## Lab Protocol Article Template

Title	Prognostic value of combining 24-hour ASPECTS, and
	hemoglobin to red cell distribution width ratio on the
	THRIVE-score in predicting in-hospital mortality among
	ischemic stroke patients treated with intravenous
	thrombolysis.
Metadata	The file labeled 'data set' is stored in Microsoft Excel
	Worksheet (.xlsx) format and includes information
	gathered from January 1, 2015, to July 31, 2022, at
	Saraburi Hospital in Thailand. Its most recent
	modification occurred on August 12, 2023.
Funding	The research was supported by a particular grant (MC013-
	2566) from the Medical Education Center at Saraburi
	Hospital. The funding source did not participate in the
	design of the study, data collection and analysis, decision-
	making regarding publication, or manuscript preparation.
Competing interests	The authors have no conflicts of interest directly relevant
	to the content of this article.
Data availability	The datasets used and/or analyzed during the current
This should include, where applicable, links to data and code produced by the protocol or necessary to interpret the outputs.	study are available from the corresponding author upon
	request.
Associated content Minimum include DOI for protocol on	-
Abstract	Background: Acute ischemic stroke (AIS) emerges as a
	significant global health issue, directly impacting
	mortality and disability. The Total Health Risk In Vascular
	Events (THRIVE) score, appreciated for its simplicity and
	ease of use, consistently validates its ability to accurately
	predict stroke clinical outcomes. However, the THIRVE

score is currently lacking laboratory and neuroimaging data, which limits its ability to provide precise prediction of stroke outcomes. Our study evaluates the impact of integrating the 24-hour Alberta Stroke Program Early CT Score (ASPECTS) and hemoglobin-to-red cell distribution width (HB/RDW) ratio into the THRIVE score using the multivariable fractional polynomial (MFP) method (combined THRIVE-MFP) compared to the THRIVE-c model. We aim to assess their added value in predicting in-hospital mortality (IHM) prognosis. Methods: A retrospective study from January 2015 to July 2022 examined consecutive AIS patients receiving intravenous thrombolysis. Data on THRIVE scores, 24hour ASPECTS, and HB/RDW levels were collected upon admission. The model was constructed using logistic regression and the MFP method. The prognostic value was determined using the augmented area under the receiver operating characteristic (AuROC). Ischemic cerebral lesions within the middle cerebral artery territory were evaluated with non-contrast computed tomography (NCCT) following 24 hours of intravenous thrombolysis completion (24-hour ASPECTS).

Introduction	Ischemic stroke remains a significant cause of
	global mortality, ranking second, and continues to be a
	leading cause of disability, despite ongoing advancements
	in treatment. <sup>1</sup> Global ischemic stroke burden has shifted
	from developed to developing countries in recent decades.
	<sup>2</sup> Thailand's disability mortality rate varies from 10% to
	50%. The Ministry of Public Health implements
	innovative technology policies to reduce associated costs
	and improve outcomes. <sup>3</sup> Intravenous recombinant tissue
	plasminogen activator (rt-PA) is the main treatment for
	AIS within 3–4.5 hours of symptom onset. While it
	improves outcomes, it doesn't lower mortality rates. <sup>4</sup>
	Early detection, vigilant monitoring, and appropriate risk
	stratification measures are essential for optimizing stroke
	unit management and improving prognosis in AIS
	patients.
	Recently, numerous mortality prediction models
	for AIS have emerged <sup>5–7</sup> ; nevertheless, the utilization of
	these prediction models in routine clinical practice for all
	patients has not been thoroughly investigated. Therefore,
	the applicability of these models remains limited.
	Consequently, there is a continued need for practical
	clinical risk tools that accurately predict mortality in

ischemic stroke, overcoming these limitations. The Totaled Health Risks in Vascular Events (THRIVE) score is an easily applicable stroke outcome prediction tool that considers clinical predictors available at the time of stroke presentation, including age, the National Institutes of Health Stroke Scale (NIHSS), and Chronic Disease Scale (CDS) factors including hypertension (HTN), diabetes mellitus (DM), or atrial fibrillation (AF). It was developed using data from the MERCI and Multi-MERCI trials.<sup>8</sup> Continuous variables, like age, are categorized into dichotomized or trichotomized groups to simplify score calculation and facilitate the use of predictive scores without relying on computer-based tools. However, these simplifications may compromise the accuracy of outcome prediction.<sup>9</sup> In a previous study, Alexander C. Flint et al. showed that the THRIVE-c calculation, using continuous variables and a logistic equation, outperformed the traditional THRIVE score in predicting poor outcomes (modified Rankin Scale [mRS] 3-6 at 90 days) with a higher area under the curve (AuROC) (AuROC = 0.785, 95% CI: 0.777-0.793 vs. AuROC = 0.746, 95% CI: 0.737-(0.755) and a significant *p*-value of less than  $(0.001)^{10}$ 

The Alberta Stroke Program Early CT Score (ASPECTS) is a reliable and clinically applicable system for assessing brain computed tomography (CT) images. It accurately identifies the location of early-stage acute ischemic stroke, particularly in cases involving middle cerebral artery occlusion.<sup>11</sup> Previous research has shown that the 24-hour ASPECTS, assessed through non-contrast computed tomography (NCCT) after administering rt-PA at 24 hours, demonstrates superior predictive ability compared to the pre-thrombolysis ASPECTS in predicting the functional stroke outcome at 3 months.<sup>12,13</sup> Although Diffusion-weighted imaging ASPECTS demonstrates superior sensitivity in the early detection of ischemic changes after stroke onset compared to NCCT-ASPECTS <sup>14</sup>, the utilization of CT perfusion and magnetic resonance imaging for prompt diagnosis and management of AIS patients remains limited in routine clinical practice in Thailand due to resource and cost constraints. Low blood oxygen saturation is linked to increased RDW, potentially impacting ischemic stroke development by reducing brain oxygen delivery. RDW

severity, indicating its potential predictor for AIS

also exhibits a significant statistical association with AIS

prognosis.<sup>15,16</sup> Currently, the hemoglobin/red cell distribution width (HB/RDW) ratio has emerged as a significant prognostic tool for predicting mortality in heart failure, cardiovascular hospitalizations<sup>17</sup>, survival outcomes in cancer patients<sup>18</sup>, and the risk of death in patients with atrial fibrillation and AIS.<sup>19</sup> Therefore, the 24-hour ASPECTS and HB/RDW ratio may serve as easily applicable, non-invasive predictors that are costeffective and should be considered to enhance the efficacy of clinical prediction and encompass the diverse clinical characteristics of patients in predicting in-hospital mortality.

With widespread computer and internet access, simplifying tools by setting cut-off values for continuous predictors is unnecessary. Multivariable fractional polynomials (MFP) algorithms are used for continuous predictors to identify optimal polynomial transformations, ensuring a good fit in the binary logistic model and generating regression coefficients. These coefficients create a logistic equation for individualized prediction, facilitating outcome probability analysis. Utilizing computer or mobile applications enables the calculation of probabilities based on equations derived from the

	implementation of the MFP procedure, which may		
	provide greater accuracy than using continuous variables		
	and the logistic equation. However, limited data exists on		
	the clinical significance of 24-hour ASPECTS and		
	HB/RDW ratio regarding in-hospital mortality (IHM) in		
	AIS patients treated with rt-PA.		
	This study aimed to evaluate the prognostic added		
	value of incorporating 24-hour ASPECTS and HB/RDW		
	into the THRIVE score through the MFP procedure		
	(combined THRIVE-MFP), as compared to the THRIVE-		
	c model. Additionally, it sought to develop a novel clinical		
	prediction model by combining the THRIVE score with		
	24-hour ASPECTS and HB/RDW to predict IHM in AIS		
	patients treated with rt-PA.		
Materials and Methods	Study population		
	We employed a retrospective cohort design for		
	our study, gathering data from electronic medical records		
	our study, gathering data from electronic medical records of 345 patients diagnosed with AIS who received		
	our study, gathering data from electronic medical records of 345 patients diagnosed with AIS who received intravenous thrombolysis within the 3-4.5-hour window		
	our study, gathering data from electronic medical records of 345 patients diagnosed with AIS who received intravenous thrombolysis within the 3-4.5-hour window from symptom onset or last known normal. The research		
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2019 early management guidelines for AIS.<sup>20</sup> The inclusion criteria were: (1) age  $\geq 18$  years; (2) a diagnosis of acute anterior circulation ischemic stroke (AACIS); and (3) patients treated with rt-PA only. Exclusion criteria included: (1) diagnosis of transient ischemic attack and minor stroke; (2) being pregnant or lactating; (3) posterior circulation ischemic strokes; (4) poor-quality NCCT scans; (5) referred patients with untracked treatment data; (6) incomplete clinical information for THRIVE score; (7) deceased patients within 24 hours after the initiation of rt-PA; and (8) patients received EVT. Patients who had EVT were excluded from our study due to the lack of a neurointerventionalist at our facility. Patients meeting the criteria for EVT were not referred to another center due to reimbursement constraints in Thailand's public health policy during the study period, thereby hindering eligible EVT candidates from accessing this treatment elsewhere. The study protocol (Approval ID. EC034/2566) was approved by the Institutional Review Board and Ethics Committee of Saraburi Hospital. **Data collection** 

Patient demographics (gender, age), vascular risk factors (diabetes mellitus [DM], hypertension [HTN],

AF), HB/RDW, and 24-hour ASPECTS were obtained
from medical records. Moreover, upon hospital admission,
hemoglobin (Hb) levels and red cell distribution width
(RDW) were also assessed. The Sysmex XN-3000
automated analyzer was used to measure complete blood
count parameters. Upon admission, the National Institutes
of Health Stroke Scale (NIHSS) was employed to evaluate
the severity of strokes. Certified neurologists and
neuroradiologists evaluated the 24-hour ASPECTS, and
discrepancies were resolved through consultation and
consensus to ensure accurate results.
THRIVE-c and combined THRIVE-MFP calculation
The THRIVE-c calculation was a multivariable
logistic regression (MVLR) model constructed by
entering continuous inputs of age and NIHSS and
combines them with the dichotomous inputs for HTN,
DM, and AF for estimates outcome probability for
individual patients.
The combined THRIVE-MFP calculation utilized
the MVLR with MFP model, which employed MFP
algorithms to optimize the transformation of continuous
predictors (age, NIHSS, 24-hour ASPECTS, and
HB/RDW). This model also incorporated dichotomous

	inputs for HTN, DM, and AF, resulting in the most
	accurate calculation of the predicted outcome probability.
	Outcomes assessment
	The primary endpoint of this study focused IHM
	included death from all causes, which refers to the
	occurrence of death during hospitalization after
	intravenous thrombolysis. Patient survival information
	was obtained from discharge summaries, and admissions
	were classified as either non-survivors or survivors.
Expected results This should include information about the likely outcome of the protocol (for example, likely yield of protein, typical microscopy images, etc.). We encourage authors to include one set of data from an experiment that worked using the protocol. If applicable, include advice on how to interpret and analyze raw data	The addition of 24-hour ASPECTS on NCCT and
	HB/RDW to the THRIVE score might significantly
	enhance its predictive performance in determining IHM
	among AIS patients treated with rt-PA.
Ethics declarations	The research obtained ethical authorization from the
	human research ethics committee at Saraburi Hospital on
	July 24, 2023, with Certificate No. EC034/2566.
Supporting information The protocol in PDF format available from protocols.io must be provided as Supporting Information file 1, with the caption: S1: Step-by-step protocol, also available on protocols.io	-
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	work and have approved the final version of the		
	manuscript. Concept and design: SK. Acquisition of data:		
	SK. Statistical analysis: SKd. Interpretation of data: SK,		
	WN, SS and NA. Interpretation of ASPECTS on NCCT:		
	SK, NA. Writing original draft: SK, WN, and SS. Writi		
	review	w and editing: all authors.	
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