

## Appendix

### Appendix S1. The complete list of CNSR-III members and sites.

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## **Appendix S2. Baseline information and discharge information**

### **Baseline Information**

- Demographics (including: age, gender, education, ethnicity, marital status, occupation, occupation)
- Pre-hospital care (onset date, first aid transport mode, general situation, intravenous thrombolytic therapy, endovascular treatment)
- Past-history (smoking, alcohol consumption, hypertension, diabetes, dyslipidemia, lifestyle, cerebrovascular history, heart disease, acute infection, other diseases) /family history
- Previous medication: regular or not (anti-platelet therapy; lipid-lowering therapy; anticoagulant; antihypertensive drugs; hypoglycemic agents; others)
- Previous disability (mRS):
- Physical examination at admission
- Primary diagnosis: cerebral infarction / TIA
- Admission risk factor assessment (blood pressure: the average blood pressure within 24 hours after admission, excluding other factors)
- Admission score: mRS score; NIHSS score; ABCD2 score
- Laboratory tests: completed within 24 hours (blood / biochemical / renal function)

### **Discharge Information (discharge $\pm$ 7 days)**

- Hospital interventions for stroke:
  - Auxiliary examination during hospitalization:
  - Laboratory tests: blood lipids, coagulation, infectious diseases, glycosylated hemoglobin
  - Standard etiological examination and evaluation:
- Cerebrovascular events during hospitalization
  - The definitions of cerebrovascular events are presented in Appendix A.
- Inpatient treatment

- Medication therapy (anti-platelet / anticoagulant / lipid lowering / hypoglycemic / antihypertensive / expansion / dehydration / anti-infection): including administration time and dose
- Vascular related operations and surgical procedures (Carotid stenting/Carotid endarterectomy/ Intracranial arterial stenting/ Decompressive craniectomy)
- Other KPI: swallowing function evaluation / deep vein thrombosis / rehabilitation training

Evaluation at Discharge

- The final diagnosis: cerebral infarction (SSS-TOAST subtype) or transient ischaemic attack
- Other diagnoses: hypertension; blood glucose abnormalities; lipid metabolism disorders; heart disease; respiratory diseases; liver disease; urinary system diseases; peripheral vascular disease; haemorrhage (detail information is presented in Appendix B); epilepsy.

Discharge

- NIHSS score;
- mRS score;
- health guidance;
- application of secondary prevention drugs;
- evaluation of hospitalization quality of stroke.

Inpatient medical burden: hospitalization time, medical expenses.

### **Appendix S3. Definitions of cerebrovascular events**

**Ischaemic stroke:** An acute focal infarction of the brain or retina. Criteria: ① Acute onset of a new focal neurological deficit or worsening of an existing focal neurological deficit (NIHSS score increased by 4 points or above), lasting more than 24 hours. ② Acute onset of a new focal neurological deficit or worsening of an existing focal neurological deficit (NIHSS score increased by 4 points or above), lasting less than 24 hours, imaging confirmed (CT or MR) new infarction lesions or expansion of the original infarction. The above two conditions are required to be excluded for fever, drug effects, infection and any other etiology. Brain imaging (CT or MR) is needed for the exception of cerebral edema, bleeding and haemorrhage transformation etc.

**Transient ischaemic attack:** Rapid onset of a focal neurological deficit attributed to focal brain or retinal ischemia lasting less than 24 hours, without evidence of associated acute focal infarction on imaging (CT or MR).

**Haemorrhagic stroke:** An acute extravasation of blood into the brain parenchyma or subarachnoid space with associated neurological symptoms.

**Cerebral haemorrhage:** cerebral bleeding caused by parenchymal blood vessel rupture, confirmed by imaging (CT or MR). Here mainly refers to non-traumatic bleeding, including primary and secondary cerebral haemorrhage.

**Subarachnoid haemorrhage:** intracranial vascular rupture of blood flow into the subarachnoid space, confirmed by imaging (CT or MR), here is mainly to non-traumatic spontaneous subarachnoid haemorrhage.

**Haemorrhagic transformation after cerebral infarction:** any non-traumatic haemorrhage within the scope of the known ischaemic stroke infarction.

- ❑ Symptomatic: Imaging evidence of haemorrhage in the infarct region (CT or MRI); symptoms associated with haemorrhagic transformation; NIHSS score increased by 4 points or more after an ischaemic event; death.

- ❑ Non-symptomatic: imaging evidence of haemorrhage in the infarcted area (CT or MRI); no symptoms, or clinical deterioration resulting in NIHSS score increase of less than 4 points caused by haemorrhagic transformation.

## **Bleeding Type**

### **❑ Major bleeding**

- Intracranial haemorrhage;
- Pericardial bleeding (cardiac tamponade);
- Hypotensive shock and severe low blood pressure caused by bleeding, requiring compression therapy or surgical treatment;
- Significant clinical manifestations of haemorrhage or hb drop  $> 50\text{g / l}$ ;
- Blood transfusion  $\geq 4$  units due to bleeding (whole blood or red blood cell suspension).

### **❑ Major bleeding-other**

- Bleeding accompanied by significant function impairment (e.g. bleeding eyes leading to Persistent visual loss);
- Hb fell more than 30-50g/L;
- Transfusion 2-3 units due to bleeding (whole blood or red blood cell suspension).

### **❑ Moderate bleeding**

Bleeding requiring medical intervention or treatment (such as epistaxis which needs tamponade therapy in hospital)

### **❑ Minor bleeding**

Bleeding does not require intervention or treatment (such as bruising, bleeding gums, staxis at the injection site)

#### Appendix S4. Abbreviations and explanation of biomarkers

<b>Biomarker</b>	<b>Full name</b>	<b>Unit</b>	<b>Calculation or explanation</b>
Cre	Creatinine	µmol/L	Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass).
CysC	Cystatin C	mg/L	Cystatin C is a 122 amino acid protein with a molecular mass of 13 kDa. Cystatin C has been thought of as produced at a constant rate by a “housekeeping” gene expressed in all nucleated cells. Cystatin C is freely filtered at the glomerulus because of its small size and basic pH.
eGFR <sub>CKD_EPI_Cre</sub>	Estimate Glomerular filtration rate_CKD-EPI creatinine equation	ml/min/1.72m <sup>2</sup>	$141 \times \min(\text{Scr}/\kappa, 1)^\alpha \times \max(\text{Scr}/\kappa, 1)^{-1.209} \times 0.993^{\text{Age}} \times 1.018$ [if female], where Scr is serum creatinine, $\kappa$ is 0.7 for females and 0.9 for males, $\alpha$ is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/ $\kappa$ or 1, and max indicates the maximum of Scr/ $\kappa$ or 1.
eGFR <sub>CKD_EPI_CysC</sub>	Estimate Glomerular filtration rate_CKD-EPI cystatin C equation	ml/min/1.72m <sup>2</sup>	$133 \times \min(\text{Scys}/0.8, 1)^{-0.499} \times \max(\text{Scys}/0.8, 1)^{-1.328} \times 0.996^{\text{Age}} \times 0.932$ [if female], where Scys is serum cystatin C, min indicates the minimum of Scys/ $\kappa$ or 1, and max indicates the maximum of Scys/ $\kappa$ or 1.
<u>eGFR</u> <sub>CKD_EPI_Cr_e_CysC</sub>	Estimate Glomerular filtration rate_CKD-EPI creatinine–cystatin C equation	ml/min/1.72m <sup>2</sup>	$135 \times \min(\text{Scr}/\kappa, 1)^\alpha \times \max(\text{Scr}/\kappa, 1) - 0.601 \times \min(\text{Scys}/0.8, 1)^{-0.375} \times \max(\text{Scys}/0.8, 1)^{-0.711} \times 0.995^{\text{Age}} \times 0.969$ [if female], where Scr is serum creatinine, Scys is serum cystatin C, $\kappa$ is 0.7 for females and 0.9 for males, $\alpha$ is -0.248 for females and -0.207 for males, min indicates the minimum of Scr/ $\kappa$ or 1, and max indicates the maximum of Scr/ $\kappa$ or 1.
ACR	Albumin-to-creatinine ratio	mg/g	Albuminuria is established by one measurement of albumin-to-creatinine ratio
NGAL_B	Plasmic neutrophil gelatinase-associated lipocalin	ng/ml	NGAL is a protein that in humans is encoded by the LCN2 gene. It is expressed in neutrophils and in low levels in the kidney, prostate, and epithelia of the respiratory and alimentary tracts.
NGAL_U	Urinary neutrophil gelatinase-associated lipocalin	ng/ml	NGAL is a protein that in humans is encoded by the LCN2 gene. It is expressed in neutrophils and in low levels in the

			kidney, prostate, and epithelia of the respiratory and alimentary tracts.
UROM	Uromodulin	µg/ml	Uromodulin, also known as Tamm–Horsfall glycoprotein, is a glycoprotein that in humans is encoded by the UMOD gene. Uromodulin is the most abundant protein excreted in ordinary urine.
tHcy	Total homocysteine	µmol/L	Total homocysteine is defined as the sum of all homocysteine species in serum, including free and protein-bound forms.
FA	Folic acid	nmmol/L	Folic acid is a form of vitamin B-9 that can dissolve in water. It is a key ingredient in the making of the nucleic acid that forms part of all genetic material.
VitB12	Vitamin B12	pg/ml	Vitamin B12, also called cobalamin, is a water-soluble vitamin that is involved in the metabolism of every cell of the human body: it is a cofactor in DNA synthesis, and in both fatty acid and amino acid metabolism.
MMA	Methylmalonic acid	µg/mg	Methylmalonic acid is a dicarboxylic acid that is a C-methylated derivative of malonate.
UA	Uric acid	µmol/L	Uric acid is a heterocyclic compound of carbon, nitrogen, oxygen, and hydrogen with the formula C <sub>5</sub> H <sub>4</sub> N <sub>4</sub> O <sub>3</sub> . It forms ions and salts known as urates and acid urates, such as ammonium acid urate. Uric acid is a product of the metabolic breakdown of purine nucleotides, and it is a normal component of urine.
Kim-1	Kidney Injury Molecule-1	ng/ml	KIM-1 is a type I cell membrane glycoprotein which contains, in its extracellular portion, a novel six-cysteine immunoglobulin-like domain, two N - glycosylation sites and a T/SP rich domain characteristic of mucin-like O -glycosylated proteins. The structure of the protein led us to initially believe it had adhesion molecule properties
AGEs	Advanced Glycation End Products	ng/ml	
sRAGE	soluble receptor for AGEs	pg/ml	
esRAGE	endogenous secreted receptor for AGEs	ng/ml	

Fib	Fbrinogen	mg/dl	Fbrinogen, the clotting factor I, is a protein synthesized by the liver with coagulation function. The plasma content is 2.0-4.0 g /L. It converts to fibrinin under the action of thrombin and makes the blood coagulates.
D-D	D-dimner	μg/ml	D-dimer comes from cross-linked fibrin clots dissolved by fibrinolytic enzymes, which mainly reflect fibrinolytic function.
IL-1β	interleukin-1β	pg/mL	IL-1β is a promoter of inflammatory response and can directly damage the vascular endothelium. It promotes the release of inflammatory factors such as TNF-α, IL-6, and initiates and maintains the inflammatory response of atherosclerosis.
IL-6	interleukin-6	pg/mL	IL-6 is mainly secreted by mononuclear macrophages and related to plaque formation and instability by promoting platelet aggregation, enhancing the expression of CRP, fibrinogen and other inflammatory factors.
IL-8	interleukin-8	pg/mL	IL-8 is a chemotactic cytokine that promotes chemokines of inflammatory cells.
TNF-α	tumor necrosis factor -α	pg/mL	TNF-α promotes the occurrence and development of atherosclerosis by mediating endothelial cell injury, inhibiting fibrinolysis, promoting blood coagulation and matrix metalloproteinase expression.
hs-CRP	Hypersensitive c-reactive protein	mg/L	hs-CRP is a sensitive indicator of inflammatory response, mainly stimulated liver synthesis by IL-6.
MCP-1	monocytechemoattractant protein-1	pg/mL	MCP-1 is a key factor in the early development of atherosclerosis and can promote plaque rupture and affect the stability of atherosclerotic plaques.
ICAM-1	Intercellular adhesion molecules 1	pg/mL	ICAM-1 mediates the rolling adhesion of inflammatory cells along vascular endothelial cells and permeates to the inner and subcutaneous, causing endothelial cell damage.
MMP-9	matrix metalloprotein-9	ng/ml	MMP-9 belongs to MMP family and has the effect of dissolving and collapsing the surface fibers of plaques, leading to plaque rupture and thrombosis
sCD40L	Soluble CD40 Ligand	pg/mL	CD40L is a kind of transmembrane glycoprotein, most of which is located inside platelet. When activated, platelets are

			transferred to the surface of blood to form sCD40L, which stimulates smooth muscle cells, vascular endothelial cells and macrophages to produce adhesion molecules and interleukin.
t-PA	Tissue-type plasminogen activator	pg/mL	t-PA is synthesized and secreted by vascular endothelial cells and has a high affinity with fibrin, which can start the fibrinolysis system to dissolve the formed thrombus.
PAI-1	plasminogen activator inhibitor-1	ng/ml	PAI-1 is synthesized and secreted by vascular endothelial cells and specifically bound to t-PA, making it rapidly inactivated and playing an antifibrinolytic role.
vWF-A2	Von Willebrand factor A2	pg/mL	vWF is an important plasma component that plays an important role in hemostasis.
IL-1RA	interleukin-1 receptor antagonist	pg/ml	IL-1RA is an agent that binds non-productively to the cell surface interleukin-1 receptor (IL-1R), the same receptor that binds interleukin 1 (IL-1), preventing IL-1 from sending a signal to that cell.
vWF	von Willebrand factor	ng/ml	vWF is a blood glycoprotein involved in hemostasis. It is deficient or defective in von Willebrand disease and is involved in a large number of other diseases, including thrombotic thrombocytopenic purpura, Heyde's syndrome, and possibly hemolytic-uremic syndrome. Increased plasma levels in a large number of cardiovascular, neoplastic, and connective tissue diseases are presumed to arise from adverse changes to the endothelium, and may contribute to an increased risk of thrombosis
MCP-1	monocyte chemotactic protein 1	pg/ml	MCP-1 is a small cytokine that belongs to the CC chemokine family. CCL2 recruits monocytes, memory T cells, and dendritic cells to the sites of inflammation produced by either tissue injury or infection.
VCAM-1	vascular cell adhesion molecule 1	ng/ml	VCAM-1 is a protein that in humans is encoded by the VCAM1 gene. VCAM-1 functions as a cell adhesion molecule.
PCSK9	Proprotein convertase subtilisin/kexin type 9	ng/mL	Proprotein convertase subtilisin/kexin type 9 (PCSK9) is an enzyme encoded by the PCSK9 gene in humans on chromosome 1.

TMAO	Trimethylamine-N-oxide	μmol/L	TMAO is a metabolite produced from gut microbiota metabolism of dietary trimethylamine (TMA)-containing nutrients such as choline, carnitine and phosphatidylcholine.
Carnitine		μmol/L	Carnitine, a trimethylamine abundant in red meat, is metabolized by intestinal microbiota also producing TMAO.
Choline		μmol/L	Choline is a precursor of TMAO metabolized by gut flora and choline is required for VLDL synthesis in liver.
Betaine		μmol/L	Betaine is generated from choline. And it serves as a methyl donor in a reaction converting homocysteine to methionine, catalyzed by the enzyme betaine-homocysteine methyltransferase.
γ-Butyrobetaine		μmol/L	γ-butyrobetaine (γBB) is produced as an intermediary metabolite by gut microbes from carnitine
TMAVA	N,N,N-Trimethyl-5-Aminovaleric Acid	μmol/L	TMAVA is generated from TML by gut flora.
PC	Phosphocholine	μmol/L	Phosphocholine is required for VLDL synthesis.
TML	Trimethyllysine	μmol/L	TML is abundant in both plant- and animal-derived foods and serve as a dietary precursor for gut microbiota-dependent generation of TMAO in vitro.
Creatinine		μmol/L	
CEC	Cholesterol efflux capacity	%	