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Sepsis biomarkers reprofiling to predict stroke-associated infections

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ABSTRACT

We aimed to evaluate the usefulness of sepsis biomarkers to predict stroke-associated infections. Soluble triggering receptor expressed on myeloid cells-1 (sTREM-1), mid-regional pro-adrenomedullin (MR-proADM), presepsin (sCD14), and soluble urokinase-type plasminogen activator receptor (suPAR), were explored in 125 blood samples collected at different time-points. At baseline, MR-proADM was an independent predictor of infection [> 0.94 pg/mL, OR = 3.63 (1.16–11.33), p = 0.026], as well as suPAR at 24 h [> 2185.8 pg/mL, OR = 5.81 (1.05–32.26), p = 0.044]. Both MR-proADM and suPAR were raised in patients with infections throughout the first week after stroke. These results are especially relevant for MR-proADM given its early elevation, which would allow early preventive interventions.

1. Introduction

Infections represent one of the most frequent complications after acute stroke, with an estimated prevalence of 30% (Westendorp et al., 2011). Although the effect of infections on stroke outcome has been discussed, at least respiratory tract infections (RTI) have a major effect on stroke outcome, accounting for one third of all deaths and increasing the likelihood of extended care needs by 70% (Heuschmann et al., 2004; Katzan et al., 2007). The main reasons accounting for infections after stroke arise from both higher microbial exposure, increased use of medical devices or dysphagia on one hand and, besides, from the endogenous immunosuppression which is noted after central nervous system injuries. This immunosuppressive state consists mainly of down-regulation of the systemic cellular immune response, manifested by functional deactivation of monocytes, T helper and natural killer T cells (Klehmet et al., 2009; Wong et al., 2011). Reflecting this dual mechanism, the use of blood biomarkers related to the immune response, in combination with clinical information, has been proposed for prediction of post-stroke infections (Salat et al., 2013). However, from all the explored biomarkers, none of them has demonstrated clear evidence for the prediction or diagnosis of post-stroke infections so far (Smith et al., 2015).

Sepsis biomarkers, such as procalcitonin (PCT), represent potential candidates for the prediction of post-stroke infections. However, for this marker, both positive (Xie et al., 2011; Fluri et al., 2012) and negative results (Hug et al., 2011) have been reported, questioning therefore its validity. Some evidences suggest new promising candidates, such as soluble triggering receptor expressed on myeloid cell-1 (sTREM-1), which acts as a trigger for the release of pro-inflammatory cytokines and chemokines, stimulating neutrophil and monocyte inflammatory response. sTREM-1 has been reported to be predictive of poor outcome in patients with stroke-associated pneumonia (Xie et al., 2015). Presepsin (sCD14) acts as a co-receptor for the detection of bacterial lipopolysaccharide. Mid-regional pro-adrenomedullin (MR-proADM) is a stress marker which acts as a hypotensive and vasodilatator agent. Both have been described as useful markers when measured within the first 24 h after intensive care unit admission among septic patients (Enguix-Armada et al., 2016). Moreover, MR-proADM has shown to improve outcome prediction over clinical variables in stroke patients (Seifert-Held et al., 2013). Soluble urokinase-type plasminogen activator receptor (suPAR) plays a role in localizing and promoting plasmin formation, and mediates the proteolysis-independent signal transduction activation effects of U-PA, being increasingly used for the monitoring of systemic inflammation and sepsis (Donadello et al., 2012).

It has been suggested that about one half of the cases of stroke-associated pneumonia occur within the first 48 h after stroke onset (Finlayson et al., 2011). Consequently, for the prediction of post-stroke infections, differences between patients with and without infections in the biomarkers' levels should be present as early as possible after stroke, in order to start preventive strategies. Therefore, we aimed to test the

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predictive ability of blood biomarkers at different time-points after stroke (first 6 h, first 24 h and a complete serial profile within the first week) for stroke-associated infections among a panel of candidates coming from the sepsis literature.

2. Methods

The present exploratory study was carried out with 125 blood samples from stroke patients collected in previous studies on stroke biomarkers at Vall d'Hebron Hospital. The study was divided into 3 different phases. In the first one, a 4-biomarker panel was tested in baseline (< 6 h from stroke onset) blood samples. For those biomarkers not associated with post-stroke infections at baseline, a second measurement was performed in samples obtained 24 h after stroke in the second phase. Finally, the selected candidates from the previous phases were measured in a new cohort including a five time-point serial profile.

2.1. Phase 1: sepsis biomarkers in baseline samples

From August 2012 to June 2014, 399 ischemic strokes were included and blood samples collected within 6 h after symptoms onset. This cohort was recruited as a part of the Stroke-Chip study (Bustamante et al., 2017) in our centre and continued after the cessation of the multicentre study under the protocol PR_AG_157-2011. From this cohort, we included all patients with post-stroke pneumonia according to CDC criteria (27 patients). Additionally, 27 patients without any post-stroke infection during the first week, matched by age, and NIHSS, 13 patients with respiratory tract infections (RTI) not fulfilling CDC criteria for pneumonia and 11 patients with urinary tract infections (UTI) were also included.

2.2. Phase 2: sepsis biomarker in 24 hour samples

This cohort was based on a previously published study (Salat et al., 2013), conducted under the protocol PR_HG_89-2003. All patients from this study with samples available for analysis obtained 24 h after stroke (39 patients) were included. Post-stroke infections were diagnosed in 14 patients, being 11 of them RTI and fever of unknown origin the remaining three.

2.3. Phase 3: complete serial profile

From September 2011 to November 2012, stroke patients were included within 12 h after symptom onset to collect blood samples at different time-points after stroke for the study of blood biomarkers. These patients were enrolled in the phase 0 clinical trial ASY11714, in the non-thrombolysis arm (Alessi et al., 2016), and additional plasma samples were obtained under the protocol PR_HG_89-2003. For this study, we measured blood biomarkers obtained at baseline (< 12 h), 24, 48 and 72 h and 1 week after stroke, in 4 patients suffering RTI during the first week and in 4 patients not suffering any infection.

In all three studies, stroke diagnosis was made according with the World Health Organization definition (Hatano, 1976). Stroke severity was assessed with the National Institutes of Health Stroke Scale (NIHSS). Stroke was also classified according to the Oxfordshire Classification Stroke Project (OCSP) into total (TACI) or partial (PACI) anterior circulation infarct, posterior circulation infarct (POCI) or lacunar infarct (LACI). Stroke etiology was classified according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification. Infections were diagnosed in all three studies according to the Centers for Disease Control and Prevention (CDC) criteria (Horan et al., 2008). Briefly, the diagnosis of infection required the presence of fever (body temperature > 37.5 °C) or elevated white blood cell count ($> 1.2 \times 10^{10}$/L) and evidence of organ-specific involvement (purulent sputum, pulmonary infiltrate, abnormal urine sediment, etc.) or a positive culture. An abnormal chest X-ray was required for the diagnosis of stroke-associated pneumonia. The study protocols were approved by Vall d'Hebron Clinical Research Ethics Committee (PR_HG_89-2003 and PR_AG_157-2011) and all patients or relatives signed informed consent.

2.4. Biomarker measurement

We measured a 4-blood biomarkers panel including soluble triggering receptor expressed on myeloid cells-1 (sTREM-1), mid-regional pro-adrenomedullin (MR-proADM), presepsin (sCD14) and soluble urokinase-type plasminogen activator receptor (suPAR). In all studies, samples were centrifuged at 1500*g* for 15 min at 4 °C and serum and plasma were frozen at − 80 °C until biomarker measurement. MR-proADM (B·R·A·H·M·S MR-proADM KRYPTOR™ — Thermo Fisher Scientific, MA USA) and sTREM-1 (Quantiquine ELISA kit, R & D Systems Inc., MN, USA) were measured in plasma EDTA samples, while sCD14 (Human Magnetic Luminex Screening assay, R & D Systems Inc., MA USA) and suPAR (Quantiquine ELISA kit, R & D Systems, MA USA) were measured in serum. All biomarkers but 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, 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.

2.5. Statistical analyses

Statistical analyses were conducted with Statistical Packages for Social Sciences (SPSS), version 22. Comparisons were performed between patients with any infection regardless of its origin and patients without any infection. 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 Kolmogorov-Smirnoff test. As biomarkers were not normally distributed, comparisons on biomarker levels between groups were performed with Mann-Whitney U test. Differences in temporal profiles were assessed with Friedman test. Post hoc analysis with Wilcoxon signed-rank tests was performed with Bonferroni correction. For phases 1 and 2, logistic regression analyses were conducted, considering the presence of any infection as dependent variable. Variables associated with infections in univariate analysis with a p value < 0.1 were included as covariates and used for adjustment. For inclusion in the logistic regression analysis, biomarkers were dichotomized by the cut-off with the highest predictive accuracy for infections, which was calculated in receptor operating characteristic (ROC) curves. A p value < 0.05 was considered statistically significant.

3. Results

3.1. Phase 1: biomarkers for prediction of infection in baseline samples

The 78 patients included in this experiment had a median age of 82 (75.5–87) and 42.3% were male. As being matched by age and stroke severity, there were no significant differences on baseline characteristics between patients with and without infections, other than a higher prevalence of infections in TACIs and a trend towards a higher percentage of infections in men. Complete data from this cohort are available at Table 1. From the explored biomarkers, just MR-proADM showed a trend towards higher levels in patients with infections (0.96 (0.79–1.16) vs. 0.86 (0.74–0.95) nmol/L, p = 0.134). No significant differences were noted between the different types of infections included (data not shown). A cut-off point of 0.94 nmol/L showed a sensitivity of 51% and a specificity of 74.1% for the prediction of