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Plasma Glycoproteomic Study of Therapeutic Hypothermia Reveals Novel Markers Predicting Neurologic Outcome Post-cardiac Arrest

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Abstract

Therapeutic hypothermia (TH) is a neuroprotective treatment post-cardiac arrest but is grossly underutilized. After TH induction, traditional biomarkers and parameters can no longer predict clinical outcome due to a lack of understanding of hypothermic response. Innovative approaches to better understand the clinical effect of TH will help to prognosticate outcome and expand beneficial population. Protein glycosylation is an important extracellular post-translational modification, regulating various extracellular signaling pathways. Here, we used glycoproteomics to investigate the association of plasma glycoproteins with the prognosis of TH-treated cardiac arrest patients. Using lectin affinity chromatography and mass spectrometry, we identified 640 glycoproteins in the plasma of cardiac arrest patients undergoing TH treatment, of which 23 were up-regulated and 14 were down-regulated in good outcome patients as compared with poor outcome ones. Notably, two glycoproteins with antioxidant activity, ceruloplasmin (CP) and haptoglobin (HP), were found to be associated with favorable neurologic outcome. This was further supported by ELISA assay in a large patients cohort, in which glycosylated CP and HP enriched by concanavalin A (ConA) and wheat germ agglutinin (WGA) lectins were significantly increased in patients developing good outcome (ConA-CP: $p = 0.033$; ConA-HP: $p = 0.04$; WGA-HP: $p = 0.021$). Furthermore, ROC analysis demonstrated the predictive potential of ConA-CP, ConA-HP, and WGA-HP (ConA-CP: AUC = 0.732, $p = 0.031$; ConA-HP: AUC = 0.746, $p =$

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

0.022; WGA-HP: AUC = 0.714, $p = 0.046$) and combination of them improved the predictive power (AUC = 0.830, $p = 0.002$). Our results suggested that glycosylated CP and HP as well as other glycoproteins may play critical roles in neuroprotection and serve as sensitive prognostic markers for TH treatments.

Keywords

Therapeutic hypothermia; Anoxic brain injury; Cardiac arrest; Glycoproteomics

Introduction

Anoxic brain injury occurs frequently following cardiac arrest and is the major cause of high morbidity and mortality in post-cardiac arrest patients. Therapeutic hypothermia (TH) is an efficacious treatment to prevent brain injury caused by insufficient blood flow and improve survival and brain functions [1–3]. However, in clinical practice, TH is grossly underutilized due to fear of various complications and strict exclusion criterion. Moreover, the sedation status induced by TH also confound the early prognostic evaluation with traditional clinical parameters and biomarkers [4, 5]. Therefore, utilizing innovative approaches to better understand hypothermic response with respect to neurological function would be of immense value to monitor therapeutic efficacy at the bedside and optimize therapeutic strategies.

Protein glycosylation is an important extracellular post-translational modification (PTM), extensively involved in regulating immune response, coagulative cascades, redox hemostasis, and extracellular matrix integrity in blood [6–8]. We therefore hypothesize that the changes in extracellular glycosylation pattern following TH administration may also play a crucial role in regulating neurologic injury post-cardiac arrest, while screening out the most affected glycoproteins would advance our understanding of the efficacy of TH treatment and provide novel prognostic biomarkers.

Recent advances in lectin affinity chromatography enable in-depth characterization of glycoproteome [9]. Different lectins bind to different glycan motifs and can be used for enriching glycoproteins with specific glycan structure. For example, concanavalin A (ConA) lectin specifically binds to alpha-linked mannose and terminal glucose residues, while wheat germ agglutinin (WGA) lectin selectively recognizes *N*-acetyl glucosamine and sialic acid groups. Prior studies suggested that brain glycoproteins with altered affinity for ConA and WGA were correlated with the pathology and progression of neurological disease [10]. Therefore, in the current study, we combined ConA and WGA lectin affinity chromatography with mass spectrometry analysis to investigate the plasma glycoproteome in TH-treated cardiac arrest patients with different clinical outcomes and explore prognostic markers with putative clinical value.

Materials and Methods

Population

Thirty in- and out-of-hospital cardiac arrest patients undergoing TH treatment in the Emergency Department at Massachusetts General Hospital were recruited in accordance with an institutional review board (IRB)-approved protocol. Patients and their family were informed about the study, and written consent was obtained for all patients when possible. Venous blood was collected at 24 h after hypothermia induction. Clinical outcomes were determined by standard neurological examination at 6 months after cardiac arrest. Sixteen patients with normal cerebral function or moderate disability were defined with good outcome, while the other 14 patients with severe disability, vegetative state, or death were defined with poor outcome. Clinical features of enrolled patients are listed in Table 1.

Glycoproteomics

Venous blood was collected in EDTA-treated tubes. After centrifuging at 1500g for 15 min, supernatant plasma was stored at -80°C before use. Three samples associated with good neurological outcome, and three samples associated with poor neurological outcome were used for discovery glycoproteomics screening. Glycoproteins were enriched by lectin affinity chromatography as previously described [11]. Briefly, agarose-bound ConA or WGA was respectively packed into a spin column and equilibrated with binding buffer before incubated with plasma sample. After washing off the nonspecific binding protein with binding buffer (20 mM Tris, 0.15 M NaCl, pH 7.4), glycoproteins were eluted with elution buffer (0.4 M alpha methyl mannopyranoside and 0.4 M *N*-acetylglucosamine in 20 mM Tris and 0.5 M NaCl, pH 7.0). Eluant was reduced by dithiothreitol (DTT) and alkylated by iodoacetamide (IAA), followed by trypsin digestion. Tryptic peptides were then loaded onto C18 column ($0.1 \times 150 \text{ mm}^2$, C18 AQ particles, 5 mm, 200 Å) and analyzed by a linear ion-trap mass spectrometer (model LTQ; Thermo Scientific) in positive ion mode.

Label-Free Quantification

A label-free quantification method is employed to identify differential glycosylation status [12]. For each protein, its peptide spectral count X_{good} in the patients with good outcome was transformed into Y_{good} with formula $Y_{\text{good}} = \log_2(X_{\text{good}} - 1)$, whereas the X_{poor} of the patients with poor outcome was transformed into Y_{poor} with the same formula. $M = Y_{\text{good}} - Y_{\text{poor}}$ was defined as the differential protein abundance between two samples, and $A = (Y_{\text{good}} + Y_{\text{poor}})/2$ was defined as the average protein abundance. Based on these formulas, proteins were plotted as a scatter chart (Fig. 1b, c; M is distributed on the y -axis, and A is distributed on the x -axis). For a particular protein, spectral counts of neighboring proteins with an A value located within a window covering 25% of the entire length of the A -axis (with the focal protein right in the middle) were summed separately for the two samples. The significance of the expression change of the focal protein between the two samples was evaluated by Fisher's exact test with reference to the whole abundance of neighboring proteins.

ELISA Assay

ELISAs for ceruloplasmin (CP) and haptoglobin (HP) were done in all 30 enrolled patients. ConA-enriched glycoproteins and WGA-enriched glycoproteins were respectively prepared from each patient by lectin affinity chromatography as above, and the abundance of CP and HP was measured by ELISA.

Statistical Methods

Student's *t* test was applied to compare the differential ConA-CP, ConA-HP, and WGA-HP levels as well as age, glucose, and lactate between cardiac arrest patients with good and poor outcome following TH treatment. Logistic regression model was built with and without adjustment of confounders. ROC analysis was performed to evaluate the predictive ability of potential biomarkers and determine the optimal cut off values. Bootstrap-corrected Harrell's *C*-index were used to assess the model's discriminatory ability by bootstrapping with 100 resamples to estimate an unbiased measure of the ability of our predictive model [13].

Results

Plasma Glycoproteomics of Cardiac Arrest Patients Undergoing TH

Plasma glycoproteins were enriched through ConA and WGA lectin affinity chromatography followed by mass spectrometry identification. Altogether, we identified 506 glycoproteins from ConA eluant and 217 from WGA eluant (Fig. 1a). Interestingly, a large proportion of the proteins could only be identified in patients with good or poor outcomes alone, probably as a result of their low expression levels. We took the number of spectral counts assigned to each protein as quantitative information and identified differential protein glycosylation between the patients with different outcomes using a label-free quantification approach [12] (Fig. 1b, c and Table 2). As compared with the patients with poor outcomes, 17 ConA-enriched glycoproteins were significantly up-regulated and 11 ConA-enriched glycoproteins were down-regulated in patients with good outcomes (Fig. 1b, c and Table 2). In addition, 11 up-regulated and 5 down-regulated WGA-enriched proteins were identified to be associated with good neurological outcomes (Fig. 1b, c and Table 2).

Alterations in protein glycosylation may influence key cellular processes and yield valuable information to differentiate the clinical status of individuals in response to TH treatment. We then investigated the functions of differentially expressed glycoprotein between patients with good and poor outcomes using DAVID bioinformatics resources [14]. As shown in Fig. 3 and Table 2, most of the differentially expressed proteins are involved in redox homeostasis, complement activation, coagulation, and fibrinolysis (see discussion). Notably, we observed up-regulated expression of CP and HP in patients with good neurological outcome. In blood, CP and HP oxidize toxic Fe^{2+} to a less toxic form (Fe^{2+}) and reduce ROS generation (Fig. 1d) [15, 16]. ROS-induced oxidative damage is a major reperfusion injury post-cardiac arrest. It turns on apoptotic pathways; damages cellular DNA, proteins, and plasma membrane; and induces acute injury to the brain. Accordingly, increased CP and HP during TH treatment might protect brain from severe oxidative damage and facilitate neurological recovery.

ELISA Assay of CP and HP

We then studied the changes of glycosylated CP enriched by ConA lectin (ConA-CP) and glycosylated HP enriched by both ConA and WGA lectins (ConA-HP, WGA-HP) in all 30 enrolled patients, and glycosylation levels of CP and HP were also measured in eight healthy controls and four cardiac arrest patients without TH treatment. As shown in Fig. 2a, as compared with healthy controls, no significant changes of ConA-CP, ConA-HP, and WGA-HP would be observed in cardiac arrest patients. However, following TH treatment, ConA-CP, ConA-HP, and WGA-HP levels were largely increased in patients developing good neurological outcomes, while patients bearing unfavorable outcome failed to induce the production of glycosylated CP and HP. A significant difference of all three glycosylated proteins were observed between patients with good and poor outcomes (ConA-CP: 5.73 ± 1.99 vs. 3.82 ± 2.68 ng/ml, p value = 0.033; ConA-HP: 11.74 ± 12.74 vs. 3.84 ± 5.47 ng/ml, p value = 0.04; WGA-HP: 95.96 ± 54.41 vs. 56.57 ± 28.1 ng/ml, p = 0.021), which is fully consistent with the glycoproteomics results above.

The prognosis of cardiac arrest is also associated with the location of cardiac arrest, early cardiopulmonary resuscitation as well as glucose and lactate levels during acute phase. We therefore performed multivariable logistic regression to adjust the influence of these cofactors. We also took age, gender, and medical history of hypertension, hyperlipidemia, diabetes, and coronary artery disease into consideration, which are all risk factors of cardiac arrest. As listed in Table 3, ConA-CP, ConA-HP, and WGA-HP were the only independent predictors of different neurological outcomes following TH treatment even after controlling for the influence of the confounders (ConA-CP: OR: 19.98, 95% CI: 1.51–264.5, p -value: 0.023; ConA-HP: OR: 2.21, 95% CI: 1.03–4.76, p value: 0.043; WGA-HP: OR: 1.04, 95% CI: 1–1.09, p value: 0.029).

Moreover, receiver operating characteristic (ROC) analysis also indicated significant discriminatory value for glycosylated CP and HP (Fig. 2b). The area under curve (AUC) was yielded as 0.732 for ConA-CP (p value: 0.031; 95% CI: 0.532–0.932), while ConA-HP and WGA-HP had AUC values of 0.746 (p value: 0.022; 95% CI: 0.569–0.922) and 0.714 (p value: 0.046; 95% CI: 0.530–0.899) respectively. Internal validation of the prognostic model by bootstrapping with 100 resamples resulted in corrected C-index of 0.717 for ConA-CP, 0.749 for ConA-HP, and 0.725 for WGA-HP, indicating good discriminatory performance of the model. As shown in Fig. 2b, ConA-CP with concentration above 4.26 ng/ml was predictive of good neurological outcome with a sensitivity of 81.3% and a specificity of 71.4%. For ConA-HP, a concentration higher than 3.52 ng/ml may indicate a good outcome at 68.8% sensitivity and 78.6% specificity. Also, WGA-HP has predictive sensitivity of 37.5% and specificity of 100% when >128 ng/ml. In addition, combination of ConA-CP, ConA-HP, and WGA-HP improved the predictive power with AUC as 0.830 (p value: 0.002; 95% CI: 0.683–0.977), sensitivity as 87.5% and specificity as 71.4% (Fig. 2c).

Discussion

TH treatment was proposed in the 1950s as an efficacious neuroprotective treatment for cardiac arrest patients. However, little mechanistic information is currently available at molecular level for monitoring treatment efficacy and predicting neurological outcomes,

which largely restricted the clinical application of TH. Recent advances in molecular biology and animal studies have suggested several putative neuroprotective mechanisms of TH involving dampening neurotoxicity, altering mitochondrial functions and apoptotic pathways, reducing free radical response, and interacting with multiple intracellular signaling pathways including PKC and MAPkinase [17, 18]. While there has been a wealth of knowledge establishing the mechanistic importance of TH, however, less attention has been placed on secreted factors that can be easily translated into clinical measurements. Neuroprotective factors such as BDNF have been reported to work synergistically with TH in animal models [19] but demonstrated little clinical predictive value in patients [5]. Blood S-100B and NSE are up to now the only promising prognostic markers for TH, but they only show moderate sensitivity of prediction, and conflicting results have been reported by different studies [5, 20, 21]. It is therefore necessary to use innovative approaches to investigate other extracellular signaling and get better understanding of peripheral response of TH.

In the current study, for the first time, we profiled the plasma glycoproteome associated with different neurological outcomes following TH treatment. Notably, our results support the roles of glycosylated CP and HP as prognostic biomarkers for TH treatment. Cardiac arrest patients with high concentrations of ConA-CP, ConA-HP, and WGA-HP were more likely to develop favorable neurological outcomes and benefit more from TH treatment, whereas the failure to induce the glycosylation of these proteins may expose brain cells to more ischemic damage and finally contribute to severe neurological function loss and death. Also, in patients with good outcome, we also observed a coordinated downregulation of several glycoproteins involved in complement activation including the key factor complement component 3 and 4B (C3 and C4b), as well as immunoglobulin heavy constant mu, alpha 1 and alpha 2 (IGHM, IGHA1, and IGHA2) (Fig. 3 and Table 2). Decreased complement activation may inhibit downstream inflammatory reaction and therefore reduce local cerebral injury caused by the immunologic response and decrease the risk of infection during TH treatment [22]. Moreover, we found activation of both the coagulation and fibrinolytic systems during TH treatment. A significantly high level of coagulation factor XII (F12) was observed in the patients with good outcome. F12 initiates the generation of fibrin clot by increasing the abundance of thrombin (F2) and fibrinogen (FGA, FGB) and also triggers fibrinolysis by catalyzing plasminogen (PLG) into active plasmin. Also, reduced level of two plasmin inhibitors, alpha-2 antiplasmin (A2AP) and alpha-1 antitrypsin (A1AT), was also observed in patients with good outcome, which could further facilitate fibrin degradation (Fig. 3 and Table 2). This is consistent with prior observations that simultaneous activation of both coagulation and fibrinolysis occurs during hypothermia [23]. The balance between coagulation and fibrinolysis is related to the bleeding complication of TH and therefore leads to different treatment outcomes.

The present findings suggest that protein PTMs may comprise a key mechanism for TH, and glycosylated CP and HP may serve as good prognostic biomarkers. However, there are a few caveats to keep in mind. First, the number of patients examined in this initial study is relatively small. More patients will be recruited not only to explore the prognostic biomarkers but also to understand extracellular glycosylation alteration in response to cardiac arrest and TH treatment. Also, in addition to glycosylation, many other PTMs such

as phosphorylation are also crucial for protein function regulation. Therefore, further exploration on the changes of other PTMs will gain a better understanding of anoxic brain injury and TH treatment. Further, to improve our power in this discovery study, we restricted our glycoproteomic profiling at a single time point. Given the complex protein metabolism in blood, it is necessary to expand the comparison to a serial time points and also before the induction of hypothermia to see if the beneficiary population could be indicated at the very beginning and also for how long the neuroprotective effect is maintained. Finally, in addition to hypothermia (32–34 °C), normothermia (i.e., the prevention of hyperthermia) has also been reported to improve survival and neural functions after cardiac arrest by managing targeted temperature near normal level (<37.5 °C). In the current study, we did not compare the two treatments, mainly because the normothermia-treated patients were likely excluded by hypothermia protocol (non-coma, > 6 h admission, infection, bleeding). These differences will introduce more confounding clinical factors and mislead our current analysis within a small patient cohort. Therefore, an expanded patient cohort is essential in the future to adjust the confounders and perform a systematic comparison between different treatment strategies.

In summary, our study suggests an important role of protein glycosylation in regulating the efficacy of TH treatment. The changes of glycoproteins were indicative of the prognosis of patients and would be useful to define beneficiary patient population and expand the clinical utilization of TH treatment.

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Fig. 1.

A summary of glycoproteomics results. **a** Venn diagram depicting the number of proteins identified from ConA and WGA affinity chromatography, and the overlap of protein identifications between the patients with good and poor outcomes after TH treatment. Changes of **b** ConA- and **c** WGA-enriched glycoproteins identified by a label-free quantification method with the number of peptides assigned to each glycoprotein as quantitative information. Significantly up-regulated proteins (p value <0.05) are in *red dots*, and down-regulated proteins (p value <0.05) are in *green*. **d** Biological pathway involved with ceruloplasmin (CP) and haptoglobin (HP). *Red color* indicates the up-regulated proteins in patients with good outcome

Fig. 2.

ELISA analysis of ConA-CP, ConA-HP, and WGA-HP in TH-treated patients. **a** Levels of ConA-CP, ConA-HP, and WGA-HP in healthy controls, cardiac arrest patients not treated with TH (CA) as well as TH-treated patients with good and poor neurological outcome. **b** ROC curves for discriminating cardiac arrest patients with good and poor outcomes by ConA-CP, ConA-HP, and WGA-HP alone. **c** ROC curve for discriminating cardiac arrest patients with good and poor outcomes by ConA-CP, ConA-HP, and WGA-HP in combination

Fig. 3. Biological pathways involved with significantly changed glycoproteins. *Red color* indicates the up-regulated proteins in patients with good outcome, and *blue color* indicates the down-regulated proteins in those patients

Table 1

Clinical characteristics of enrolled patients

Patient characteristic	Good outcome (n = 16)	Poor outcome (n = 14)	p value
Age (years) $\pm$ SD	54.69 $\pm$ 13.39	60.14 $\pm$ 18.57	0.36
Male gender	9 (56%)	8 (57%)	0.96
Location of cardiac arrest			1.00
• Out-of-hospital	14 (88%)	12 (86%)	
• In-hospital	2 (13%)	2 (14%)	
Bystander-initiated CPR	11 (69%)	10 (71%)	1.00
Presenting rhythm			0.51
• Asystole	2 (13%)	4 (29%)	
• PEA	5 (31%)	4 (29%)	
• VF/VT	8 (50%)	4 (29%)	
• Unknown	1 (6%)	2 (14%)	
Glucose (mg/dL)	212.31 $\pm$ 90.49	257.36 $\pm$ 92.33	0.19
Lactate (mmol/L)	5.52 $\pm$ 3.41	5.86 $\pm$ 3.5	0.79
Medical history			
• Hypertension	10 (63%)	6 (43%)	0.28
• Hyperlipidemia	5 (31%)	2 (14%)	0.51
• Diabetes	3 (19%)	2 (14%)	1.00
• Coronary artery disease	2 (13%)	2 (14%)	1.00

Table 2

Differential glycoproteins between good and poor clinical outcome of cardiac patients post-TH treatment

Symbol	Protein name	Expression change (<i>p</i> value)		Biological functions
		ConA	WGA	
APOL1	Apolipoprotein L, 1	↑ (0.003)	NA	Lipid metabolism
FCN3	Ficolin 3 (Hakata antigen)	— (0.20-2)	↑ (0.007)	Immune response complement activation
CP	Ceruloplasmin (ferroxidase)	↑ (0.023)	— (0.08)	hemostasis
F2	Coagulation factor II (thrombin)	↑ (0.038)	NA	Coagulation/fibrinolysis
HP	Haptoglobin	↑ (<0.0-01)	↑ (0.028)	Hemostasis
HPR	Haptoglobin-related protein	↑ (0.01)	— (0.19)	Hemostasis
PLG	Plasminogen	— (0.23-3)	↑ (<0.0-01)	Coagulation/fibrinolysis
F12	Coagulation factor XII (Hageman factor)	— (0.09)	↑ (0.004)	Coagulation/fibrinolysis
A1AT	Alpha-1 antitrypsin	↓ (0.006)	— (0.26-9)	Inflammatory response hemostasis
A2M	Alpha-2-macroglobulin	↑ (<0.0-01)	↑ (0.034)	Coagulation/fibrinolysis complement activation
C3	Complement component 3	↓ (0.001)	NA	Immune response complement activation
KNG1	Kininogen 1	↓ (0.005)	— (0.18-4)	Coagulation/fibrinolysis hemostasis
LV101	Ig lambda chain V-I region VOR	↓ (0.044)	— (0.17-3)	Immune response
IGHG4	Immunoglobulin heavy constant gamma 4	↑ (0.004)	— (0.22-9)	Immune response
IGHM	Immunoglobulin heavy constant mu	— (0.46-4)	↓ (0.033)	Immune response
IGHA1	immunoglobulin heavy constant alpha 1	↓ (<0.0-01)	↓ (<0.0-01)	Immune response
IGHA2	Immunoglobulin heavy constant alpha 2	↓ (<0.0-01)	↓ (<0.0-01)	Immune response
HBD	Hemoglobin, delta	↑ (<0.0-01)	NA	Transport
APOE	Apolipoprotein E	↑ (<0.0-01)	↑ (0.004)	Stress response
FGA	Fibrinogen alpha chain	↓ (0.009)	— (0.23-1)	Coagulation/fibrinolysis hemostasis
FGG	fibrinogen gamma chain	↑ (0.003)	— (0.26-8)	Coagulation/fibrinolysis hemostasis
SAA	Serum amyloid A	↓ (0.029)	— (0.27-3)	Inflammatory response hemostasis
FN1	Fibronectin 1	↓ (0.002)	NA	Cell morphogenesis immune response
ORM1	Orosomucoid 1	— (0.42-6)	↑ (0.016)	Inflammatory response
ALB	Albumin	— (0.45-4)	↑ (0.039)	Stress response
APOB	Apolipoprotein B	↑ (<0.0-01)	↑ (<0.0-01)	Cholesterol homeostasis
SHBG	Sex hormone-binding globulin	↑ (0.045)	↑ (0.036)	Reproductive process
CFH	Complement factor H	— (0.42-9)	↓ (<0.0-01)	Immune response complement activation
A2AP	Alpha-2 antiplasmin	— (0.05)	↓ (0.01)	Inflammatory response coagulation/fibrinolysis
C4A	Complement component 4A	↑ (<0.0-01)	NA	Immune response complement activation
C4B	Complement component 4B	↓ (<0.0-01)	NA	Immune response complement activation
C7	Complement component 7	↑ (0.003)	NA	Immune response complement activation
ORM2	Orosomucoid 2	↑ (0.041)	— (0.07-8)	Inflammatory response
ITIH1	Inter-alpha (globulin) inhibitor H1	↓ (0.031)	— (0.27-3)	Cell activation
PZP	Pregnancy-zone protein	↑ (<0.0-01)	NA	pregnancy
SZT2	Seizure threshold 2 protein homolog	— (0.5)	↑ (0.021)	Neural development
ESYT1	Membrane-bound C2 domain-containing protein	↑ (0.038)	NA	

↑ indicates up-regulated in Good, ↓ indicates down-regulated in Good; *p* value indicates the significance of the change

Logistic regression analysis of the association of ConA-CP, ConA-HP, and WGA-HP with clinical outcomes of TH treated cardiac arrest patients

Variables	ConA-CP		ConA-HP		WGA-HP	
	Univariable OR (p value) 95% CI	Multivariable OR (p value) 95% CI	Univariable OR (p value) 95% CI	Multivariable OR (p value) 95% CI	Univariable OR (p value) 95% CI	Multivariable OR (p value) 95% CI
Glycosylated protein	3.29 (0.029) 1.13 ~ 9.58	19.98 (0.023) 1.51 ~ 264.5	1.8 (0.035) 1.04 ~ 3.12	2.21 (0.043) 1.03 ~ 4.76	1.02 (0.04) 1 ~ 1.04	1.04 (0.029) 1 ~ 1.09
Age		1.02 (0.743) 0.92 ~ 1.12		1.01 (0.898) 0.93 ~ 1.09		1 (0.926) 0.92 ~ 1.08
Gender		0.18 (0.246) 0.01 ~ 3.28		0.28 (0.28) 0.03 ~ 2.84		0.2 (0.217) 0.02 ~ 2.58
Location of cardiac arrest (in- or out-of-hospital)		8.84 (0.276) 0.17 ~ 446.4		1.87 (0.695) 0.08 ~ 42.67		1.02 (0.99) 0.05 ~ 22.73
Bystander-initiated CPR		0.05 (0.137) 0 ~ 2.52		1.78 (0.624) 0.18 ~ 18.03		1.38 (0.798) 0.12 ~ 16.05
Glucose		0.98 (0.145) 0.96 ~ 1.01		0.99 (0.081) 0.97 ~ 1		0.99 (0.065) 0.97 ~ 1
Lactate		0.99 (0.938) 0.69 ~ 1.42		1.06 (0.686) 0.79 ~ 1.42		1.09 (0.626) 0.78 ~ 1.53
Hypertension		0.11 (0.21) 0 ~ 3.43		1.83 (0.65) 0.13 ~ 25.01		3.18 (0.449) 0.16 ~ 63.46
Hyperlipidemia		0.01 (0.128) 0 ~ 3.82		0.29 (0.417) 0.01 ~ 5.88		0.13 (0.203) 0.01 ~ 2.98
Diabetes		0.06 (0.192) 0 ~ 4.28		0.11 (0.281) 0 ~ 6.09		0.41 (0.635) 0.01 ~ 16.84
Coronary artery disease		1.82 (0.758) 0.04 ~ 80.31		1.61 (0.79) 0.05 ~ 53.44		6.04 (0.405) 0.09 ~ 416.6