INTRODUCTION

As a common cerebral disease, stroke attacks one in six people worldwide annually, not to mention the big number of acute ischemic stroke (AIS) survivors suffering from a damaged quality of life and disability.1 The AIS accounts for more than 80 percent of all stroke cases, it presents with an increase in incidence due to the aging of the population, especially in the developing countries, which obviously complicates the national health burden due to AIS is a main cause of mortality and disability.2 Mainstay of AIS management is to rehabilitate the blocked artery and recover the perfusion in the affected brain tissue, which is mainly implemented by the thrombolysis therapy.3 AIS could damage the brain tissue profoundly, therefore, to eliminate the irreversible...
brain injury, AIS demands intervention as soon as possible, which, however, is difficult to achieve in the clinical practice. This situation is resulted from that the proportion of patients who can be timely treated with effective therapy, such as intravenous tissue plasminogen activator, is very low due to many delays pre-hospital and in-hospital.

Sirtuin 2 (SIRT2) is a NAD⁺ dependent deacetylase that belongs to the sirtuin family, which is a protein family with capacity of deacetylation that participates in various activities in human body including response to stress, cell apoptosis, gene fixing, and so on.⁵ Above all, although like the other sirtuin family members, SIRT2 expresses universally in all the systems in human, evidence suggests that SIRT2 has the most abundant expression in the brain.⁵⁻⁷ Besides, previous reports reveal that SIRT2 is involved in the mediation of various processes that are related to cerebral diseases, such as oxidative stress and neuron-inflammation.⁸⁻⁹ Moreover, several findings elucidate that SIRT2 plays a harmful role in the brain-related diseases, for instance, the SIRT2 polymorphism rs10410544 may be correlated with increased risk of Alzheimer’s disease.¹⁰ In terms of stroke, previous study illuminates that the SIRT2 expression is enhanced by microglia after stroke, which results in an inhibition of anti-inflammation function of the infiltrating regulatory T Cells.¹¹ Based on these facts, we hypothesized that SIRT2 may have potential clinical value in AIS management.

Hence, this study aimed to investigate the correlation of SIRT2 with AIS risk, severity, inflammation, and prognosis.

2 | METHODS

2.1 | Participants

During the period of July 2015 and August 2016, a total of 164 first episode AIS patients who admitted to Tongren Hospital, Shanghai Jiao Tong University School of Medicine were consecutively recruited in this study. At the same period, 164 non-AIS patients who complicated with high-stroke-risk factors were enrolled as controls. The inclusion criteria of AIS patients were: (1) diagnosed as AIS based on the clinical history, neurological symptoms, cranial computed tomography (CT) scan, magnetic resonance imaging (MRI) and/or diffusion weighted imaging; (2) age≥45 years old; (3) admitted to the hospital and received treatment within 12 hours of stroke onset. The exclusion criteria of AIS patients were: (1) recurrent stroke, intracranial hemorrhage, or transient ischemic stroke; (2) with a preexisting neurological disease that would confound the neurological evaluations; (3) history of hematological malignancies or solid tumors; (4) history of severe infections or systemic inflammatory disease, and received immunosuppressive drugs with 3 months before enrollment; (5) pregnant or lactating woman. The age- and gender-matched controls were screened from high-stroke-risk population, and they had at least 2 risk factors of ischemic stroke listed in World Health Organization (WHO) recommendations on stroke prevention, diagnosis, and therapy.¹² The controls who had history of stroke, hematological malignancies, solid tumors, or severe infections were excluded. The Ethics Committee of Tongren Hospital, Shanghai Jiao Tong University School of Medicine approved this study, and written informed consents were obtained from the participants or their family members.

2.2 | Clinical data collection

The age, gender, body mass index (BMI), current smoke status, and common complications (such as hypertension, hyperlipidemia, hyperuricemia, diabetes mellitus, and chronic kidney disease (CKD)) of all participants were recorded. The severity of AIS was assessed using National Institute of Health stroke scale (NIHSS) score. The total NIHSS score ranges from 0 to 42, and a higher score was represented as a more severe stroke.¹³

2.3 | Blood collection and processing

Peripheral blood samples of AIS patients were collected on the day of admission. Besides, peripheral blood samples were also collected from controls on enrollment. After collection, all peripheral blood samples were centrifuged at 2000 g for 10 min to separate serum. Then, the serum samples were stored at −80°C until further detection.

2.4 | SIRT2 detection

The level of SIRT2 in serum samples of AIS patients and controls was detected by enzyme-linked immunosorbent assay (ELISA). The human ELISA kit of SIRT2 was designed by Shanghai Enzyme-linked Biotechnology Co., Ltd. (Shanghai, China). The procedures of ELISA were conducted according to the instructions of manufacturer. In brief, firstly, the serum samples were added to the 96-cell plate. Then the samples were mixed with antibody. After incubation, the wells were washed to remove unbound material, subsequently, tetramethylbenzidine substrate was added to the wells to generate blue coloration. This reaction was then stopped by addition of Stop Solution. Finally, the intensity was measured at 450 nm wavelengths on microplate reader (BioTek, Winosky, USA).

2.5 | Inflammatory cytokines detection

The inflammatory cytokines including tumor necrosis factor (TNF-α), interleukin-6 (IL-6), and interleukin-17 (IL-17) in AIS patients’ serum samples were detected by ELISA, which were carried out referring to the instructions of ELISA Kits. The brief procedures were the same as SIRT2 detection. The ELISA Kits used in the study were as follows: human TNF-α ELISA Kit (Abcam, Cambridge, USA), human IL-6 ELISA Kit (Abcam, Cambridge, USA), and human IL-17 ELISA Kit (Abcam, Cambridge, USA).
2.6 | Follow-up

AIS patients were continually followed up to 36 months or death. Recurrence-free survival (RFS) was calculated from the date of admission to the date of stroke recurrence or death. Overall survival (OS) was calculated from the date of admission to the date of death. The patients who lost to follow-up were censored at the last visit date.

2.7 | Statistical analysis

Comparison of characteristics between AIS patients and controls was determined by Student’s t test or Chi-square test. Comparison of SIRT2 expression level between AIS patients and controls, or between recurrent/dead AIS patients and non-recurrent/dead AIS patients, or between dead AIS patients and non-dead AIS patients was determined by Wilcoxon rank sum test. Correlation of SIRT2 expression level with NIHSS score or inflammatory cytokines was determined by Spearman’s rank correlation test. Receiver-operating characteristic (ROC) curve was plotted and the area under the curve (AUC) with 95% confidence interval (CI) was used to assess the ability of SIRT2 in discriminating AIS patients from controls. RFS and OS were displayed using Kaplan-Meier curve, and the comparisons of RFS and OS between SIRT2 high expression AIS patients, and SIRT2 low expression AIS patients were analyzed by log-rank test. Statistical analysis was performed using SPSS 24.0 software (IBM, Chicago, USA). Figure was plotted using GraphPad Prism 7.01 software (GraphPad Software Inc., San Diego, USA). P value <0.05 was considered statistically significant.

3 | RESULTS

3.1 | Study flow

Initially, 213 AIS patients and 196 non-AIS patients (controls) with high-stroke-risk were invited to participate in this study, 49 AIS patients were excluded including 38 exclusions and 11 patients who refused to participate, and 32 non-AIS patients were excluded which consisted of 21 exclusions and 11 patients who declined to participate (Figure 1). In the remaining 164 eligible AIS patients, peripheral
blood samples on admission were collected followed by the detection of SIRT2, TNF-α, IL-6, and IL-17 in serum, while in the 164 eligible non-AIS patients (controls) who were recruited, blood samples on admission were also collected and then the SIRT2 expression was detected in serum. Afterward, the AIS patients were followed up to 36 months or death, and in the 164 AIS patients, there were 27 (16.5%) patients who were lost to follow-up, 17 (10.4%) patients who had stroke recurrence and 22 (13.4%) patients who died. In addition, all the 164 AIS patients and 164 non-AIS patients (controls) were included in the final analysis.

### 3.2 Characteristics in AIS patients and controls

The 164 AIS patients had an age of 65.8 ± 9.6 years in average and 164 controls had an age of 65.0 ± 9.9 years in average (p = 0.435) (Table 1). The numbers of female and male were 47 (28.7%) and 117 (71.3%) in AIS patients, and were 38 (23.2%) as well as 126 (76.8%) in controls (p = 0.723). Mean value of BMI was 24.2 ± 2.3 kg/m² in AIS patients and was 24.1 ± 2.8 kg/m² in controls (p = 0.373). In addition, the numbers of current smokers in AIS patients and controls were 76 (46.3%) and 68 (41.5%), respectively (p = 0.373). Information about percentages of hypertension (p = 0.095), hyperlipidemia (p = 0.374), hyperuricemia (p = 0.152), diabetes mellitus (p = 0.123), and CKD (p = 0.105) between AIS patients and controls can be seen in Table 1. Moreover, no difference in characteristics was found between AIS patients and controls.

### 3.3 Value of SIRT2 in differentiating AIS patients and controls

SIRT2 expression was upregulated in AIS patients compared to controls (p < 0.001) (Figure 2A), and ROC curve disclosed that the SIRT2 expression could differentiate AIS patients from controls with a good AUC of 0.890 (95%CI: 0.854-0.926) (Figure 2B). Besides, the SIRT2 expression at the best cut-off point was 15.479 ng/μL, with a sensitivity of 78.7% and a specificity of 91.5%.

### 3.4 Correlation of SIRT2 with disease severity and inflammation cytokines in AIS patients

In AIS patients, SIRT2 expression was positively correlated with NIHSS score (p < 0.001, r = 0.426) (Figure 3). As for the pro-inflammatory cytokines, SIRT2 expression was positively associated with TNF-α (p < 0.001, r = 0.346) (Figure 4A), IL-6 (p = 0.012, r = 0.196) (Figure 4B) and IL-17 (p < 0.001, r = 0.464) (Figure 4C) serum expressions. These data indicated that SIRT2 was positively correlated disease severity and inflammation level in AIS patients.
3.5 | Correlation of SIRT2 with prognosis in AIS patients

In AIS patients, SIRT2 expression was elevated in recurrent/dead patients compared to non-recurrent/dead patients \((p = 0.025)\) (Figure 5A), and RFS was less prolonged in patients with SIRT2 high expression compared to patients with SIRT2 low expression \((p = 0.029)\) (Figure 5B). Moreover, SIRT2 expression was also increased in dead patients compared to survivors \((p = 0.006)\) (Figure 6A), and the OS was shorter in patients with SIRT2 high expression compared to patients with SIRT2 low expression \((p = 0.049)\) (Figure 6B). These data implied that SIRT2 overexpression was correlated with worse prognosis in AIS patients.

4 | DISCUSSION

The initial process of AIS is not difficult to follow, namely the ischemia in brain tissue caused by occlusion of its supplying artery, which could derive from the neck blood vessel or the intracranial vessel. Nonetheless, pathogenetic processes after the stroke is quite intricate, so that the understanding of an overwhelming mechanism is still limited. However, through decades of investigation, many mechanisms are relatively established in the pathogenesis of AIS, which involves oxidative stress, damaged mitochondrial function, post-stroke inflammation, and so on.\(^{14,15}\) Meanwhile, based on increasing findings of the AIS pathology, development in AIS diagnosis and therapy has never stopped. In this study, the expression of SIRT2 and its association with disease severity, inflammation as well as prognosis in AIS patients were determined, then the results displayed that: (1) SIRT2 was upregulated in AIS patients and had good value in differentiating AIS patients from controls. (2) SIRT2 was positively correlated with disease severity and pro-inflammatory cytokines in AIS patients. (3) Increased SIRT2 associated with unfavorable prognosis in AIS patients.

The sirtuin family plays a critical role in maintaining human body homeostasis, as a family member, SIRT2 is essential in many metabolic activities that may participate in many diseases, such as mediation of cell cycles and tumorigenesis.\(^{16-20}\) Interestingly, SIRT2 is also indicated in many cerebral diseases. For example, an in vitro and in vivo experiment conducted in Parkinson’s disease animal and cell models reveals that, SIRT2 collaborates with and deacetylates aSyn, a main protein that results in Parkinson’s disease, and SIRT2 knockdown inhibits the accumulation and toxicity of aSyn; which indicates that SIRT2 plays a role in aggravating the progression in Parkinson’s disease.\(^{21}\) In addition, another study elucidates that SIRT2 activation enhances male specific neuronal injury and functional damage post experimental cardiac arrest by regulating the Transient receptor potential melastatin 2 (TRPM2) Ion channels in mouse model, suggesting that SIRT2 also plays a role in promoting the progression of brain injury caused by ischemia.\(^{22}\) Most importantly, there is now evidence showing that SIRT2 might be involved in the regulation of stroke pathology. For instance, SIRT2 knockdown maintains the neurological function in mice experience experimental stroke after 48 h after the stoke.\(^{23}\) Additionally, a study illustrates that inhibiting SIRT2 contributes to neuroprotection after ischemic stroke via repressing forkhead box O3 (FOXO3) and mitogen-activated protein

**FIGURE 3** SIRT2 was positively correlated with AIS disease severity. SIRT2, sirtuin 2; AIS, acute ischemic stroke; NIHSS, National Institute of Health stroke scale

**FIGURE 4** SIRT2 positively correlated with inflammation in AIS. Correlation of serum SIRT2 expression with serum TNF-\(\alpha\) (A), IL-6 (B) and IL-17 (C) expressions in AIS patients. SIRT2, sirtuin 2; AIS, acute ischemic stroke; TNF-\(\alpha\), tumor necrosis factor-\(\alpha\); IL-6, interleukin-6; IL-17, interleukin-17
kinases (MAPK) signaling pathways in vitro and in vivo. These two studies uncover a pejorative role of SIRT2 in ischemic stroke, and both reveal possible mechanistic processes. In this study, we found that SIRT2 was upregulated in AIS patients and had good value in separating AIS patients from controls. Moreover, increased SIRT2 was correlated with more severe disease assessed by NIHSS score and worse prognosis in AIS patients. These results might be related to the following possible reasons: (1) SIRT2 might deteriorates neuron dysfunction after AIS via interacting with multiple factors, such as the TRPM2, FOXO3, and MAPK signaling pathways, which resulted in an upregulated SIRT2 expression in AIS patients, and its positive correlation with disease severity. (2) As for the correlation of SIRT2 expression with prognosis in AIS patients, a possible reason was that an increased SIRT2 expression probably impaired the neuron function in patients after AIS, which delayed the recovery and led to worse disease severity, which finally resulted in a worse prognosis.

Moreover, SIRT2 also functions in the regulation of inflammation, which has been reported by several prior studies. A study elucidates that SIRT2 aggravates inflammation in allergic asthma presented as positively modulating cellular recruitment, CC chemokine ligand 17 (CCL17), production, and goblet cell hyperplasia after Aspergillus fumigatus (DRA) challenge in cellular and mouse models. And a previous study reveals that the cell-permeative protein of SIRT2, PEP-1-SIRT2, results in an elevated inflammation in rabbit articular chondrocytes by enhancing the cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2) expressions. These data indicate that SIRT2 is a promoter in inflammation. As one of the most crucial pathological processes in AIS, inflammation and its correlation with SIRT2 expression was also assessed in our study, which displayed that, SIRT2 expression was positively associated with pro-inflammatory cytokines expressions, including TNF-α, IL-6 and IL-8, in AIS patients. In addition, we chose these three inflammatory cytokines to detect due to that
they were among the most commonly reported and crucial inflammatory cytokines in stroke. As for possible explanations to our results, we presumed that it was possible that SIRT2 promoted the pro-inflammatory cytokines via interacting with various factors as described in the previous studies, such as COX-2 and PGE2.

There were some limitations in the present study. The first limitation was that the other types of stroke (such as hemorrhagic stroke) were not included in this study to exclude their possible interference to the results. Therefore, the findings of this study could not be applied in other stroke types, which should be explored in the future. The second limitation was that the sample size was relatively small which should be enlarged in future studies to enhance the statistical power. Third, the controls of low-stroke-risk cases or healthy cases were not included in our study, if possible, these cases will be included in our future studies. Fourth, the inflammatory cytokines expressions were not detected in the controls (high-stroke-risk cases) of our study, thus, the correlation between SIRT2 and inflammatory cytokines could not be validated in the controls; therefore, we would like to conduct this analysis in our future studies if possible. Last, mechanistic experiments were not conducted to evaluate the function of SIRT2 in AIS in our study, which should be performed in the future.

In summary, our findings support a role of SIRT2 in facilitating the disease monitoring and prognosis in AIS patients.

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CONFLICT OF INTERESTS

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author, [Yan Zhang], on reasonable request.

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