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Original Article

Circulating serum amyloid A: a potential biomarker for infection after aneurysmal subarachnoid hemorrhage

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Abstract: Objectives: One of the complications after Aneurysmal Subarachnoid Hemorrhage (aSAH) is infection. An accurate biomarker for the early diagnosis of the infection may lead to a better management of aSAH. This study is to investigate the role of circulating serum amyloid A (SAA) in identifying high risk of infection in Chinese aSAH patients. Methods: Circulating levels of SAA were evaluated in 126 aSAH patients during hospitalization (at Day 1/3/5, respectively), using ELISA. Multivariable logistic regression, receiver operating characteristic (ROC) curve, and multivariate stepwise linear regression analysis were performed to assess the association between SAA and post-aSAH infection. Results: The patients with post-aSAH infection presented a higher circulating SAA level (Day 1) (2084.76 ± 588.76 ng/ml, $P < 0.001$) than the ones without infection (1580.95 ± 566.11 ng/ml). Meanwhile, infection status presented a significantly upward trend (40.5%, 45.2%, and 90.5%, $P < 0.001$), as SAA concentration elevated among its tertiles. Compared with the lowest tertile of SAA, the highest revealed an association with the presence of infection (odds ratio = 2.461, 95% confidence interval [CI] [1.210-5.003], $P = 0.013$) after controlling age, gender, Body Mass Index, C-reactive protein and Erythrocyte sedimentation rate. Furthermore, ROC curve of SAA was developed to predict the presence of infection (Area under ROC = 0.736 [95% CI 0.648-0.824], $P < 0.001$). Conclusions: It supports circulating SAA as a potential biomarker for the early diagnosis of post-aSAH infection.

Keywords: Aneurysmal subarachnoid hemorrhage, serum amyloid A, biomarker, infection, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), the World Federation of Neurosurgical Societies Scale (WFNS), the Glasgow Coma Scale (GCS)

Introduction

Aneurysmal Subarachnoid Hemorrhage (aSAH) is the subsequent accumulation of blood in the subarachnoid space, resulted from the rupture of an intracranial aneurysm [1-4]. One of the main complications in aSAH is post infection, including pneumonia, urinary tract infections etc. The infection not only prolongs hospitalization, but also results in a principal cause of mortality and morbidity [5, 6].

Currently, the diagnosis of infection tends to depend on patients' symptoms, e.g. fever, and clinical parameters, e.g. higher levels of white blood cell [7, 8]. And then, a bacterial culture is performed for suspected infection, even though the procedure usually takes more than 48 hours. Thus, an accurate biomarker for the early diagnosis of the infection could lead to a better management of aSAH, via identifying the patients with a higher risk of infection [8, 9].

Recently, Azurmendi et al. [10] performed quantitative mass spectrometry to discover biomarkers for the prediction of infection in aSAH patients. They identified 17 potential proteins, and among these, circulating serum amyloid A (SAA) presented significantly different levels between aSAH patients with infection and ones without infection. It suggested measuring SAA could be an efficient way to identify aSAH patients susceptible of developing infection during hospitalization.

As an acute phase protein, higher levels of SAA have been found in patients with infections, autoimmune diseases and cancers [11-13].

Moreover, previous studies support it as a diagnostic parameter for neonatal sepsis [14]. This study, thus, is to investigate the role of circulating SAA in identifying high risk of post-aSAH infection in Chinese patients.

Subjects and methods

Subjects

Subjects in the study were selected from the aSAH patients who hospitalized at Suqian People's Hospital from January 2013 to March 2015. The criteria for enrollment included: 1) confirmed diagnosis of aSAH supported by completed radiological results; 2) admission within 48 hours of the initial hemorrhage; 3) aged above 18 years old. Participants were excluded if they had viral/drug-induced/autoimmune liver diseases, malignant tumor, severe cardiopulmonary disorders, renal dysfunction, inflammatory diseases, and thyroid dysfunction. All subjects gave written informed consent before participation. This study was approved by the Ethics Committee of Suqian People's Hospital, in accordance with the Helsinki Declaration, revised in 2008.

Clinical evaluation and treatment

At admission, each patient's severity was evaluated by the World Federation of Neurosurgical Societies scale (WFNS: good status 1-2, and poor status 3-5) [15] and the Glasgow Coma Scale (GCS: minor brain injury ≥ 13, moderate brain injury 9-12, and severe brain injury ≤ 8) [16, 17]. Plasma levels of C-reactive protein (CRP) and Erythrocyte sedimentation rate (ESR) were evaluated at Day 1. Infection status was confirmed based on the International Sepsis Forum Consensus Conference on Definitions of Infection in the Intensive Care Unit [18]. Antibiotherapy started on the condition that clinical and biological evidence supported bacterial infection. Then bacterial culture was performed and antibacterial treatment was adjusted according to cultural results.

Serum sample

About 10 mL whole blood samples were collected from each subject during hospitalization (at Day 1/3/5, respectively), and then serum samples were separated for further analysis (stored at -80°C). Circulating SAA was evaluated using a commercial enzyme-linked immunosorbent assay (ELISA) (Catalogue No. CEA885Hu; Uscn life Science, Cloud-Clone Corp., Houston, TX, USA; Intra-assay coefficient of variation (CV) < 10%; Interassay CV < 12%) [19]. Furthermore, circulating SAA levels were validated by ELISA from the other provider (Catalogue No. ab18713; Abcam Ltd., Cambridge, UK) and western blotting (Anti-Serum Amyloid A, Catalogue No. ab18713; Abcam Ltd., Cambridge, UK and β-Actin Rabbit mAb, Catalogue No. 8457; Cell Signaling Technology, Inc., Danvers, MA).

Statistical analysis

Power of sample size was calculated by G*Power (version 3.1, Heinrich-Heine-Universität Düsseldorf, Germany) [20]. Normally distributed variables were presented as mean ± standard deviation (SD). Normality of distribution was tested with the Kolmogorov-Smirnov test. The Student's t test for continuous variables and Chi-square test for categorical variables were used to compare the parameters between two groups. Comparisons among various groups used One-way ANOVA, followed by post-hoc test (Bonferroni). To assess the relationship between circulating SAA and infection, we calculated the adjusted odds ratio (OR) and 95% confidence interval (CI) with a multivariable binary logistic regression. Receiver operating characteristic (ROC) curve of circulating SAA was developed to predict the presence of infection. Multivariate stepwise linear regression analysis was conducted for SAA (dependent variable), including the related variables as independent variables. All statistical analyses and plotting were performed using SPSS (version 21.0, SPSS, Inc., Chicago, IL, USA) and GraphPad Prism (version6.0, GraphPad Software, Inc., San Diego, CA, USA). A two-sided $P < 0.05$ was considered statistically significant.

Results

Validation of ELISA and power of sample size

The validation of SAA levels presented a fine consistency (n = 50, $r = 0.6241$, $P < 0.001$) between two ELISA evaluations (Supplementary Figure 1A). Representative western blot detection of SAA in eight serum samples revealed a

Table 1. Characteristics of subjects according to infection status

Parameters	No Infection	Infection	*P* for trends
No. of subjects	52	74	
Age (years)	52.75 ± 13.51	56.03 ± 10.39	0.126
Male, n (%)	35, 67.3%	46, 62.2%	0.577
BMI (kg/m^2)	22.77 ± 2.89	25.04 ± 3.62	< 0.001
Diabetes, n (%)	8, 15.4%	18, 24.3%	0.268
Hypertension, n (%)	7, 13.5%	17, 23.0%	0.250
SAA at Day 1 (ng/ml)	1580.95 ± 566.11	2084.76 ± 588.76	< 0.001
SAA at Day 3 (ng/ml)	2153.56 ± 769.98	2873.94 ± 790.45	< 0.001
SAA at Day 5 (ng/ml)	1860.11 ± 729.14	2262.55 ± 746.17	0.003
CRP (mg/L)	12.47 ± 5.13	16.75 ± 6.88	< 0.001
ESR (mm/h)	7.57 ± 2.73	9.02 ± 2.95	0.006
FPG (mmol/L)	6.09 ± 2.48	6.22 ± 2.18	0.768
WFNS, n (%)			0.003
1-2	28, 53.8%	20, 27.0%	
3-5	24, 46.2%	54, 73.0%	
GCS, n (%)			< 0.001
≥ 13	20, 38.5%	13, 17.6%	
9-12	25, 48.1%	27, 36.5%	
≤ 8	7, 13.5%	34, 45.9%	
Treatment			0.002
Surgery	13, 25.0%	40, 54.1%	
Embolization	35, 67.3%	27, 36.5%	
No treatment	4, 7.7%	7, 9.5%	
EVD, n (%)	13, 25.0%	36, 48.6%	0.009
LCD, n (%)	23, 44.2%	47, 63.5%	0.045

Data presents as mean ± SD for continuous variables.

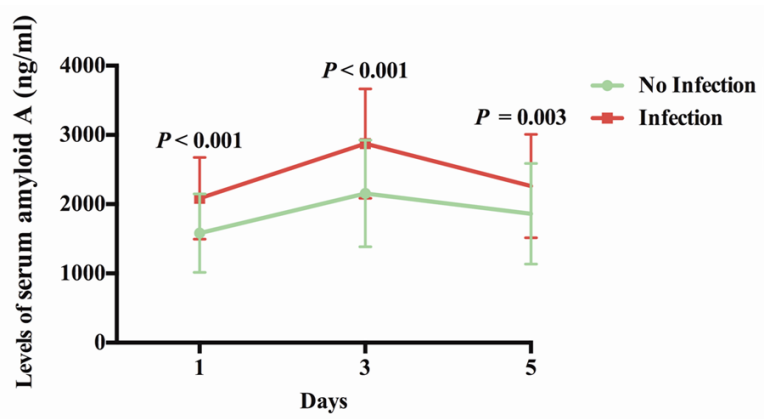

Figure 1. Comparisons of SAA levels (mean ± SD) according to infection status during Day 1 to Day 5. Day 1: No infection (1580.95 ± 566.11 ng/ml) vs. Infection (2084.76 ± 588.76 ng/ml), $P < 0.001$; Day 3: No infection (2153.56 ± 769.98 ng/ml) vs. Infection (2873.94 ± 790.45 ng/ml), $P < 0.001$; Day 5: No infection (1860.11 ± 729.14 ng/ml) vs. Infection (2262.55 ± 746.17 ng/ml), $P = 0.003$.

consistent trend with the ELISA result (Supplementary Figure 1B).

Given the serum SAA concentrations and the numbers of subjects, the power of the sample size was 0.997 (Effect size d = 0.872, Critical t = 1.979), using G*Power that is a widely accepted software to calculate sample power (Supplementary Figure 2).

Characteristics of subjects according to infection status

The characteristics of the 126 subjects in the study are revealed in **Table 1**. The concentration of SAA in subjects with infection (n = 74) at Day 1 (2084.76 ± 588.76 ng/ml, $P < 0.001$) was higher than it in subjects without infection (n = 52) (1580.95 ± 566.11 ng/ml, **Table 1**; **Figure 1**). The similar higher levels appeared, when it came to Day 3 ($P < 0.001$) and Day 5 ($P = 0.003$).

The Body Mass Index (BMI) between two groups witnessed a significant difference ($P < 0.001$), whereas the proportions of diabetes ($P = 0.268$) or hypertension ($P = 0.250$) presented no difference. The levels of CRP ($P < 0.001$) and ESR ($P = 0.006$) at Day 1 were differed between two groups, while it appeared similarly in terms of fasting plasma glucose (FPG) ($P = 0.768$). Furthermore, WFNS ($P = 0.003$) and GCS ($P < 0.001$), two indicators of patients' severity at hospital admission, indicated that the subjects with infection tended to present poor clinical state and severe grade of brain injury, compared with the non-infection. In terms of treatment (P =

Table 2. Characteristics of subjects according to SAA (Day 1) tertiles

SAA (ng/ml)	SAA (Day 1) Tertiles			*P* for trends
	T1 (1180.71 ± 231.30)	T2 (1860.76 ± 168.57)	T3 (2589.05 ± 326.62)	
Infection, n (%)	17, 40.5%	19, 45.2%	38, 90.5%	< 0.001
WFNS, n (%)				< 0.001
1-2	35, 83.3%	13, 31.0%	0, 0.0%	
3-5	7, 16.7%	29, 69.0%	42, 100.0%	
GCS, n (%)				< 0.001
≥ 13	30, 71.4%	3, 7.1%	0, 0.0%	
9-12	12, 28.6%	31, 73.8%	9, 21.4%	
≤ 8	0, 0.0%	8, 19.0%	33, 78.6%	
Treatment				< 0.001
Surgery	12, 28.6%	12, 28.5%	29, 69.9%	
Embolization	23, 54.3%	28, 66.7%	11, 26.2%	
No treatment	7, 16.7%	2, 4.8%	2, 4.8%	
EVD, n (%)	12, 28.6%	14, 33.3%	23, 54.8%	0.032
LCD, n (%)	20, 47.6%	22, 52.4%	28, 66.7%	0.188

Data are mean ± SD for continuous variables; *P* values for the overall comparisons across SAA tertiles.

0.002), the infection indicated a higher proportion of surgery (54.1%), whereas more subjects without infection underwent Embolization (67.3%). Besides, subjects with infection indicated higher percentages of External ventricular drain (EVD) ($P = 0.009$) and Lumbar cistern drainage (LCD) ($P = 0.045$).

Characteristics of subjects according to SAA tertiles

All 126 subjects were then divided into 3 groups, according to the tertiles of SAA concentrations at admission, as indicated in **Table 2**. Infection status presented a significant difference ($P < 0.001$) that its proportion increased (40.5%, 45.2%, and 90.5%), as SAA concentration elevated.

As far as severity and treatment, WFNS ($P < 0.001$), GCS ($P < 0.001$), Treatment ($P < 0.001$) and EVD ($P = 0.032$) witnessed difference among the tertiles, except LCD ($P = 0.188$).

Odds ratios of infection

Binary logistic regression analysis (**Table 3**) reveals that compared with the 1st tertile, the 2nd tertile of SAA indicated no association with the presence of infection (adjusted OR = 1.170, 95% CI [0.263-5.200], $P = 0.837$), after adjustments.

In the comparison between the 1st and the 3rd, the adjusted OR was 2.461 (95% CI [1.210-5.003], $P = 0.013$), after adjustments of age, gender, BMI, CRP and ESR. However, the association failed to appear (adjusted OR = 2.493 (95% CI [0.575-10.806], $P = 0.222$), after controlling age, gender, BMI, WFNS and GCS.

ROC curve

ROC curve of SAA (Day 1) was developed to predict the presence of infection (**Figure 2**). Area under ROC (AUROC) was 0.736 (95% CI 0.648-0.824, $P < 0.001$), with the sensitivity of 62.2%, the specificity of 69.2% and the accuracy of 64.29%, when the cut off value chose 1879.30 ng/mL.

AUROC of SAA (Day 3) was 0.737 (95% CI 0.649-0.826, $P < 0.001$), while AUROC of SAA (Day 5) was 0.658 (95% CI 0.561-0.755, $P = 0.003$), as shown in Supplementary Figures 3 and 4.

Multivariable linear regression analysis

In the multivariable linear regression analysis (**Table 4**), the best model (corrected $r^2 = 0.749$, $P < 0.001$) that predicted circulating SAA level included GCS, ESR and WFNS as predictive variables.

Discussion

This study found that the circulating SAA level in patients with post-aSAH infection was higher than it in patients without infection. Circulating level of SAA indicated a close association with the presence of post-aSAH infection, supported by the results of logistic regression and ROC curve.

Recent advance suggests that SAA, an acute-phase protein, plays a key role in inflammation [11]. Given its pleotropic feature, SAA shows multiple functions that include attracting

Table 3. ORs and 95% CIs of the presence of infection in relation to SAA tertiles

		T1 vs. T2			T1 vs. T3		
	Adjustments	ORs	95% CIs	*P* for trends	ORs	95% CIs	*P* for trends
Infection	Model 1	1.215	0.511-2.886	0.659	3.738	2.051-6.812	< 0.001
	Model 2	1.113	0.435-2.847	0.823	3.351	1.811-6.200	< 0.001
	Model 3	0.525	0.171-1.609	0.259	2.461	1.210-5.003	0.013
	Model 4	1.170	0.263-5.200	0.837	2.493	0.575-10.806	0.222

Model 1: unadjusted; Model 2: adjusted for age, gender, and BMI; Model 3: adjusted for age, gender, BMI, CRP and ESR; Model 4: adjusted for age, gender, BMI, WFNS and GCS.

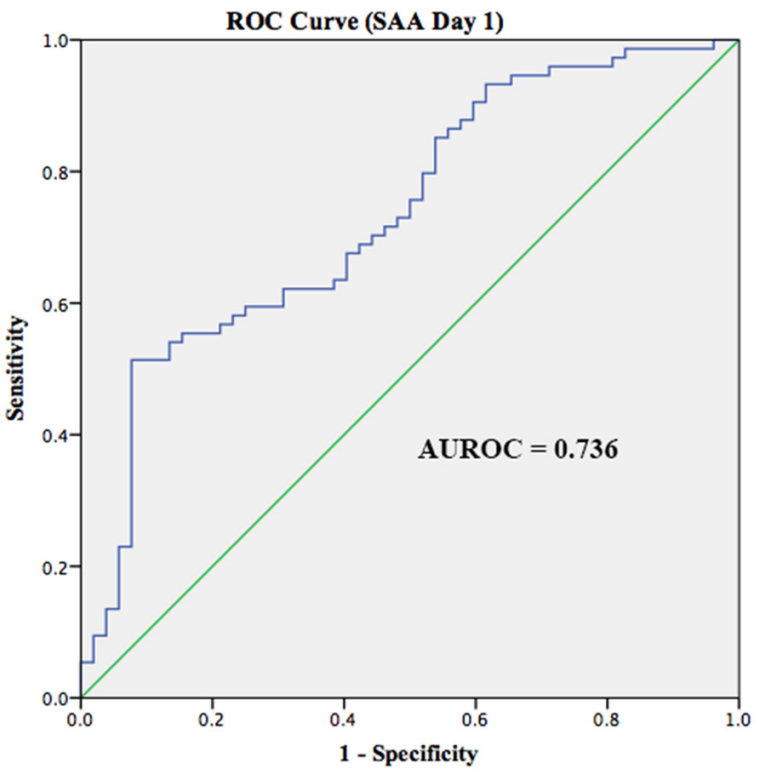

Figure 2. ROC curve of circulating SAA was developed to predict the presence of post-aSAH infection (AUROC = 0.736 [95% CI 0.648-0.824], $P < 0.001$).

migrating phagocytes to inflammatory site and triggering a series of transcription factors and inflammatory cytokines [21]. Previously, O'Hara et al. [22] demonstrated that SAA associated with increased joint damage in rheumatoid arthritis, which may be the direct result of synovial SAA-induced effects on cartilage degradation. Vallon et al. [23] supported that SAA participated in cartilage destruction in age-induced arthritis of human and rabbit. And de Seny et al. [24] found that SAA in circulation and osteoarthritic joint cavity might play an important role in inflammatory process of osteoarthritis, which related to the involvement of Toll-Like Receptor 4. In terms of atherosclerosis, Meek et al. [25] revealed that SAA involved in lipid metabolism in atherosclerosis and played a role in defending against injurious agents. Rodent study by Wilson et al. [26] revealed that SAA modifies vascular proteoglycans in a pro-atherogenic way by stimulating Transforming growth factor beta, thereby playing a causal role in the development of atherosclerosis, while Dong et al. [27] performed Apolipoprotein E-deficient mice study suggested SAA is not only a potential biomarker for atherosclerosis, but also an essential regulator in atherogenesis. Ludwigshafen Risk and Cardiovascular Health study by Zewinger et al. [28] revealed that SAA alters the biological effects of High-density lipoprotein-cholesterol in various cardiovascular conditions. When it came to Type 2 Diabetes, Region of Augsburg S4 study [29] suggested SAA protein precedes the onset of type 2 diabetes independently of parameters of glucose metabolism. Marzi et al. [30] revealed the regulation of SAA in inflammatory processes of diabetes and its close associations with leptin and other acute-phase proteins. Moreover, Niederauet al. [31] proved circulating SAA is a more useful indicator for the activity of Inflammatory Bowel Disease than conventional markers, e.g. C-reactive protein, sedimentation rate and platelet count.

Table 4. Multiple regression analyses with SAA as dependent variable

	Variable	r^2	β	*P* value
Model 1		0.631		< 0.001
	GCS		-0.796	< 0.001
Model 2		0.735		< 0.001
	GCS		-0.579	< 0.001
	WFNS		0.390	< 0.001
Model 3		0.749		< 0.001
	GCS		-0.557	< 0.001
	WFNS		0.363	< 0.001
	ESR		0.135	0.005

Values are corrected r^2 (r^2), standardized coefficients (β) and associated *P* values.

Interestingly, previous studies supported circulating SAA a potential role in subarachnoidal hemorrhage. Jernaset al. [32] found that plasma levels of SAA were higher at 7 to 9 days after subarachnoidal hemorrhage in comparison with admission or recovery, which supported that acute-phase protein may involve in the development of insulin resistance during critical illness. And Onda et al. found [33] the upregulated expression of SAA in vasospastic arteries suggested that inflammatory reaction might be involved in the development of cerebral vasospasm after subarachnoid hemorrhage.

In this present study, the concentrations of circulating SAA at Day 1/3/5 in patients with post-aSAH infection were all higher than them in patients without infection. Among SAA tertiles at admission, infection status presented a significantly upward trend, as SAA concentration elevated. As far as patients' severity, WFNS and GCS also witnessed difference among the tertiles. Compared with the 1st tertile of SAA, the 3rd revealed an association with the presence of infection after adjustments of age, gender, CRP, ESR and BMI. However, when took patients' severity into consideration, the association failed to appear. To predict the presence of infection, ROC curves of SAA (Day 1/3/5) were developed that AUROC were 0.736/0.737/0.658, respectively.

Consistent with the previous study by Azurmendi et al. [10], this Chinese study revealed that the levels of circulating SAA at multiple days during hospitalization increased in aSAH patients with infection, compared with the non-infection. Both two studies suggested circulating SAA level as a potential biomarker for post-infection in aSAH patients. Moreover, in this study, multiple linear regression analysis indicated a predicting model, including WFNS, ESR and GCS, explained 74.9% of the total variability of circulating SAA levels.

In terms of the strengths, to begin with, we measured circulating SAA level with ELISA kits from two providers and also performed western blotting detection to validate the reliability, which presented a fine consistency. Besides, the power of sample size analysis suggested that the current size was enough to analyze the difference of circulating SAA between the two groups.

Some limitations of the study merit comment. Firstly, a prospective cohort study in multiple centers is required in the future. Secondly, the significant diversity of BMI between two groups was not further analyzed in the study. Lastly, the category of infection, the result of bacterial culture, and the variables of inflammation and immunity, e.g. interleukin, failed to be collected to analysis.

In summary, this clinically observational study demonstrates that the circulating SAA increased in patients with post-aSAH infection, compared with patients without infection. Furthermore, circulating SAA presented an association with higher odds of infection. Lastly, the severity of patient at admission related to circulating level of SAA. It supports circulating SAA as a potential biomarker for the early diagnosis of post-aSAH infection.

Disclosure of conflict of interest

None.

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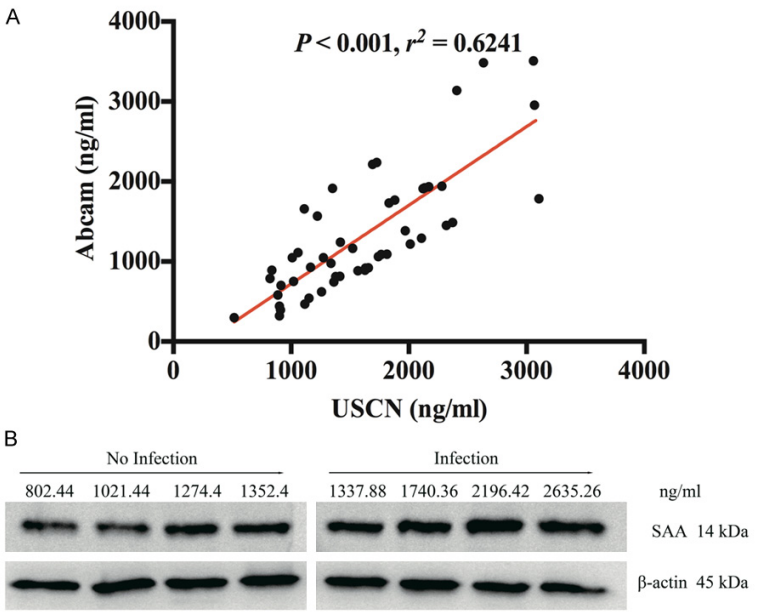
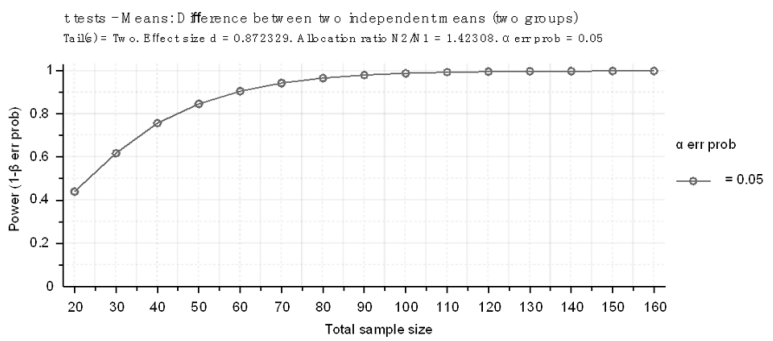
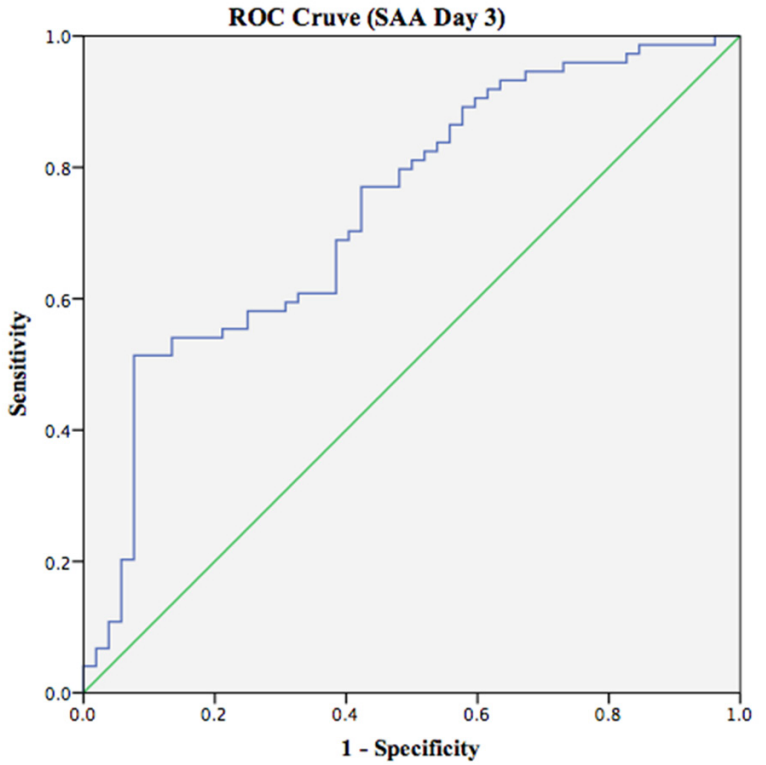


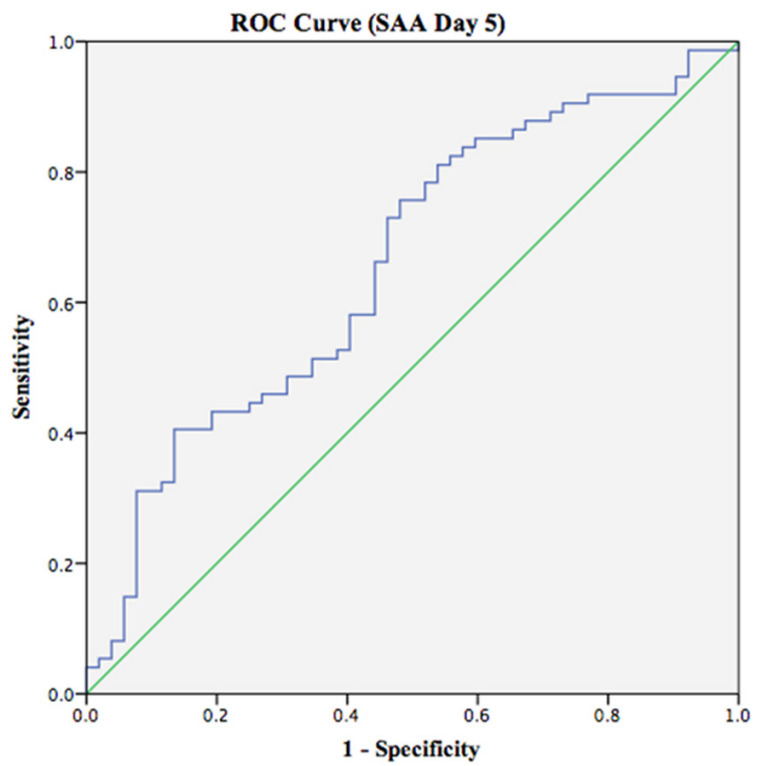
Supplementary Figure 1. A: Correlation of circulating SAA levels between USCN ELISA and Abcam ELISA (n = 50, r^2 = 0.6241, P < 0.001); B: Representative western blot detection of circulating SAA in eight serum samples.



Supplementary Figure 2. Given the serum SAA concentrations and the numbers of subjects, the power of the sample size was 0.997 (Effect size d = 0.872, Critical t = 1.979), using G*Power that is a widely accepted software to calculate sample power.



Supplementary Figure 3. AUROC SAA Day 3. AUROC of SAA (Day 3) was 0.737 (95% CI 0.649-0.826, *P* < 0.001).



Supplementary Figure 4. AUROC SAA Day 5. AUROC of SAA (Day 5) was 0.658 (95% CI 0.561-0.755, *P* = 0.003).