Evaluation of Chemotherapy Response with Serum Squamous Cell Carcinoma Antigen Level in Cervical Cancer Patients: A Prospective Cohort Study

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Abstract

MRI does not always reflect tumor response after chemotherapy. Therefore, it is necessary to explore additional parameters to more accurately evaluate tumor response for the subsequent clinical determination about radiotherapy or radical surgery. A training cohort and an external validation cohort were used to examine the predictive performance of SCC-ag to evaluate tumor response from teaching hospital of Harbin Medical University. The study included 397 women with SCC (age: 28–73 years). Patients consecutively enrolled between August 2008 and January 2010 (n = 205) were used as training cohort. Patients consecutively enrolled between February 2010 and May 2011 (n = 192) were used as validation cohort. A multivariate regression analysis of the data from the training cohort indicated that serum SCC-ag level is an independent factor for neo-adjuvant chemotherapy (NACT) response. Analysis of the data from the validation cohort suggested that chemotherapy response could be more accurately predicted by SCC-ag than by magnetic resonance imaging (MRI) (sensitivity (Se): 0.944 vs. 0.794; specificity (Sp): 0.727 vs. 0.636; positive predictive value (PPV): 0.869 vs. 0.806; negative predictive value (NPV): 0.873 vs. 0.618; the area under ROC curve (AUC): 0.898 vs. 0.734). Combining SCC-ag with MRI was more powerful than MRI alone (Se: 0.952 vs. 0.794; Sp: 0.833 vs. 0.636; PPV: 0.916 vs. 0.806; NPV: 0.902 vs. 0.618; AUC: 0.950 vs. 0.734). Our study indicates that serum SCC-ag level is a sensitive and reliable measure to evaluate cervical cancer response to chemotherapy. Using SCC-ag in combination with MRI findings further improves the predictive power.

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Introduction

Neoadjuvant chemotherapy (NACT) could create chances for curative resection of initially non-resectable tumors [1–4]. However, approximately 30% of the patients with squamous cervical cancer (SCC) are non-responsive to chemotherapy [5,6]. For patients not responding to neoadjuvant chemotherapy, attempt to remove the tumor with surgery could be disastrous.

MRI is the golden standard to evaluate tumor response to chemotherapy. For cervical cancer patients receiving neoadjuvant chemotherapy, MRI findings are used to determine eligibility of the patients for subsequent resection [7–9]. MRI is prone to false-positive results, i.e. tumor appears to be decreased in size upon MRI imaging, but actually did not change or even have increased in size based on post-surgical pathological examination, or false-negative results, e.g. in patients with “no residual disease” as judged by MRI imaging, histologic examination detected lesions that measured >1 cm in 8% of the case [10]. Integrated 18F-fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) imaging improves the evaluation accuracy of tumor volume after chemotherapy [11]. The expense, however, has limited its use, especially in developing countries.

The squamous cell carcinoma antigen (SCC-ag), which serves as a serological marker for squamous cell cervical cancer, is a sub-fraction of the tumor antigen TA-4, which is a 48 kDa glycoprotein that was first isolated by Kato and Torigoe [12]. This antigen is reported to be closely related to clinical staging or tumor spread as well as the tumor response of advanced squamous disease to radiation or chemotherapy [13–15] and can be used to predict the survival and tumor recurrence during the follow-up period [16–20].

In the current study, we examined the sensitivity and reliability of using serum SCC-ag level to evaluate response to chemotherapy in patients with cervical cancer. The study included a training cohort of 205 subjects and an external validation cohort of 192
Figure 1. Conventional and DW-MRI of the same lesion from a 55-year-old woman undergoing NACT. (A)–(C): pretreatment axial (A) and sagittal (B) conventional MR images and diffusion-weighted MR image (C). (D)–(F): preoperative axial (D) and sagittal (E) conventional MR images and diffusion-weighted MR image (F). The red circles in (B)–(F) indicate the largest pretreatment and preoperative lesion as measured in different planes and using different MRI techniques.
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Figure 2. Number of patient enrollment.
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397 patients were performed radical surgery underwent pathological, MRI and SCC-ag examinations

205 patients enrolled between August 2008 and January 2010 were considered as training cohort

192 patients enrolled between February 2010 and May 2011 were considered as validation cohort

145 patients with post-operative pathological examination confirmed response

60 patients with post-operative pathological examination confirmed non-response

126 patients with post-operative pathological examination confirmed response

66 patients with post-operative pathological examination confirmed non-response
subjects. A random forest model was used to test the hypothesis that SCC-ag level in combination with MRI improves the evaluation of response to chemotherapy.

Materials and Methods

Inclusion Criteria

Patients were enrolled in this study if they satisfied all following inclusion criteria: 1) a diagnosis of stage IB2-IIB SCC (FIGO classification); 2) no prior hysterectomy, pelvic radiotherapy, systemic chemotherapy or medical contraindications to chemotherapy. All patients have signed up the written informed consent. The study was approved by the Institutional Review Board. All patients received NACT treatment following radical dissection, and underwent MRI and SCC-ag examinations. NACT regimen consisted of three cycles of paclitaxel and carboplatin treatment. On the first day of each cycle, patients received paclitaxel at 150 mg/m² intravenously (IV) over a period of 3 hours plus carboplatin (area under the serum concentration-time curve: 5) over a period of 30 minutes. Blood pressure, ECG and blood oxygen saturation were monitored during the infusion. Cycles were separated by 3 weeks.

Training and Validation Cohorts

The training cohort includes all patients with a diagnosis of stage IB2-IIB SCC between August 2008 and January 2010. The validation cohort included all patients diagnosed with stage IB2-IIB SCC between February 2010 and May 2011.

Magnetic Resonance Imaging

All patients underwent MRI scans at the initial visit using a 1.5-T NVi/CVi magnetic resonance machine (GE, Waukesha, Wisconsin, USA) with a standard phased array torso coil. An additional MRI scan was carried out upon completion of NACT. Briefly, a sagittal T1- and T2-weighted fast spin echo acquisition was obtained with a 24 x 24 cm field of view (FOV), an echo time (TE) of 97.6 cm, and a repetition time (TR) of 1600 ms. Slices (4 mm) were acquired with 1-mm gap using a 256 x 256 matrix. The scan ranged from the iliac crests to the pubic symphysis. Based on the sagittal view, axial slices were obtained through the tumor using a T2-weighted fast recovery fast spin echo sequence with an FOV of 32 x 32 cm, a TE/TR of 83.6/4520 ms, 3-mm slices (no gap) and a 256 x 256 matrix. Once the tumor was fully visualized, diffusion-weighted images (DWIs) were acquired in straight axial and sagittal planes (planned on the T2-weighted sagittal scan) and centered through the middle of the tumor using an epi-based diffusion tensor imaging sequence. The epi-based sequence was limited to straight axial slices. All slices were acquired with a 40 x 40-cm FOV, a 2-mm slice thickness, a TR/TE of 6800 ms/70 ms, and a 160 x 256 matrix. Data were acquired with a b value of 1000. The ADC value was obtained from diffusion tensor images on each slice.

Clinical Response Evaluation and Imaging Analysis

Clinical response was evaluated using the RECISR criteria for solid tumors (version 1.1).9 Complete response (CR) was defined as complete disappearance of all lesions; partial response (PR) was defined as at least a 30% decrease in the sum of the largest diameter (LD) of the targeted lesions; stable disease (SD) was defined as neither shrinkage that qualified as PR nor sufficient increase that qualified as progressive disease (PD); and PD was defined as at least a 20% increase in the sum of the LD of the target lesions. The overall response was defined as CR plus PR. The clinical response was evaluated based on imaging only. The images were evaluated by two experienced radiologists aware of patient diagnosis and treatment but not the results of other

| Characteristics | Training cohort (n = 205) | Validation cohort (n = 192) |
|----------------|---------------------------|---------------------------|
|                | Non-response | Response | p value | Non-response | Response | p value |
| Age            | Mean±SD       | n = 60    | n = 145  |       | n = 66    | n = 126  |       |
| Mean±SD        | 47.65±8.39    | 49.1±8.42 | 0.2615   |        | 46.85±8.45| 46.28±9.39| 0.6870 |
| Min-max        | 32–73         | 28–68     |          |        | 30–71     | 25–72    | |
| Menses         |               |           |          |        |           |          | |
| Non-menopause  | 25(41.67)     | 64(44.14) | 0.7453   |        | 24(36.36)| 41(32.54)| 0.5949 |
| menopause      | 35(58.33)     | 81(55.86) |          |        | 42(63.64)| 85(67.46)| |
| FIGO Stage     |               |           |          |        |           |          | |
| IB2            | 16(26.67)     | 24(16.55) | 0.0719   |        | 23(34.85)| 22(17.46)| 0.0389 |
| IIA            | 12(20.00)     | 47(32.41) |          |        | 18(27.69)| 44(34.92)| |
| IIB            | 32(53.33)     | 74(51.03) |          |        | 25(38.46)| 60(47.62)| |
| Lymph node metastasis | |            | |        |           |          | |
| Negative       | 11(18.33)     | 46(31.72) | 0.0515   |        | 35(53.03)| 59(46.83)| 0.4140 |
| Positive       | 49(81.67)     | 99(68.28) |          |        | 31(46.97)| 67(53.17)| |
| Differentiation|               |           |          |        |           |          | |
| Well           | 9(15.00)      | 22(15.28) | 0.9895   |        | 9(13.64)| 32(25.4) | 0.1668 |
| Moderate       | 29(48.33)     | 68(47.22) |          |        | 34(51.52)| 57(45.24)| |
| Poor           | 22(36.67)     | 55(37.50) |          |        | 23(34.85)| 37(29.37)| |

The values in the parentheses represented the percentage frequency; doi:10.1371/journal.pone.0054969.t001
imaging modalities. Upon discrepancy between the two readings, a third, independent experienced radiologist served as the final arbitrator. Representative MRI and DWI of a 55-year-old patient are shown in Figure 1.

SCC-ag Assay
Fasting serum samples were collected in duplicate at the initial visit and upon the completion of NACT. SCC-ag level was measured using an IMx SCC-ag microparticle enzyme immunoassay kit (Abbott Laboratories, Abbott Park, IL).

Pathological Assessment
All specimens removed by surgery were submitted for pathological analysis that included macroscopic measurement of the lesion size and microscopic determination of the lesion boundary based on frozen tissue.

Statistical Analysis
Categorical data are described as frequency counts and percentages, and the quantitative data were presented as mean ± standard deviation (X ± SD). Comparisons between the clinopathological characteristics, e.g., FIGO staging, lymph node metastasis and differentiation in responsive and non-responsive patients, were performed using Pearson’s chi-square test. Bland-Altman analysis was used to assess the agreement between MRI examinations and pathological findings and the Bland-Altman plot was used to visualize this agreement [21–23]. Univariate and multivariate logistic regression analyses were conducted to determine whether SCC-ag level was an independent factor in the evaluation of the NACT response. Random forest (RF) analysis [24–27] was carried out in the validation cohort to verify the predictive performance and compare the evaluation accuracy of MRI findings in combination with SCC-ag vs. MRI results or SCC level alone. Sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and the area under receiver operating curve (AUC) were calculated using pathological results as reference standard.

All statistic analyses were carried using SAS version 9.1.3 (SAS, Cary, NC), with the exception of random forest analysis (R version 2.12).

Results

Demographic and Clinical Characteristics
Between August 2008 and October 2010, 446 subjects were enrolled. Among these, 397 patients received NACT followed by radical surgery (Figure 2). The demographic and clinical data were summarized in Table 1. The baseline characteristics were comparable across the datasets, with the exception of FIGO stage

### Table 2. Univariate and Multivariate Logistic Analysis of SCC-ag Level and Response to Neoadjuvant Chemotherapy in a Prospective Cohort.

| Variable | Univariate Analysis | Multivariate Analysis |
|----------|---------------------|-----------------------|
|          | OR 95% CI          | \( p \) value | OR 95% CI | \( p \) value |
| ΔMRI*    |                    |                 |           |                 |
| <0.30    | 1.00               | –               | –         | –               |
| ≥0.30    | 13.30              | 6.40–27.63      | <0.0001   | 10.28           | 3.86–27.37      | <0.0001 |
| ΔSCC-ag* |                    |                 |           |                 |
| <0.30    | 1.00               | –               | –         | –               |
| (0.30, 0.50) | 4.63               | 1.53–13.98  | 0.0158    | 3.62           | 1.01–2.93       | 0.0210 |
| (0.50, 0.70) | 30.06              | 10.35–87.36 | 0.0048    | 31.70          | 9.28–108.25     | 0.0025 |
| ≥0.70    | 112.54             | 28.18–449.40   | <0.0001   | 75.26          | 17.00–333.16    | <0.0001 |

*ΔMRI indicated the decrease percentage in tumor size before and after NACT with MRI.
+ΔSCC-ag indicated the decrease percentage in SCC-ag level before and after NACT.

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in the validation cohort. Although the training cohort had many more lymph node positive metastases, there was no significantly statistical difference (\(p = 0.0515\)).

**Agreement between MRI data and the Postsurgical Pathological Findings**

The Bland-Altman plot was used to visualize the agreement between pretreatment or posttreatment MRI and the postsurgical pathological finding. In the Bland-Altman plot, the limit of agreement was the acceptably maximal difference of MRI and postsurgical pathological results, known from the clinical point of view, approximately 10 mm. The Bland-Altman analysis showed that the tumor size measured by pretreatment MRI and postsurgical pathology exhibited good agreement (Figure 3A) because 95% plots lies in the limits, whereas approximately 40% of the plots were out of the limit of agreement (Figure 3B), indicating a poor agreement in tumor size measured by posttreatment MRI and postsurgical pathological examinations results.

**SCC-ag Level and the Response to NACT**

As mentioned above (see Introduction), SCC-ag is closely related to the extent of the disease as well as the response to treatment and can be used to predict the survival and tumor recurrence during the follow-up period. However, to our knowledge, few studies were performed to investigate the role of SCC-ag in the evaluation performance to chemotherapy response. Therefore, we tried to assess whether serum SCC-ag level measured before and after NACT can be a parameter for evaluation of tumor response. In this study, the 30, 50, and 70% decreases in SCC-ag levels were chosen as cutoffs to categorize the

**Table 3.** Tumor sizes before and after neoadjuvant chemotherapy with the percent of SCC-ag decrease.

| Group                      | Percent of SCC-ag decrease after NACT | Pretreatment | Posttreatment |
|----------------------------|---------------------------------------|--------------|---------------|
|                            | \(X \pm SD\) | Median(range)  | \(X \pm SD\) | Median(range)  |
| Training cohort (n = 205)  |                                      |              |               |
| <0.30                     | 42.93±13.77  | 44(20–75)    | 35.78±14.95   | 37(0–61)       |
| [0.30, 0.50)               | 43.13±12.84  | 40(24–73)    | 30.25±14.75   | 28(0–61)       |
| [0.50, 0.70)               | 45.85±10.33  | 44.5(25–68)  | 26.92±14.36   | 28.5(0–51)     |
| \(\geq 0.70\)             | 47.49±12.07  | 47(28–80)    | 24.01±13.01   | 23.5(0–67)     |
| Validation cohort (n = 192)|                                      |              |               |
| <0.30                     | 42.68±12.05  | 40(24–68)    | 36.51±16.79   | 40(0–65)       |
| [0.30, 0.50)               | 41.00±10.53  | 40(24–60)    | 28.43±14.08   | 25(0–54)       |
| [0.50, 0.70)               | 44.56±11.05  | 44(20–68)    | 27.47±12.82   | 25(0–50)       |
| \(\geq 0.70\)             | 48.89±11.41  | 50(24–80)    | 24.02±13.56   | 22(0–67)       |

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![Figure 4. The empirical (A) and smooth (B) AUCs in the validation cohort. MRI in combination with serum SCC-ag vs. MRI or SCC-ag alone, respectively.](https://www.plosone.org/doi/10.1371/journal.pone.0054969.g004)
continuous pre- and post-treatment SCC-ag levels and find out the correlation between SCC-ag level and chemotherapy response in a more clear way. Univariate and multivariate logistic regression analyses of the chemotherapy response evaluation based on the MRI findings or SCC-ag levels alone were shown in Table 2. The mean and median of pre-treatment and post-treatment tumor sizes as well as range with the percent of SCC-ag decrease were presented in Table 3. Both MRI findings and SCC-ag level are independent factors for response to chemotherapy and the percent of MRI examination and SCC-ag level decrease positively correlated with chemotherapy response. From the multivariate logistic regression, we found that the percent of MRI above 30% increase the likelihood of chemotherapy response for patients by 10.28 times compared with 30% or less. The percent of SCC-ag decrease after chemotherapy exceeding 30, 50, 70% increase the likelihood of chemotherapy response by 3.62, 31.70, 75.26 times compared with 30% or less. The percent of SCC-ag decrease positively correlated with chemotherapy response for patients by 10.28 times compared with 30% or less. The percent of SCC-ag decrease after chemotherapy exceeding 30, 50, 70% increase the likelihood of chemotherapy response for patients by 3.62, 31.70, 75.26 times compared with 30% or less.

Validation

Analysis of the data from the validation cohort using a random forest model demonstrated that chemotherapy response could be more accurately predicted by SCC-ag than by MRI in Table 4 (Se: 0.944 vs. 0.794; Sp: 0.727 vs. 0.636; PPV: 0.969 vs. 0.806; NPV: 0.873 vs. 0.618; AUC: 0.950 vs. 0.734). Combining SCC-ag with MRI was more powerful than MRI alone (Se: 0.952 vs. 0.794; Sp: 0.833 vs. 0.638; PPV: 0.916 vs. 0.806; NPV: 0.902 vs. 0.618; AUC: 0.950 vs. 0.734). Figure 4 presented the empirical and smooth AUCs for the external validation cohort with MRI findings further improves the predictive power. Chemotherapy response evaluation may benefit from this serum biomarker and help the oncologists to make optimal decision for cervical cancer patients.

Discussion

The current study revealed good agreement of post-surgical pathological findings with MRI findings prior to, but not after NACT. Possible factors that contributed to the poor agreement after chemotherapy include necrosis, granuloma formation, hyaline degeneration, tiny lesions and inflammation in the target lesions after the treatment. Diffusion-weighted MRI (DW-MRI) could improve the accuracy, but only to limited degree [28,29].

Serum SCC-ag, which is one of the most common biomarkers of cervical cancer, has been used to monitor the disease recurrence. Few studies, however, have investigated the role of the SCC-ag levels in the evaluation of NACT response in SCC patients. Hashimoto et al investigated the role of SCC-ag as a biomarker of chemotherapy response in patients with metastatic cervical cancer and reported that the patients with reduction in serum SCC-ag levels may be responsive to chemotherapy [30] and Kim et al demonstrate that there is a linear correlation between percent decrease in SCC-ag and that of tumor volume in cervical cancer patients [31], which are consistent with our results. Besides, we also demonstrates that the accuracy of the percent of SCC-ag decrease alone in the chemotherapy response evaluation is better than that of MRI alone, and SCC-ag level together with MRI examination is more powerful than MRI alone. Additionally, Reesink-Peter et al recently reported that in the early-stage cervical cancer, serum SCC-ag level is more refined preoperative estimation of the likelihood for adjuvant radiotherapy [32]. SCC level may be useful in the evaluation of primary and recurrent SCC of cervix to radiation and chemotherapy [15], Ohara et al found that postradiotherapy SCC-ag can be used to predict tumor recurrence and not useful for evaluation radiotherapy response [33]. In this present study, we did not mention the relationship between radiotherapy response or metastasis and decrease percent of SCC-ag level, which needs to be further studied.

The ADC values from the DW-MRI data at each assessment (Table 5) did not differ between non-responsive and responsive patients, suggesting the limited value of DW-MRI.

In theory, the findings from the current study could be reasonably extrapolated to all malignant tumors that originate from squamous tissue. Such a speculation, however, requires extensive studies in the future.

In conclusion, our study indicates that serum SCC-ag level is a sensitive and reliable measure to evaluate cervical cancer response to chemotherapy. Using SCC-ag in combination with MRI findings further improves the predictive power. Chemotherapy response evaluation may benefit from this serum biomarker and help the oncologists to make optimal decision for cervical cancer patients.

**Table 4.** The Accuracy Estimation of NACT Response in an External Validation Cohort.

| Evaluation indicator | ∆MRI | ∆SCC | MRI plus SCC |
|----------------------|------|------|-------------|
|                      | Estimator | 95%CI | Estimator | 95%CI | Estimator | 95%CI |
| Sensitivity          | 0.80  | 0.72–0.87 | 0.94  | 0.90–0.98 | 0.95  | 0.91–0.98 |
| Specificity          | 0.64  | 0.52–0.74 | 0.73  | 0.62–0.83 | 0.83  | 0.74–0.92 |
| PPV                  | 0.81  | 0.73–0.87 | 0.87  | 0.81–0.92 | 0.92  | 0.86–0.96 |
| NPV                  | 0.62  | 0.50–0.74 | 0.87  | 0.78–0.95 | 0.90  | 0.82–0.97 |
| AUC                  | 0.73  | 0.66–0.81 | 0.90  | 0.85–0.95 | 0.95  | 0.91–0.99 |

Abbreviation: ∆MRI: the decrease percentage in tumor size before and after NACT with MRI; ∆SCC-ag: the decrease percentage in SCC-ag level before and after NACT; PPV: positive predictive value; NPV: negative predictive value; AUC: area under the ROC curve; The confidence interval was estimated based on exact test of binominal distribution.

| Evaluation indicator | Sensitivity | Specificity | PPV | NPV | AUC |
|----------------------|-------------|-------------|-----|-----|-----|
|                      | Se          | Sp          | PPV | NPV | AUC |
| MRI                  | 0.84        | 0.72        | 0.87 | 0.80 | 0.89 |
| SCC-ag               | 0.90        | 0.83        | 0.93 | 0.90 | 0.92 |

*compare with pre-treatment ADC value, p>0.05; doi:10.1371/journal.pone.0054969.t005

**Table 5.** Apparent diffusion coefficient (ADC) values in the response and non-response patients before and after chemotherapy treatment (mm²-s).

| N  | Pre-treatment | Post-treatment |
|----|---------------|---------------|
| Response | 271 | 0.92±0.22×10⁻² | 1.03±0.16×10⁻³ |
| Non-response | 126 | 0.93±0.18×10⁻³ | 1.01±0.24×10⁻³ |

*compare with pre-treatment ADC value, p>0.05; doi:10.1371/journal.pone.0054969.t005
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Author Contributions
Designed the software used in analysis: FS. Conceived and designed the experiments: MY YH CC XZ GL KL. Performed the experiments: MY YH HL, JZ XC KL CI. Wrote the paper: MY YH KL GL.