Squamous cell carcinoma (SCC) antigen as a novel candidate marker for amniotic fluid embolism

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Short title: Diagnostic marker for AFE

Abstract

Aim: We aimed to evaluate the clinical usefulness of serum squamous cell carcinoma (SCC) antigen for the diagnosis of amniotic fluid embolism (AFE).

Methods: Sera and information of 20 patients with AFE (autopsy-proven AFE, 4 cases; clinical AFE, 16 cases) were obtained from the Japan Amniotic Fluid Embolism Registration Center at Hamamatsu University School of Medicine. As controls, we included 74 gestational age-matched healthy women who gave birth to healthy newborns during the period from December 2012 to January 2014. Receiver operating characteristic (ROC) curves were used to evaluate the diagnostic performance of SCC levels for prediction of AFE.

Results: Serum SCC antigen levels in women with autopsy-proven AFE (112.0 ± 169.4 ng/mL, $P = 0.001$) and clinical AFE (9.5 ± 10.3 ng/mL, $P = 0.004$) were significantly higher than those in healthy controls with normal delivery (4.4 ± 2.2 ng/mL). On ROC analysis, the optimal cutoff value for SCC antigen levels was 7.15 ng/mL, for which the sensitivity and specificity for AFE prediction was 60.0% and 89.2%, respectively (area under the ROC curve, 0.785; 95% confidence interval, 0.663–0.908; $P < 0.001$).

Conclusion: Serum SCC antigen may be a promising predictor of the entry of amniotic fluid into the maternal circulation, potentially serving as a candidate marker for

noninvasive diagnosis of AFE.

Key words: amniotic fluid embolism; amniotic fluid-specific protein; diagnosis; squamous cell carcinoma (SCC) antigen

Introduction

Amniotic fluid embolism (AFE) is a catastrophic complication of pregnancy, causing cardiopulmonary collapse with sudden onset. AFE is difficult to predict or prevent because of its rare prevalence and broad spectrum of clinical manifestations.[1] While the etiology of AFE remains unknown, early diagnosis of AFE is of critical importance. Therefore, it is relevant to pursue the discovery of AFE biomarkers with clinical application. Diagnostic criteria for AFE have been proposed by the Japan Association of Obstetricians and Gynecologists, but these criteria are not sufficient for the diagnosis of AFE in the early phases. [2] Two conventional serum markers have been identified to date, namely zinc-coproporphyrin-1 (ZnCP1) and sialyl Tn (STN), which are preferentially overexpressed in amniotic fluid and meconium.[3-7] Both ZnCP1 and STN tests are commonly used in Japan for supporting the diagnosis of AFE, as well as for predicting AFE.[7] Although serum levels of ZnCP1 and STN are indeed higher in some patients with AFE, the efficacy of these biomarkers remains far from optimal. A downside of ZnCP1- and STN-based diagnosis is low sensitivity and specificity. A recent report from French indicated that the serum levels of insulin-like growth factor binding protein-1 (IGFBP-1) may represent a valuable biomarker for AFE diagnosis.[8] However, this marker was studied in a limited number of AFE cases. Therefore, serum tests for

confirming the AFE diagnosis have not become universally accepted to date. A rational approach to detecting AFE biomarkers is to identify amniotic fluid-specific proteins/peptides in the maternal serum.

We previously conducted a basic research investigation involving high-throughput proteomics to identify gene products that are specifically present only in the amniotic fluid and not in the maternal serum, as well as proteins/peptides that are present in the amniotic fluid at concentrations much higher than those in the maternal serum. [9-14] We identified a variety of amniotic fluid-specific antigens. Among the candidate markers identified, we focused on the squamous cell carcinoma (SCC) antigen.[15]

In the present study, we aimed to determine the role of serum SCC antigen in supporting or predicting the AFE diagnosis.

Methods

Medical record survey and AFE definition

Since 1992, patients who had fatal or nonfatal AFE have been registered at the Japan AFE Registration Center, at the Hamamatsu University School of Medicine. The AFE Registration Center database is a public repository that archives the medical records and clinical characteristics of the patients, diagnostic and treatment outcomes,

survival and quality of life outcomes, and laboratory results including serum biomarker data. A panel of selected, relevant experts reviewed all cases of suspected AFE before registration. We were informed by the Registration Center investigators that blood specimens remained from 20 cases, including 4 patients with autopsy-proven AFE and 16 patients with suspected AFE based on clinical manifestations. Therefore, we obtained the sera and information of these 20 patients, who had been registered between 2008 and 2013.

The enrollment criteria were as follows: i) at least one relevant symptom mentioned in the patient's record, including cardiac arrest (acute hypoxia and hypotension), respiratory arrest (dyspnea), or consumptive coagulopathy (severe obstetric hemorrhage); ii) the relevant signs and symptoms occurred first during pregnancy, labor, cesarean section, or within 12 hours postpartum; and iii) absence of other illnesses that could explain the observed signs and symptoms.

The histological findings of AFE on autopsy were: presence of embolic particles of fetal squamous cells or amniotic fluid materials in the maternal pulmonary circulation, pulmonary edema, and alveolar hemorrhage. Of the 4 patients with a histologically confirmed diagnosis of AFE (i.e., autopsy-proven AFE), 3 experienced symptoms after vaginal delivery (up to 2 hours post-delivery), and 1 experienced symptoms during labor.

The remaining 16 patients included in the study had a clinical diagnosis of AFE, based on the Japanese Consensus Criteria for the Diagnosis of AFE.[6] They experienced symptoms after vaginal delivery.

Healthy controls

We also analyzed serum samples collected from 74 gestational age-matched healthy women who gave birth to healthy newborns in Nara Medical University Hospital between December 2012 and January 2014. Blood samples were taken from all women within two hours after normal delivery, and serum aliquots were stored at −20 °C until analysis. No woman included as a healthy control had a comorbid condition such as malignancies, hypertension, diabetes, asthma, congenital heart disease, kidney disease, connective tissue disorders, or autoimmune disease. All neonates had normal anatomies. This study was approved by the local Institution Review Board (#411-2), and written informed consent was obtained from each subject.

Measurement of serum SCC antigen levels

The serum levels of SCC antigen were measured using chemiluminescence immunoassays performed by BIO MEDICAL LABORATORIES corporation (BML, Inc.,

Tokyo, Japan).

Statistical analysis

All statistical analyses were performed by SPSS version 22 (IBM Corp., Armonk, NY). Between-group comparisons were performed using the *t*-test, χ^2-test, Mann-Whitney U test (pairwise comparisons), and Kruskal Wallis test (comparison among three groups). Data were expressed as number of observations, mean, standard deviations, median, range, and frequency. Receiver operating-characteristic (ROC) curves were constructed and the area under the curve (AUC) was calculated to estimate the predictive power of SCC antigen levels for predicting AFE. Comparison results with $p < 0.05$ were considered statistically significant.

Results

Patient characteristics and symptoms

The demographic and clinical features of healthy controls and patients with AFE are listed in **Table 1**. The autopsy-proven AFE patients were significantly older than the patients in the other two groups ($P = 0.041$). All patients with AFE had massive postpartum hemorrhage (>2,000 mL), which was significantly more substantial than that

noted in healthy controls, as was the risk of cardiopulmonary collapse. In the control group, there was no statistically significant correlation between SCC antigen levels and postpartum hemorrhage (r = -0.023, P = 0.843). Moreover, we did not find any significant difference in SCC antigen levels associated with postpartum hemorrhage; specifically, SCC antigen levels were similar (P = 0.443) between patients with ≥500 mL of blood loss (n = 21; 4.53 ± 1.81 ng/mL) and those with <500 mL blood loss (n = 53; 4.34 ± 2.37 ng/mL).

Forensic autopsy confirmed the histological diagnosis of AFE in the 4 cases with sudden maternal death. Autopsy demonstrated the presence of amniotic fluid components and fetal materials such as squames, meconium, mucin, or lanugo hairs in the pulmonary vasculatures, and established histological evidence of AFE. Among the cases with sudden maternal death, the onset of the AFE episode occurred during labor in 1 case and after delivery in 3 cases (within the first 1 hour, 1 case; within the first 2 hours, 2 cases). Of the 4 mothers who died, 2 had non-instrumental vaginal delivery, 1 had instrumental delivery, and 1 had cesarean delivery. In the autopsy-proven AFE group (n = 4), the prominent presenting features included hemorrhage and disseminated intravascular coagulation (4/4, 100%), as well as cardiac arrest and dyspnea (3/4, 75%). In the clinical AEF group (n = 16), the most prominent initial presenting features were hemorrhage and

disseminated intravascular coagulation (15/16, 93.8%), dyspnea or shortness of breath (6/16, 37.5%), and cardiopulmonary collapse (3/16, 18.8%).

Diagnostic accuracy of SCC antigen

The levels of SCC antigen (mean ± standard deviation) in patients with autopsy-proven AFE (112.0 ± 169.4 ng/mL, $P = 0.001$) and in those with clinical AFE (9.5 ± 10.3 ng/mL, $P = 0.004$) were significantly higher than those in the healthy controls (4.4 ± 2.2 ng/mL) (**Figure 1**). The median levels of SCC antigen in the autopsy-proven AFE, clinical AFE, and control groups were 35.4, 6.1 and 4.2 ng/mL, respectively. The levels of SCC antigen were significantly higher in the autopsy-proven AFE group than in the clinical AFE group ($P = 0.007$).

ROC curve analysis was used to evaluate the ability of SCC antigen to predict the presence of AFE (**Figure 2**). We used 7.15 ng/mL as the optimal cutoff value for SCC antigen levels, which had a sensitivity and specificity of 60.0% and 89.2%, respectively, and an AUC of 0.785 (95% confidence interval, 0.663–0.908, $P < 0.001$). The sensitivity and specificity of SCC antigen for prediction of autopsy-proven AFE was 100.0% and 89.2%, respectively (AUC, 0.997; 95% confidence interval, 0.986–1.000; $P = 0.001$), for prediction of clinical AFE, these values were 50.0% and 89.2%, respectively (AUC,

0.733; 95% confidence interval, 0.591–0.874; $P = 0.004$).

Discussion

AFE represents a catastrophic complication in pregnant women, and therefore early diagnosis and treatment of AFE have important prognostic significance.[16] Since the syndrome occurs acutely with a wide array of clinical manifestations, the clinical diagnosis of AFE is difficult and misdiagnosis is prone to occur.[1] Because AFE is highly heterogeneous in terms of symptoms, clinical practice would benefit highly from a battery of biological tests for diagnosing AFE or predicting its prognosis, enabling the implementation of a more objective clinical management strategy. Therefore, a definitive diagnostic test is desired. In the previous study, we found that SCC antigen was present in the amniotic fluid at concentrations much higher than that in the maternal serum.[2,15] In this study, we estimated the clinical efficacy of serum SCC antigen in diagnosing AFE. ROC curve analysis indicated 7.15 ng/mL as an optimal cut-off value of SCC antigen levels for diagnosing AFE with an overall sensitivity of 60.0% and specificity of 89.2%. For the diagnosis of autopsy-proven AFE, the sensitivity and specificity of SCC antigen were 100.0% and 89.2%, respectively. While these indicators of diagnostic capability were not as high for predicting clinical AFE, we found that women with suspected AFE

based on clinical manifestations also showed significantly higher levels of SCC antigen compared with those noted in healthy controls. To our knowledge, the present study is the first to show that maternal serum SCC antigen levels are significantly higher in patients with AFE than in healthy controls. A previous study proposed that amniotic fluid and fetal debris become trapped in the uterine vasculatures and flow into the maternal blood at the time of vaginal delivery.[6,17] The increase in serum SCC antigen levels is likely due to the entry of amniotic fluid into the maternal circulation, which supports the potential use of SCC antigen as a predictor of AFE. We speculate that AFE becomes fatal when a critical amount of amniotic fluid enters the maternal circulation. On the other hand, amniotic fluid entry into the maternal circulation during labor was proven as a physiological phenomenon, which is consistent with the finding of squamous cells in the blood of controls without symptoms of AFE.[17]

In Japan, ZnCP1 and STN are the most widely used markers in supporting the diagnosis of AFE, even though both markers lack sensitivity and specificity for early detection and thus cannot serve as efficient predictors of AFE.[6,7] To further assess the potential relevance of SCC antigen, its diagnostic performance was comparable against that of conventional markers ZnCP1 and STN.

A previous analysis of data from the same source reported a sensitivity and

specificity of 45.9% and 73.0%, respectively, for ZnCP1, and of 25.8% and 97.2%, respectively, for STN.[18] These findings suggest that serum SCC antigen, which was presently found to have a sensitivity and specificity of 60.0% and 89.2%, respectively, is superior to both ZnCP1 and STN in establishing the diagnosis of AFE. Similarly, the sensitivity of SCC antigen, ZnCP1, and STN in the diagnosis of autopsy-proven AFE was 100.0%, 75.0%, and 25.0%, respectively. Serum SCC antigen levels had also better sensitivity than those of ZnCP1 and STN levels in the prediction of suspected AFE (31.3 %, 18.8%, and 6.3%, respectively). Regarding the AFE diagnosis capabilities, while SCC antigen clearly had the highest sensitivity among these markers, no conclusions can be drawn regarding specificity because of the lack of data on ZnCP1 and STN in healthy controls.

IGFBP-1 represents another potential candidate marker for AFE, as it has already been used to diagnose AFE.[8] We could not estimate the sensitivity and specificity of IGFBP-1 because of the lack of appropriate serum samples. Nevertheless, it should be noted that the ratio of amniotic fluid levels to maternal serum levels is lower for IGFBP-1 than for SCC antigen (150 vs. 400).[19] However, it is not possible to conclude the superiority of SCC antigen over IGFBP-1 since little is known about the diagnostic capabilities of these markers in clinical practice.

Immunohistochemistry might be pivotal in the post-mortem approach to AFE diagnosis. Although available data regarding immunohistochemical stainings in AFE are sparse, it is well known in the literature that presence of keratin, mucin, or endothelin-1, as well as tryptase stainings can support the diagnosis of AFE.[5,20-24] Abnormally low levels of complement C3 and C4 levels were also identified in AFE (vs. non-AFE), suggesting that complement activation such as C5aR signaling might play a role in the etiology of AFE.[25,26] We speculate that, when traditional microscopic examination or histological stains cannot easily detect AFE-specific changes in clinical and medicolegal investigations, evaluation of immunohistochemical expression may represent a sensitive method for detecting amniotic fluid-derived SCC antigen in the maternal pulmonary vasculatures; however, since we were not able to obtain autoptic samples for immunohistochemistry, we could not confirm this hypothesis. Taken together, the diagnostic test based on SCC antigen appears to be more useful compared with the conventional tests for diagnosing AFE.

The reported diagnosis approaches, incidence, and case mortality of AFE vary widely because of a lack of uniform clinical definition. Specific biomarkers including SCC antigen, ZnCP1, STN, IGFBP-1, C3 and C4 have been described and may aid the diagnosis of AFE. However, the definition of AFE based on these markers is not widely

accepted because the prominent presenting features are not only fetal-antigen dose-dependent but also dose-independent. We believe that the presence of fetal antigens in maternal circulation alone is not sufficient to cause AFE, but the adverse reaction may be idiosyncratic, most likely against the fetal antigens. Development of a consensus on case definition would minimize inclusion of false positive cases and prevent inclusion of false negative cases.

A major limitation of this preliminary investigation is the relatively small sample size. Future studies examining large cohorts of patients with autopsy-proven AFE and clinical AFE are needed to confirm the clinical relevance of SCC antigen. Furthermore, the diagnostic power of known biomarkers of AFE was not estimated because of the lack of appropriate serum samples; therefore, conclusions regarding the superiority of SCC antigen over biomarkers such as ZnCP1 or STN should be interpreted with care.

Despite the limitations of the present study, our findings indicate that SCC antigen may be a sensitive and noninvasive marker for supporting the diagnosis of AFE. Further studies with more rigorous design are required for validating the present findings in clinical practice and to clarify whether elevated serum SCC antigen levels are associated with the severity of the disease.

Acknowledgements

The present study was supported by a grant-in-aid (No.26462497) for Scientific Research from the Ministry of Education, Science, and Culture of Japan to the Department of Obstetrics and Gynecology, Nara Medical University.

Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figure Legend

Figure1. The SCC values in the autopsy-proven AFE, clinical AFE and controls.

The results of the SCC levels in the autopsy-proven AFE group, clinical AFE group and control group are expressed as the medians, interquartiles (*boxes*) and 10th and 90th percentiles (*whiskers*).

Figure 2. Receiver operative characteristic (ROC) curves and corresponding areas under the curve (AUC) for AFE.

ROC curve shows the diagnostic values of SCC for AFE.

Figure 1.

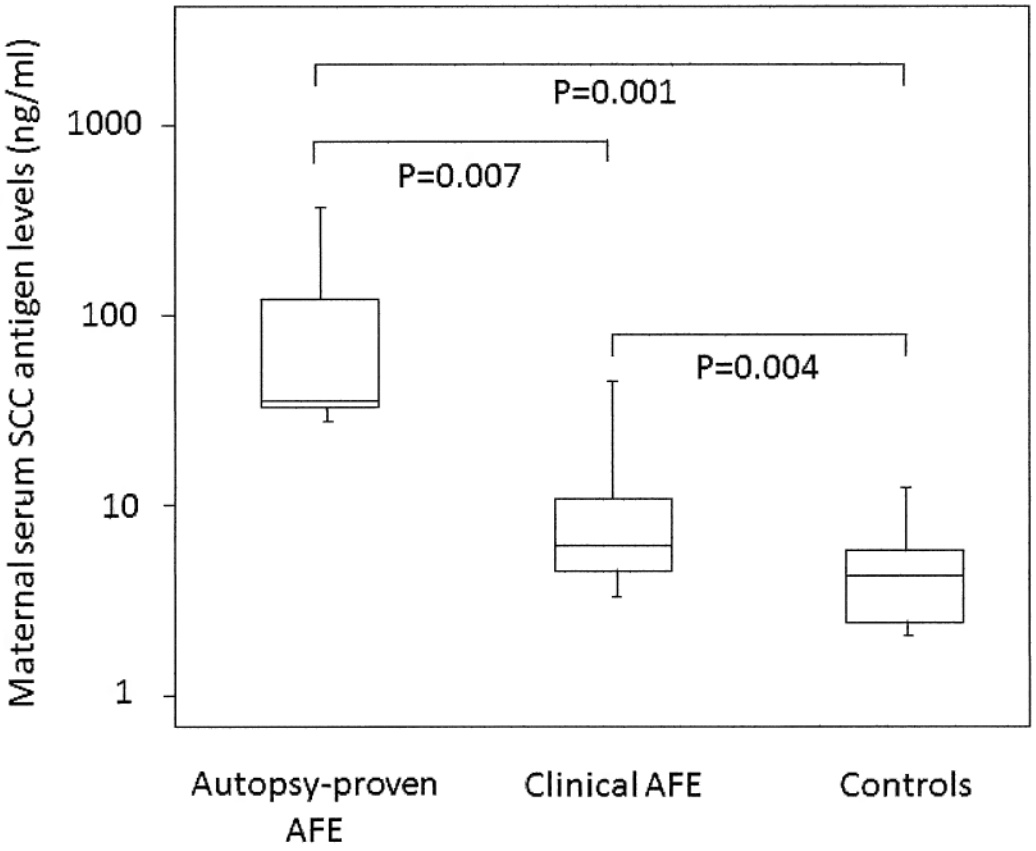
Maternal serum SCC antigen levels (ng/ml)
1000
100
10
1
P=0.001
P=0.007
P=0.004
Autopsy-proven AFE
Clinical AFE
Controls

Figure 2.

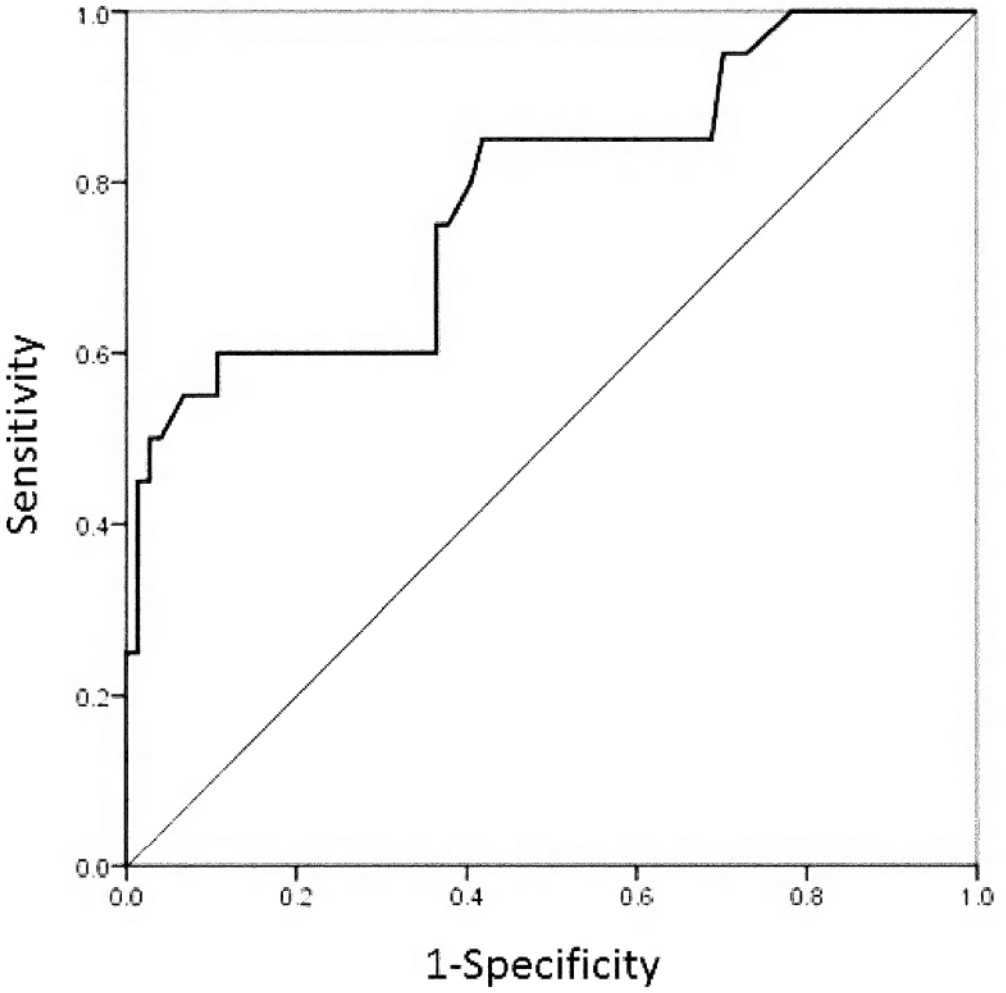

Table1. Clinical characteristics and symptoms of the autopsy-proven AFE group, clinical AFE group, and control group.

Table 1

	autopsy-proven AFE	clinical AFE	controls	
characteristics	n=4	N-16	n=74	p
age (mean ± SD)	36.8 ± 4.8[a]	33.9 ± 3.6[b]	31.5 ± 5.3[c]	0.041
(range)	(30-41)	(26-39)	(19-42)	
gestational weeks	39.3 ± 1.7	39.2 ± 1.5	39.3 ± 1.0	0.935
(range)	(37-41)	(37-41)	(37-41)	
parity				0.198
0	1	6	43	
1	2	7	22	
2	1	1	9	
>2	0	2	0	
symptoms				
postpartum hemorrhage				<0.001
0-1000ml	0	0	70	
1000-2000ml	0	0	4	
2000-4000ml	1	0	0	
4000ml≥	3	16	0	
cardiac arrest				<0.001
yes	3	3	0	
no	0	13	74	
unknown	1	0	0	
dyspnea				<0.001
yes	3	8	0	
no	0	8	74	
unknown	1	0	0	

a versus b, p=0.142; a versus c, p=0.077; and b versus c, p=0.059.