Lab Protocol Article Template

Title	Novel Clinical Prediction Model: Integrating A^2DS^2 score with 24-hour ASPECTS and Red Cell Distribution Width for Enhanced Prediction of Stroke-Associated Pneumonia following 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 October 20, 2023.
Funding	The research was supported by a particular grant (MC001-2567) 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 This should include, where applicable, links to data and code produced by the protocol or necessary to interpret the outputs.	The datasets used and/or analyzed during the current study are available from the corresponding author upon request.
Associated content Minimum include DOI for protocol on protocols.io	-
Abstract	**Background:** Stroke-associated pneumonia (SAP) is a common leading cause of death during the acute phase. The A^2DS^2 score has been widely used to predict the risk of SAP. However, 24-hour non-contrast computed tomography- Alberta Stroke Program Early CT Score (NCCT-ASPECTS) and red cell distribution width (RDW)

	were not included in this scale. The purpose of the present study was to investigate the prognostic added value of combining 24-hour NCCT-ASPECTS and RDW with the A^2DS^2 score. **Methods:** A retrospective study of thrombolyzed acute ischemic stroke (AIS) patients from January 2015 to July 2022. Data on A^2DS^2 scores, 24-hour NCCT-ASPECTS, and RDW were collected. Three logistic regression models were created: Model A used only the traditional A^2DS^2 score; Model B (A^2DS^2-c) calculated probabilities using a logistic equation; and Model C (combined A^2DS^2-MFP) used multivariable fractional polynomial logistic regression and incorporated the A^2DS^2 score, 24-hour NCCT-ASPECTS, and RDW. Ischemic brain lesions in the middle cerebral artery area were assessed using the 24-hour NCCT-ASPECTS after completing 24-hour intravenous thrombolysis.
Introduction	Ischemic stroke and post-stroke complications cause functional impairment and exhibit an elevated worldwide mortality rate. [(1)] Although endovascular thrombectomy (EVT) has advanced acute ischemic stroke (AIS) treatment, its availability in many hospitals is limited. Intravenous recombinant tissue plasminogen

	activator (IV-tPA) is widely acknowledged for efficiently treating AIS, with a 4.5-hour window for initiation. [2] Stroke-associated pneumonia (SAP), a notable AIS complication, occurs early within the first week of symptom onset, mainly in the initial three days, and negatively affects clinical outcomes at an incidence rate of 11-14%.[3,4] Previous studies have identified advanced age, atrial fibrillation, congestive heart failure, stroke severity, and dysphagia as four significant risk factors.[5] There are currently several established prediction models for early SAP detection, such as the A^2DS^2 scale[6], the ISAN scale[7], and the AIS-APS scale[8]. The predictive performance of the A^2DS^2 score is superior to other prediction scores. [4] Pretreatment non-contrast computed tomography (NCCT) or diffusion-weighted imaging (DWI) reveals early ischemic changes (EICs) and can be used to predict the efficacy of IV-tPA. [9,10] The Alberta Stroke Program Early CT Score (ASPECTS) is a standardized system used for assessing EICs. It is widely employed for patient selection in EVT treatment and can predict functional outcomes and symptomatic intracranial hemorrhage. [11] The National Institutes of Health Stroke Scale (NIHSS) is

	a well-known and medically validated tool employed to swiftly and consistently evaluate the severity of a stroke. This evaluation is crucial in predicting both SAP and functional outcomes. [12] The 24-hour NIHSS score is a better predictor of post-stroke functional outcomes in AIS patients treated with IV-tPA [13] or EVT [14] than baseline NIHSS scores. Ahmed Esmael et al. found that low ASPECTS values were more common in older patients (r= -0.70, p=0.001) and inversely correlated with initial NIHSS scores (r=-0.75, P<0.001).[15]Additionally, a prior study showed that DWI-ASPECTS can aid in predicting SAP in AIS patients (AUC = 0.743, 95% CI [0.678–0.800]).[16] In developing countries such as Thailand, in routine clinical practice, the use of CT perfusion and magnetic resonance imaging cannot be applied for diagnosing and treating all cases of AIS due to resource limitations. Therefore, assessing the 24-hour NCCT-ASPECTS after IV-tPA may provide a more accurate prediction of SAP. Red cell distribution width (RDW) reflects red blood cell (RBC) size variation, measured by automated instruments. An increase in RDW values indicates greater RBC size variability, which may be indicative of inflammation. This is associated with

	adverse clinical outcomes, impaired RBC maturation, increased oxidative stress, decreased antioxidant levels, and could lead to epithelial cell damage and lung infections. [17] The traditional A^2DS^2 (Age, Atrial fibrillation, Dysphagia, Sex, Stroke Severity) score simplifies outcome prediction by dichotomizing or trichotomizing continuous variables, enabling physicians to use predictive scores without computer-based calculators. However, this simplicity sacrifices some prediction accuracy. The widespread availability of computers and internet connectivity across devices has empowered the use of multivariable fractional polynomial (MFP) algorithms for the precise handling of continuous predictors. MFP algorithms identify optimal fractional polynomial transformations for each predictor, enhancing the fit of binary logistic models. This enables the creation of more accurate individual prediction models for predicting SAP. However, the 24-hour NCCT-ASPECTS and RDW are not included in the A^2DS^2 scoring system. Utilizing a multivariable approach to integrate data from the variables in the A^2DS^2 score along with the 24-hour

	NCCT-ASPECTS and RDW may enhance prognostic accuracy. Nevertheless, to the best of our knowledge, no studies have been conducted on this topic. Hence, the researchers hypothesize whether the inclusion of 24-hour NCCT-ASPECTS and RDW in the prediction model can enhance the predictive accuracy of the A^2DS^2 score. The primary objective is to investigate the prognostic added value of combining 24-hour NCCT-ASPECTS and RDW with the A^2DS^2 score. The secondary objective is to develop a novel prediction model based on the A^2DS^2 score in conjunction with 24-hour NCCT-ASPECTS and RDW for SAP prediction after thrombolysis.
Materials and Methods	**Study population** This retrospective study enrolled 345 AIS patients who received IV-tPA treatment within 3-4.5 hours of symptom onset at Saraburi Hospital, Thailand, between January 2015 and July 2022. Inclusion criteria were: (1) age ≥ 18 years; (2) a diagnosis of acute anterior circulation ischemic stroke (AACIS); and (3) treat with IV-tPA only. Exclusion criteria included: (1) contraindications for IV-tPA use according to the 2019 guidelines for early AIS management[17]; (2) diagnosis of transient ischemic attack and minor stroke; (3) pregnant or

	lactating women; (4) posterior circulation ischemic stroke; (5) confirmation of infection or presence of fever prior to admission or previous antibiotic treatment; (6) patients with poor-quality NCCT scans; (7) patients who were referred to other hospitals for further treatment and were unable to be tracked for treatment data; (8) patients lacking complete clinical data from A^2DS^2 score; (9) patients were discharged or deceased within three days of symptom onset; and (10) patients treated with EVT. During the study, our center faced hurdles in performing EVT, and eligible EVT candidates were not referred to other institutions due to reimbursement constraints set by Thailand's public health policy. This resulted in difficulties accessing this treatment. Informed consent for IV-tPA was obtained from either the patients or their families through the completion of consent forms. All study participants received IV-tPA at a dosage of 0.9 mg/kg, administered as a 10% bolus with the remainder infused over 1 hour. Approval for the study protocol (EC 043/2566) was obtained from the Institutional Review Board (IRB) and Ethics Committee at Saraburi Hospital. Patient consent was waived for this retrospective analysis of medical records due to the absence of patient-

	identifying details in the data collection process, ensuring no impact on treatment rights or outcomes. **Data collection** The essential clinical variables analyzed encompassed demographic characteristics (age and gender) and prior as well as present medical histories, which included AF, dysphagia, and the severity of stroke, assessed using the NIHSS. Laboratory parameters upon admission, including RDW, were assessed using the Sysmex XN-3000 automated analyzer for complete blood count measurements. Additionally, we gathered and evaluated radiological factors, with a primary focus on the 24-hour NCCT-ASPECTS (assessed after IV-tPA treatment at 24 hours). NCCT scans were performed using a 160-slice TOSHIBA Aquilion Prime CT scanner from Canon Medical Systems, Japan, covering the region from the base of the skull to the vertex. The scans produced contiguous 3 mm axial slices, using settings of 120 kV and 240 mA. The posttreatment NCCT results were interpreted using the ASPECTS system, assessing the cross-sectional images of the entire brain. ASPECTS scoring was blinded to clinical data, and any discrepancies in ASPECTS values were resolved through collaborative

	consultation between neurologists and neuroradiologists to ensure accurate ASPECTS scores. Dysphagia assessment employed the Modified Water Swallowing Test (MWST) [18] within the first 24 hours. Patients, seated upright, underwent three trials of swallowing 3 milliliters of water. Ratings followed a 5-point scale: (1) inability to swallow, (2) abnormal breathing during swallowing, (3) altered vocal quality or coughing, (4) successful swallowing with saliva pooling, and (5) normal swallowing with task repetition within 30 seconds. A rehabilitation physician conducted the evaluation, with the lowest score determining dysphagia presence if below 4. **Traditional A^2DS^2 score, A^2DS^2-c calculation, and combined A^2DS^2-MFP calculation** The traditional A^2DS^2 consisted of the following components: (1) 1 point for advanced age (≥75 years old); (2) 1 point for male sex; (3) 1 point for the presence of AF; (4) 2 points for dysphagia; (5) 3 points for an NIHSS score ranging from 5 to 15 points; (6) 5 points for an NIHSS score exceeding 16 points. The A^2DS^2-c calculation was derived from a multivariable logistic regression (MVLR) model that included continuous

	variables such as age and NIHSS as well as binary variables like AF, dysphagia, and male gender to estimate the probability of outcomes for individual patients. The combined A^2DS^2-MFP calculation enhanced outcome prediction by integrating continuous variables (age, NIHSS, 24-hour NCCT-ASPECTS, and RDW) using MVLR and MFP algorithms. It also incorporated binary factors (AF, dysphagia, and male) for the most accurate prediction of outcome probability. **Diagnostic Criteria for SAP** The diagnosis of SAP followed the criteria outlined in the modified Centers for Disease Control and Prevention guidelines [19] and/or Mann's criteria. [20] All enrolled patients were diagnosed using either Mann's criteria or the modified CDC criteria, and patients who meet the criteria for definite or probable SAP are classified as having SAP.
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	24-hour NCCT-ASPECTS and RDW might enhance the predictive value of the A^2DS^2 score for SAP after IV-tPA. The combined A^2DS^2-MFP model performed excellently in predictive performance, offering robust early SAP detection and potentially improving patient survival.
Ethics declarations	The research obtained ethical authorization from the human research ethics committee at Saraburi Hospital on October 2, 2023, with Certificate No. EC043/2566.

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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