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BRIEF COMMUNICATION

Biomarkers predictive value for early diagnosis of Stroke-Associated Pneumonia

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Abstract

To confirm the diagnostic accuracy of candidate biomarkers in stroke-associated pneumonia (SAP), we prospectively enrolled ischemic stroke patients with NIHSS ≥ 10 on admission from March-2016 to August-2017. Blood samples were collected at baseline, 24 and 48 h after stroke onset. Biomarkers (MR-proADM, suPAR, SAA) were determined by immunoassays. Regarding biomarkers, MR-proADM at 24 h ($P = 0.04$) and both suPAR and SAA at 48 h ($P = 0.036$ and $P = 0.057$) were associated with pneumonia. The combination of SAA > 25.15 mg/dL and suPAR> 3.14 ng/mL at 48 h had 80% sensitivity and 95.8% specificity when both biomarkers were above the cut-off. The evaluated biomarkers represent promising tools to be evaluated in future large, prospective studies on SAP. An accurate SAP diagnosis by thorax CT might help to reduce variability in such studies.

Introduction

Respiratory tract infections occurring within 7 days of stroke onset have been incorporated under stroke-associated pneumonia (SAP) term, after adapted definition from the Centre for Diseases and Control and Prevention (CDC)[1] by the Pneumonia in Stroke Consensus Group (PISCES).[2] However, one of the main limitations in SAP research is the lack of a gold-standard test for clinical diagnosis. Thorax high-resolution computed tomography

(THRCT) is a useful adjunct to conventional radiography and an accurate study to identify underlying findings in stroke patients, which could serve as a guide in SAP diagnosis. In fact, we have recently reported that this tool represents an accurate imaging test for the evaluation of clinical criteria.[3]

Stroke alters systemic immunity, predisposing patients to immunosuppression and infections.[4] Therefore, the use of blood biomarkers associated with the systemic inflammatory response and the immune changes would be an interesting starting point for SAP prediction. Some sepsis biomarkers, recently identified as promising predictors of stroke-associated infections,[5,6] represents interesting candidates for SAP. Soluble urokinase-type plasminogen activator receptor (suPAR), participates in immunologic functions.[7] Pro-adrenomedullin (MR-proADM), seems to correlate with the severity of pneumonia.[8] And serum amyloid A protein (SAA) with an important role in attracting leukocytes and immune cells to the sites of damage.[9] We aimed to confirm the diagnostic accuracy of mentioned candidate biomarkers related to the features on THRCT.[3]

Methods

A cohort of 45 consecutive stroke patients was included in a monocentric, prospective, observational study from March/2016-August/2017. The study was approved by our institutional review committee (ID 0103-M1-14). The patients eligible for the study were those with ischemic stroke confirmed by CT or magnetic resonance imaging (MRI) and NIHSS $\geq$ 10 on patient admission. Stroke onset to inclusion was $\leq$24 h, and informed consent signed by the patient or a relative was needed at inclusion. Mechanical ventilation pneumonia of intensive care unit transferred patients and antibiotic therapies within last 24 h previous to hospital admission were exclusion criteria. Every patient underwent a comprehensive interview, neurological and general examination on admission and every day after inclusion until the seventh day after admission by the same neurologist blinded to the patient study participation. Dysphagia was evaluated within the first 24 h with the Acute Stroke Dysphagia Screen test.[10]

Chest X-ray (CXR) and laboratory test were run following neurologist criteria during admission. A THRCT with volumetric technique acquisition was performed, without contrast, between 5th and 7th day of admission in all included patients, independently of the suspicion of respiratory infection. CT was performed in a 64 detectors rows equipment. Dosage Modulation: 120–178 Kv. Collimation: 0.5–1 mm, according the equipment. Reconstruction interval: 1 mm. PITCH could be between 0.8 and 1. Acquired data reconstruction was done with soft parts and high spatial frequency double filter. Every THRCT was evaluated by two skilled thorax radiologists, and discrepancies reviewed by another expert thorax radiologist, all blinded to the clinical data. Consensus was reached in cases with disagreement.

In this study, SAP was diagnosed by treating physician according to diagnostic categories established by the PISCES criteria.[2] PISCES Probable SAP was defined by the presence of all CDC criteria[1] but in the absence of diagnostic changes on initial CXR and repeated CXR (or where CXR not undertaken), and no alternative explanation or diagnosis. Likewise, PISCES definite SAP was diagnosed when all CDC criteria met including diagnostic CXR changes on at least one CXR.

Blood samples for biomarkers analysis were obtained at baseline, 24 and 48 h after stroke onset. Then, samples were centrifuged at 1500*g* for 15 min at 4°C and serum and plasma were frozen at −80°C until biomarker measurement.

We measured a 4-blood biomarkers panel including SAA, MR-proADM, suPAR and CRP. MR-proADM (B·R·A·H·M·S MR-proADM KRYPTOR™-ThermoFisherScientific, MA) and CRP (CardioPhase HS CRP, Siemens Healthineers Spain)were measured in plasma EDTA samples and suPAR (Quantiquine ELISA kit, R&D Systems, MA) was measured in serum. SAA1 was determined in serum with the Meso Scale Discovery (MSD) Vascular Injury Panel-I ECL assay, as per manufacturer's instructions (MSD, Gaithersburg, MD). All biomarkers except MR-proADM were assessed in duplicate and the mean intra-assay coefficient of variation (CV) was <20%. Inter-assay variation was determined testing two times in each plate with a commercial internal control (Human serum type AB, male, from clotted,

Table 1. Baseline characteristics of the stroke patients that were include in the study and received THRCT.

Variable	*n* = 41	%
Male	21	51.2
Age (years old) (mean ± range)	75 [67–82]	
NIHSS on admission	20 [15–22]	
Time to probable SAP (PISCES) (d) (Mean [range])	1.5 [1–2]	
Time to confirmed SAP on THRCT (d) (Mean [range])	2 [1.5–3.5]	
Smoker	12	29.3
Chronic obstructive pulmonary disease	8	19.5
Dysphagia	31	75.6
Urinary tract infection	5	12.1
Phlebitis	1	2.4
Clinical respiratory infection (PISCES Probable)	8	19.5
Stroke associated pneumonia on THRCT (Bronchopneumonia)	5	12.2
Stroke associated chest infection on THRCT (Bronchopneumonia + Bronchitis)	8	19.5

Sigma-Aldrich), and it was <20%. MR-proADM, as instructed, was assayed by simple. Intra-assay and inter-assay CV for this method are <10% and <20%, respectively. Biomarker measurement was performed blinded to clinical information.

Statistical analyses were conducted with Statistical Packages for Social Sciences (SPSS), version 22. Intergroup comparisons were performed using Chi-squared test for categorical variables and Mann–Whitney *U* test for continuous variables. Biomarkers were tested for normality with the Kolgomorov-Smirnoff test. As biomarkers were not normally distributed, comparisons on biomarker levels between groups were performed with non-parametric tests. Intergroup differences were assessed with Mann–Whitney or Kruskal–Wallis tests. Where relevant, biomarkers were dichotomized by the cut-off with the highest predictive accuracy for infections, which was calculated in receptor operating characteristic (ROC) curves. Sensitivity and specificity for every biomarker cut-off were evaluated. A *P* value <0.05 was considered statistically significant. Given the small sample size and the preliminary nature of the study, *P* values under 0.1 were considered as trends.

Results

Of the forty-five included patients, 3 died and one presented with psychomotor agitation that precluded

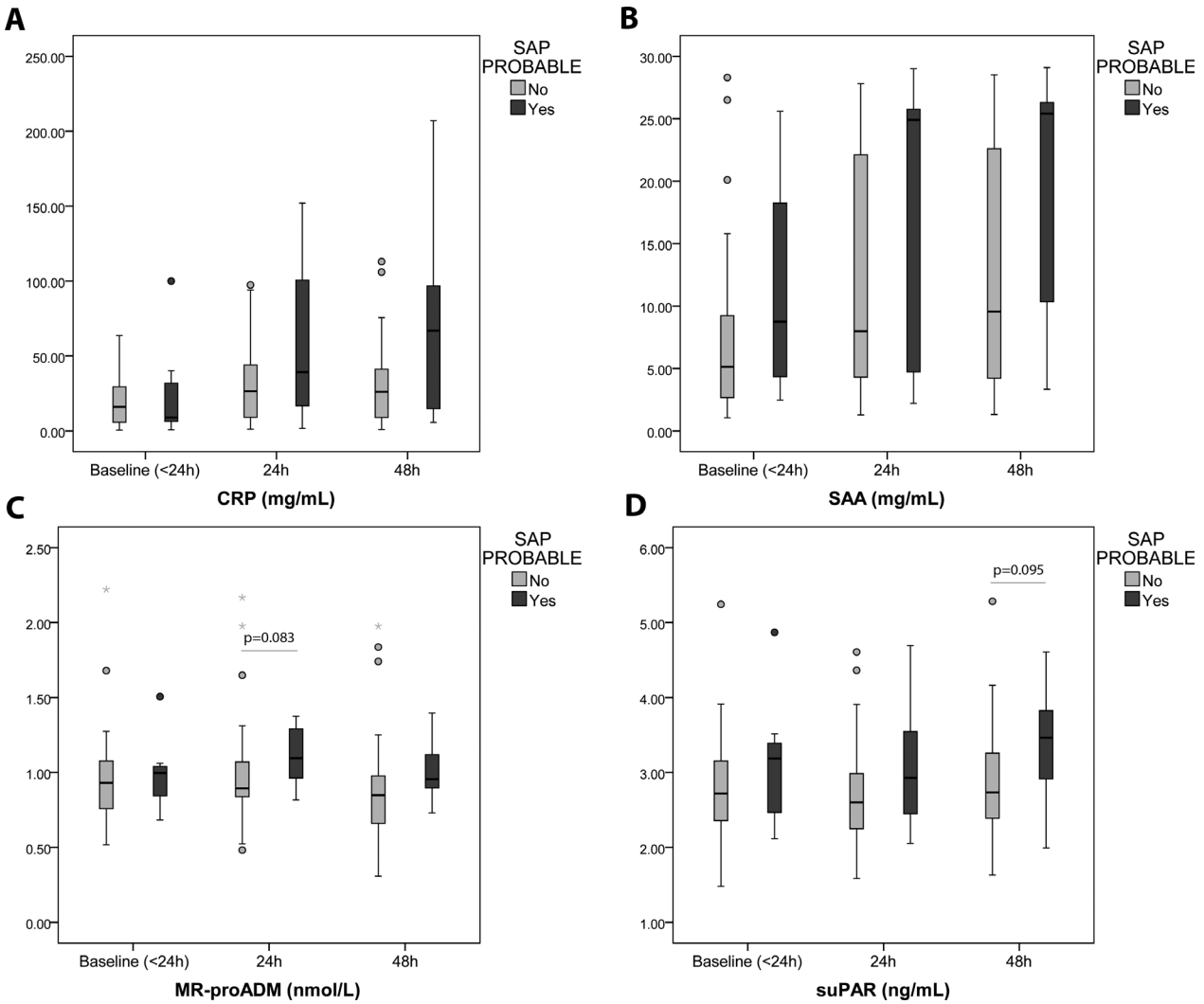

Figure 1. Biomarker level according to clinical diagnosis of SAP following PISCES criteria. Boxplots represent median and interquartile range in both no SAP and probable SAP groups. Just *P* values <0.1 are represented. CRP, C-reactive protein; SAA, serum amyloid-A protein; MR-proADM, mid-regional proadrenomedullin; suPAR, soluble urokinase-type plasminogen activator receptor.

THRCT performance. PISCES probable SAP rate was 17.8% ($N = 8$). There was no patient with PISCES definite SAP according to treating physician criteria. Moreover, chest radiography low quality was insufficient to a correct interpretation of some cases to be considered definitive SAP. Among those mentioned 8 patients, 5 (62.5%) were identified as bronchopneumonia, 2 (25%) as infectious bronchitis and 1 showed no pathology on CT. Baseline data is shown in (Table 1). According to PISCES criteria, patients with probable SAP trended to had higher levels MR-proADM at 24 ($P = 0.083$) and higher levels of suPAR at 48 h ($P = 0.095$). There were no significant differences for CRP and SAA among any visit (Fig. 1).

However, regarding THRCT findings, MR-proADM at 24 h ($P = 0.040$) and both suPAR and SAA at 48 h ($P = 0.037$ and $P = 0.057$) were associated with pneumonia. At 24 h, a cut-off of MR-proADM > 1.023 nmol/L had 80% sensitivity and 72.2% specificity for pneumonia. At 48 h, both SAA > 25.15 mg/dL (60% sensitivity, 85.7% specificity) and suPAR > 3.14 ng/mL (80% sensitivity, 57.1% specificity) were associated with infections. The combination of SAA > 25.15 mg/dL and suPAR > 3.14 ng/mL at 48 h had 80% sensitivity and 95.8% specificity when both biomarkers were above the cut-off, and 60% sensitivity and 94.1% specificity when one of them was above the cut-off (Fig. 2).

Discussion

Our results provide various key findings, about the most common complication after stroke. Employment of THRCT in SAP, could reduce diagnostic discrepancies in patients with clinical suspicion and low quality chest radiography. From the evaluated biomarkers, MR-proADM seems to be the earliest predictor of SAP, when respiratory infection is yet not suspected. The combination of SAA/suPAR improves the predictive value, but at later time-points.

The poor predictive value of previously SAP studied biomarkers[11] and the limited specificity of validated pneumonia predictive scores[12] induce uncertainty in SAP prediction and diagnosis. This leads to a late start of the antibiotic treatment, with the unfortunate consequences associated, or an overuse of antibiotics in patients without a clear diagnosis, in order to avoid these fatal outcomes, increasing antibiotic resistance in cases without real SAP. Therefore, the identification of an early reliable biomarker, could guide respiratory infection management in stroke patient.

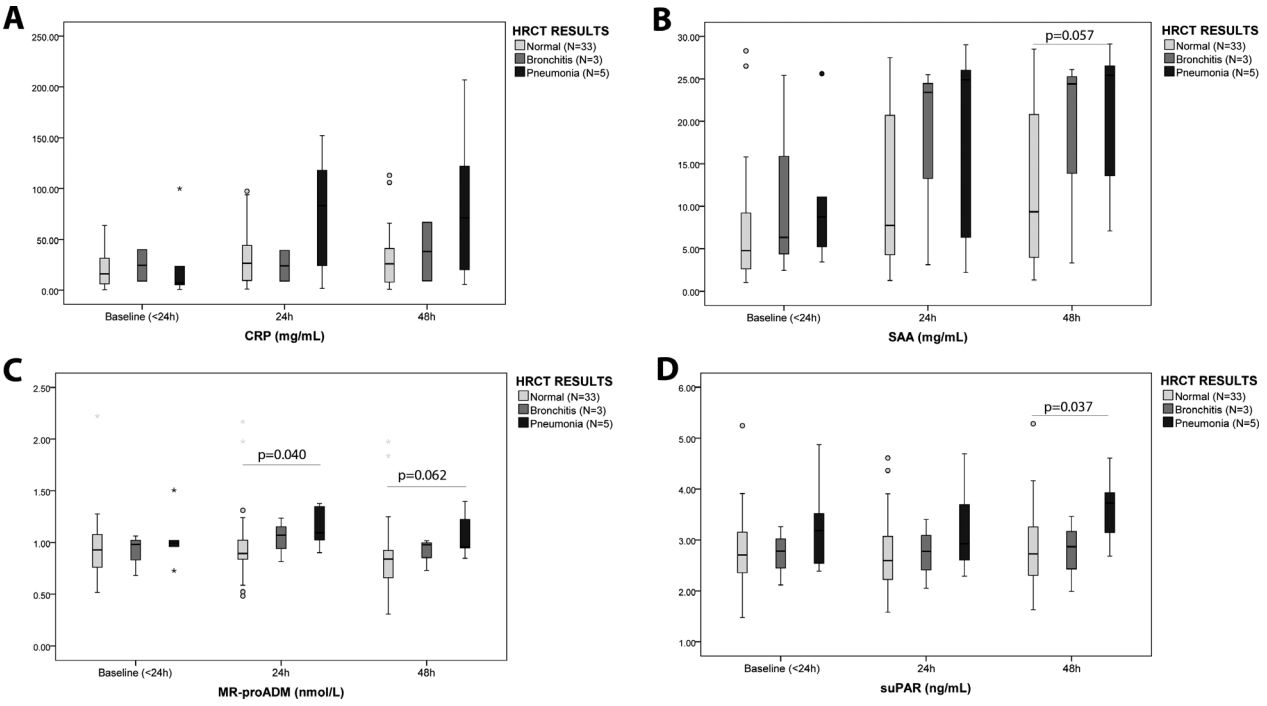

Figure 2. Biomarker level according to radiological diagnosis of SAP using thoracic HRCT findings. Boxplots represent median and interquartile range in normal, bronchitis and pneumonia groups. Just *P* values < 0.1 are represented. CRP, C-reactive protein; SAA, serum amyloid-A protein; MR-proADM, mid-regional proadrenomedullin; suPAR, soluble urokinase-type plasminogen activator receptor.

Our previous study showed for the first time THRCT radiological findings of stroke associated respiratory infections,[3] allowing us to conduct this confirmatory study to test the diagnostic accuracy of candidate biomarkers related to both clinical and radiological diagnosis. In our cohort, regarding clinical criteria, patients with probable SAP had higher levels of all evaluated biomarkers across the time-points, but without statistical significance. However, when SAP was diagnosed according to THRCT findings, we found that MR-proADM at 24 h and both suPAR and SAA at 48 h were significantly associated with pneumonia. These results may suggest a correlation between the acute-phase response and development of an infection, which would expand biomarkers scope of use because of their predictive value in SAP patients, confirmed by THRCT.

According to our results, it is interesting to highlight the insufficient association of tested biomarkers with SAP when they are explored regarding clinical criteria. Such clinical criteria have shown high sensitivity rates (87.5%) when respiratory infection suspicion is confirmed with THTCT.[3] However, high false positive rate of clinical criteria leads to discrepancies in biomarkers-SAP relationship that clearly improves when compared with THRCT findings. In fact, with the use of THRCT we would have been able to give a diagnosis of definitive SAP instead of probable SAP in seven out of the eight patients with clinical suspicion**.** Such results are consistent with low accuracy of clinical criteria; showing reliable results due to THRCT performance in this study.

Finally, for post-stroke infections prediction, to define biomarker´s cut-points in patients with SAP would help in preventive strategies, especially in early stages. In our cohort, the combination of SAA > 25.15 mg/dL and suPAR > 3.14 ng/mL at 48 h showed 80% sensitivity and 95.8% specificity. Given most SAP cases appears within first seven days after stroke onset,[2] the availability of described biomarkers, even after 48 h, could help in early detection of respiratory infection in stroke patients, with low rates of false negative results.

Therefore, these new cut-offs should now be tested in larger cohorts, since prospectively validating a cut-off is a necessary and relevant approach in the biomarkers research field that we do not usually do systematically. The main limitation of the study is that our results are based on a single cohort and our findings need to be validated in an independent and larger cohort before an intervention based on these results may be recommended. In addition, the small sample size and the low rate of events in both clinical and radiological diagnosis prevented us to perform logistic regression analysis to adjust by potential confounders, such as stroke severity or dysphagia. However, we consider that given laboratory test are compared for the first time with THRCT findings, this adds important strength to our study.

In conclusion, among explored biomarkers, MR-proADM would have an important role in SAP prediction, due to its early elevation in stroke patients suffering respiratory infections later on. In addition, the combination of SAA/suPAR improves the predictive value, especially adding specificity, but at later time-points. Further prospective studies should be developed to assess the predictive value of these results. An accurate SAP diagnosis by thorax CT might help to reduce diagnostic uncertainty in such studies.

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Conflict of Interest

None.

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