods did not meet the requirements of EP9-A2 for comparison between different methods (correlation coefficient $r > 0.975$) [19] and could not be used for subsequent regression analysis or as an indicator for the judgment of method performance [9]. Furthermore, SCCA, as a tumor marker with no national reference traceable substance or clinically acceptable levels, has led to difficulties in comparisons of the deviation between different methods. Accordingly, an SCCA reference standard substance and reference method are urgently needed to facilitate decision-making and elucidation of the clinical acceptability level for SCCA. Such standard may also reduce the differences between the results of various SCCA detection systems and enable better comparisons of SCCA results. The establishment of serum SCCA1 and SCCA2 detection systems may also contribute to improve diagnostic capability. Notably, for chemiluminescence dual target detection in the literature [20], reporting of SCCA1, SCCA2, and the SCCA2/SCCA1 ratio may further improve early diagnosis, treatment, and monitoring of SCC.

Due to the absence of previous studies about the crucial relation between SCCA detection methods and clinical diagnostic performance, our research may be the first to evaluate and compare the diagnostic efficacy of serum SCCA levels detected by ECLIA and FFIA methods in LSCC, CSCC, and HANSCC. The results show that ECLIA method is significantly superior to FFIA method in the diagnostic performance of serum SCCA level, especially in the patients with LSCC and CSCC. However, there were also some limitations for this study: (1) The patients who were diagnosed with benign lesions in cases of suspected SCC were not enrolled. (2) Further studies including a large number of relevant cases are needed to verify the conclusions of this study.

CONCLUSION

This study indicated that ECLIA method is superior to FFIA method in the diagnostic performance of serum SCCA level of patients who were diagnosed as lung squamous cell carcinoma and cervical squamous cell carcinoma.

Source of Funding:
The study was supported by Guangdong Medical Research Fund (B2018262), Shaoguan Health Bureau Research Fund (Y19042), Shaoguan Science and Technology Fund (2018sn095) and Outstanding Research Fund of YUEBEI People's Hospital.

Availability of Data and Materials:
All data generated or analyzed during this study were included in this published article. The raw data were obtained via medical laboratory in the hospital and are not publicly available due to the involvement of patients' privacy.

Authors' Contributions:
YL analyzed data and drafted paper; YC collected raw data and categorize data; SH analyzed data and revised paper; WC designed the study and revised paper. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate:
This study was approved by the ethics committee of Yuebei People's Hospital (Shaoguan, China) and conducted in accordance with The Declaration of Helsinki Principles. As a retrospective study, informed consent of research use of surplus blood after clinical laboratory test was obtained from each patient in advance.

Acknowledgment:
We sincerely thank Wencan CAI, the head of Department of Medical Laboratory of Yuebei People's Hospital, for his support in case collection.

Declaration of Interest:
None.

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