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Predisposing factors, diagnostic, and predictive markers of stroke-associated pneumonia.

Czynniki predysponujące, markery diagnostyczne i predykcyjne zapalenia płuc w przebiegu udaru mózgu.

Praca doktorska

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SPIS TREŚCI

II. WSTĘP

Zapalenie płuc występuję u ok. 10% pacjentów z udarem mózgu[1]. U chorych z ciężkim udarem mózgu hospitalizowanych w oddziałach intensywnej terapii częstość występowania zapalenia płuc jest jeszcze większa i wynosi ok. 30%[1]. Zapalenie płuc w przebiegu udaru mózgu (stroke-associated pneumonia, SAP) związane jest ze zwiększonym ryzykiem zgonu oraz niekorzystnego rokowania funkcjonalnego[2].

Postawienie wczesnego rozpoznania SAP oraz identyfikacja pacjentów zagrożonych SAP są ważne z klinicznego punktu widzenia. Nieleczone SAP może prowadzić do niewydolności oddechowej i posocznicy. Z drugiej strony, błędne rozpoznanie SAP może skutkować niepotrzebną antybiotykoterapią, która u niektórych pacjentów może być przyczyną objawów niepożądanych, zaś w skali globalnej sprzyja powstawaniu zjawiska antybiotykooporności. Obecnie stosowane kryteria PISCES (Pneumonia in Stroke Consensus)[3], będące modyfikacją kryteriów CDC (Center for Disease Control), mają szereg ograniczeń. Kryteria te nigdy nie były walidowane w badaniach prospektywnych. Objawy służące rozpoznaniu SAP zawarte w kryteriach PISCES, takie jak kaszel lub zaburzenia świadomości, mają niską wartość diagnostyczną u pacjentów z udarem mózgu. U chorych z udarem mózgu odruch kaszlowy może być upośledzony, zaś zaburzenia świadomości, a nawet gorączka mogą być bezpośrednim objawem uszkodzenia mózgu. W tej sytuacji ważna staje się identyfikacja czynników predysponujących do SAP oraz poszukiwanie nowych biomarkerów diagnostycznych i predykcyjnych dla SAP.

Badania doświadczalne przyniosły informacje o immunologicznych mechanizmach leżących u podstaw SAP. Badania te wskazują, że zjawisko przejściowej immunodepresji występujące w ostrej fazie udaru i manifestujące się hiporeaktywnością monocytów jest czynnikiem predysponującym do rozwoju infekcji[4]. Laboratoryjnym markerem immunodepresji jest obniżona produkcja czynnika martwicy nowotworów alfa (TNFα) po stymulacji in vitro leukocytów krwi za pomocą endotoksyny. Zjawisko immunodepresji jest regulowane przez cholinergiczny układ nerwowy[5]. Nadmierne pobudzenie układu cholinergicznego u myszy sprzyja powstawaniu SAP. Oprócz immunodepresji, zapaleniu płuc towarzyszy systemowa reakcja zapalna z syntezą białek ostrej fazy. Chociaż prototypowe białko z tej grupy, czyli białko C-reaktywne (CRP) ma ograniczone znaczenie diagnostyczne u pacjentów z SAP[6], to jednakże inne białka z tej rodziny (np. białko wiążące endotoksynę [LBP], sCD14), których stężenie wzrasta we krwi w przebiegu infekcji bakteryjnych Gram-dodatnich i Gram-ujemnych, mogą być potencjalnymi kandydatami na biomarkery predykcyjne SAP.

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VII. CELE PRACY

Celem pracy była identyfikacja nowych biomarkerów diagnostycznych i predykcyjnych oraz czynników predysponujących do wystąpienia SAP.

Cele szczegółowe pracy:

1. Określenie, czy profil syntetyzowanych ex vivo cytokin różni się u pacjent z SAP w porównaniu do pacjentów bez SAP i czy może on być wykorzystany do postawienia diagnozy SAP.

2. Określenie, czy pomiar we krwi białka wiążącego endotoksynę (LBP) i rozpuszczalnej formy białka CD14 (sCD14) może być wykorzystany do oceny ryzyka SAP.

3. Określenie, czy zażywanie leków wykazujących aktywność anty-cholinergiczną jest czynnikiem predysponującym do wystąpienia SAP.

VIII. TEKSTY OPUBLIKOWANYCH PRAC NAUKOWYCH BĘDĄCYCH PODSTAWĄ PRACY DOKTORSKIEJ

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Ex vivo synthesized cytokines as a biomarker of stroke-associated pneumonia

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ABSTRACT

Objectives: We aimed to determine a profile of ex vivo released cytokines in patients with stroke-associated pneumonia (SAP) and to assess the clinical utility of individual cytokines and their combination as a biomarker of SAP.

Methods: We included 279 ischemic stroke patients (median age: 69 years; 41.6% women). We collected blood samples at day 3 after the onset of stroke and stimulated them ex vivo with lipopolysaccharide (LPS). We measured the LPS-induced cytokine concentrations (TNFα, IP-10, IL-1β, IL-6, IL-8, IL-10 and IL-12p70) as well as a plasma IL-6 level as a marker of systemic inflammation. We assessed the discriminatory ability of cytokines by calculating the area under the receiver operating characteristic curve (AUC).

Results: During first 5 days after stroke pneumonia occurred in 7.2% of patients. Patients with SAP had lower ex vivo release of TNFα, IL-1β, IL-12, IP-10 and a higher level of circulating IL-6 than patients without SAP. The multimarker score composed of ex vivo synthesized IL-12, IP-10, and plasma IL-6 had better discriminatory properties than individual cytokines (AUC: 0.90).

Conclusions: Our results suggest the potential utility of ex vivo synthesized cytokines as a biomarker of SAP.

1. Introduction.

Pneumonia commonly complicates the acute phase of stroke. The overall rate of pneumonia in stroke patients is 10% and about 45% of stroke patients who are hospitalized in intensive care units develop pneumonia [1]. Stroke-associated pneumonia (SAP) is related to considerable mortality, morbidity, and cost of medical care [2,3].

A correct diagnosis of pneumonia is important because delayed administration of antibiotics might result in respiratory failure and sepsis. On contrary, the overprescribed antibiotics may lead to the development of antibiotic-resistant microorganisms. Moreover, some patients unnecessarily treated with antibiotics could suffer from side effects of these drugs.

In 2015 the Pneumonia in Stroke Consensus (PISCES) Group proposed the operational diagnostic criteria of SAP for research and clinical practice [4]. Although these criteria are an important step forward towards the standardization of diagnostic approaches, they still require rigorous prospective evaluation of their validity and reliability. Since there is a lack of a gold-standard test for the clinical diagnosis of SAP, there is an urgent need to develop novel laboratory markers aided a diagnosis of SAP.

Acute stroke is accompanied by two immune phenomena: systemic inflammation and immunodepression [5]. Systemic inflammation manifests as an elevated level of blood interleukin-6 (IL-6) and C-reactive protein (CRP) [6]. Stroke-induced immunodepression refers to transient suppression of both the innate and adaptive immune response. It manifests mainly as a monocyte deactivation, lymphopenia, and spleen atrophy [5,7]. One of laboratory hallmarks of post-stroke immunodepression is the diminished tumour necrosis factor alpha (TNFα) release after whole blood stimulation with endotoxin. Experimental studies showed that stroke-induced immunodepression predisposes to infections [8].

Both the ex vivo released and circulating cytokines might be potential candidates for SAP biomarkers. Several studies showed that both elevated blood IL-6 [9–11] and the reduced ex vivo TNFα production [12,13] are associated with post-stroke infections.

We aimed to determine a diagnostic value of ex vivo released cytokines in patients with SAP.

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2. Material and methods.

2.1. Patient selection and clinical assessment.

We prospectively recruited participants to this study from consecutive stroke patients who were hospitalized in the Department of Neurology, University Hospital, Krakow, Poland, between October 2016 and February 2019. The inclusion criteria were: (1) ischemic stroke; (2) time from the onset of stroke symptoms to admission < 24 h; (3) pre-stroke modified Rankin Scale score 0–2 (independent of daily activities); (4) National Institute of Health Stroke Scale (NIHSS) score on admission > 3; and (5) informed patient consent. The exclusion criteria were: chronic inflammatory, autoimmune or cancerous diseases; first manifestation of pneumonia occurring > 5 days after stroke onset. The study protocol was approved by the Bioethics Committee of Jagiellonian University. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

We assessed pneumonia symptoms and signs during the first 7 days of hospitalization. We diagnosed pneumonia using the PISCES criteria [4]. The diagnosis of SAP required: (A) at least 1 of the following: (1) fever (> 38 °C) with no other recognized cause; (2) leukopenia (< 4000 WBC/mm^2) or leukocytosis (> 12 000 WBC/mm^2); (3) for adults ≥ 70 year old, altered mental status with no other recognized cause, and (B) at least 2 of the following: (1) new onset of purulent sputum, or change in character of sputum over a 24 h period, or increased respiratory secretions, or increased suctioning requirements; (2) new onset or worsening cough, or dyspnea, or tachypnea (respiratory rate > 25/min); (3) rales, crackles, or bronchial breath sounds; (4) worsening gas exchange (eg. O_2 desaturation [eg $Pao_2/FiO_2 \leq 240$], increased oxygen requirements) and (C) lung infiltrate on at least one chest X-ray.

The neurological deficit on admission was assessed using the NIHSS [14]. Higher scores indicate greater impairment and more severe stroke.

2.2. Laboratory assays.

Since most post-stroke infections occur within three days of hospital admission [15], we took blood samples at day 3 after stroke symptoms onset. We collected venous blood in heparinized tubes (Sarstedt, Germany) between 7:00 AM and 7:30 AM. Subsequently, we diluted the whole blood by 1:5 in sterile RPMI 1640 medium supplemented with L-glutamine (Sigma Aldrich, St. Louis, MO) and stimulated it in sterile tubes (Lonza, Walkersville, MD) at 37 °C in 5 %CO2 with LPS (10 ng/mL, E. coli 0111:B4, Sigma Aldrich, St.Louis, MO). Based on previous publications [16–18], we performed stimulation for 4 h for TNFα and interferon-gamma-inducible protein 10 (IP-10), and 24 h for IL-1β, IL-6, IL-8, IL-10, and IL-12p70. We removed supernatants and stored them at − 80 °C.

To measure the concentrations of plasma IL-6 and ex vivo production of TNFα and IP-10, we used commercially available ELISA kits from R&D Systems (Minneapolis, MN). We measured IL-1β, IL-6, IL-8, IL-10, and IL-12p70 concentrations using a cytometric bead array immunoassay (Human Inflammatory Kit, BD Bioscience, San Diego, CA).

2.3. Statistical analysis

We used the χ^2 test to compare proportions and the Mann–Whitney U test to compare continuous variables between groups.

To check collinearity between cytokines, we calculated a Pearson's correlation coefficient and Spearman's rho using threshold of 0.7 as an indicator of high collinearity.

To evaluate the ability of models to discriminate between patients with pneumonia and patients without pneumonia, we calculated areas under receiver operator curves (AUC). An AUC of 1 indicates perfect discrimination and 0.5 no discrimination.

To construct a multimarker score, we standardized cytokine distribution to a mean of 0 and standard deviation of 1. Then we performed univariate logistic regression. To a multimarker score, we included cytokines which had the P value below 0.1 in the multivariate logistic regression model. We defined the score as:

$$\text{score} = \;(\beta_A \times \text{cytokine}\;A)\; + \;(\beta_B \times \text{cytokine}\;B)\; + \;(\beta_C \times \text{cytokine}\;C), \;\text{etc.},$$

where β_A, β_B and β_C stand for regression β-coefficients for cytokine A, B and C, respectively from logistic regression model.

The calculations were performed using the program STATISTICA for Windows (version 12.5, Statsoft, Poland).

3. Results

We included 279 patients (median age: 69 years, interquartiles: 62 – 79; 41.6% women; median NIHSS score on admission: 10, interquartiles: 5–18). We diagnosed pneumonia in 20 patients (7.2%). The mean time to pneumonia was 3.5 days. Sixty-eight per cent of patients had pneumonia within first 3 days after admission.

The baseline characteristic of patients with pneumonia and those without pneumonia are showed in Table 1.

Patients with pneumonia were older, more often suffered from atrial fibrillation and ischemic heart disease, and had more severe neurological deficit on admission.

Patients with pneumonia had lower ex vivo release of TNFα, IL-1β, IL-12, IP-10, and a higher level of circulating IL-6 (Table 2).

We found no collinearity among studied cytokines. The following pairs of ex vivo synthesized cytokines had the highest Spearman's rank correlation coefficient: IL-10 and IL-8 (R = 0.65), IL-6 and IL-1β (R = 0.64), and TNFα and IP-10 (R = 0.62).

Table 1
The characteristics of patients with pneumonia and patients without pneumonia.

	Patients with pneumonia (N = 20)	Patients without pneumonia (N = 259)	P value
Age, median (IQs)	74.5 (66.5–84.0)	68.0 (61.0–79.0)	**0.03**
Female, n (%)	7 (35.0)	109 (42.1)	0.56
Hypertension, n (%)	16 (80.0%)	203 (78.4)	0.86
Diabetes mellitus, n (%)	9 (45.0)	71 (27.4)	0.09
Atrial fibrillation, n (%)	12 (60.0)	70 (27.0)	**< 0.01**
Ischemic heart disease, n (%)	7 (35.0)	35 (13.5)	**0.01**
Previous stroke, n (%)	4 (20.0%)	30 (11.6)	0.27
NIHSS score on admission, median (IQs)	17.0 (15.5–19.5)	9.0 (5.0–17.0)	**< 0.01**
White blood cells count, x10^3/μL, median (IQs)	11.9 (10.3–14.8)	8.3 (6.8–10.0)	< 0.01
Intravenous thrombolysis, n (%)	10 (50.0)	146 (56.4)	0.58
Mechanical thrombectomy, n (%)	5 (25.0)	66 (25.5)	0.96

IQs: interquartiles.

Table 2
Cytokine levels in patients with pneumonia and patients without pneumonia.

	Patients with pneumonia (N = 20)	Patients without pneumonia (N = 259)	P value
Ex vivo stimulation			
TNFα, pg/ml.	1561 (1189–2147)	2374 (1648–3381)	**< 0.01**
IP-10, pg/mL	117 (77–254)	391 (187–680)	**< 0.01**
IL-1β, pg/mL	858 (443–1549)	1525 (1073–2204)	**< 0.01**
IL-6, pg/mL	10,440 (5741–15372)	11,905 (8310–17177)	0.31
IL-12, pg/mL	0 (0 – 0.8)	4.4 (0.4 – 8.6)	**< 0.01**
IL-10, pg/mL	57 (30–75)	53 (33–76)	0.95
IL-8, pg/mL	2329 (1490–3330)	1767 (1105–2758)	0.08
Plasma			
IL-6, pg/mL	53.1 (16.4–69.0)	4.7 (2.2–14.6)	**< 0.01**

Data are shown as medians with interquartiles.

In the univariate logistic regression analysis, the odds ratios for pneumonia were: 0.27 (95%CI: 0.11 – 0.65, P < 0.01) for ex vivo released TNFα, 0.31 (95%CI: 0.14 – 0.67, P < 0.01) for IL-1β, 0.06 (95%CI: 0.01 – 0.33, P < 0.01) for IL-12, 0.11 (95%CI: 0.03 – 0.47, P < 0.01) for IP-10 and 2.02 (95%CI: 1.45 – 2.79, P < 0.01) for plasma IL-6.

In the multivariate logistic regression analysis, the cytokines with the P value below 0.1 were: ex vivo released IL-12 (OR: 0.09, 95%CI: 0.01 – 0.75, P = 0.02), IP-10 (OR: 0.32, 95%CI: 0.09 – 1.13, P = 0.07), and plasma IL-6 (OR: 1.70, 95%CI: 1.18 – 2.45, P < 0.01).

AUC values for studied cytokines are showed in Table 3. The discriminatory ability of the mulltimarker score composed of ex vivo synthesized IL-12, IP-10 and plasma IL-6 was better than individual cytokines. The multimarker score showed good sensitivity and specificity (0.89 and 0.88, respectively).

4. Discussion

We found that patients with SAP had lower ex vivo release of TNFα, IL-1β, IL-12, IP-10, and a higher level of circulating IL-6 than patients without SAP. The multimarker score composed of ex vivo synthesized IL-12, IP-10 and plasma IL-6 had better discriminatory properties than individual cytokines and displayed the good sensitivity and specificity for SAP.

Our biomarker reflects two post-stroke pathophysiological phenomena: immunodepression (reduced synthesis of IL-12 and IP-10) and systemic inflammation (increased plasma IL-6).

The clinical utility of ex vivo released cytokines for diagnosis of SAP has not been so far examined. A few studies showed that the reduced ex vivo TNFα production after blood cells stimulation with LPS predicted an increased risk of infections after stroke [12,13].

IL-12 is a pro-inflammatory cytokine produced by macrophages, dendritic cells and B cells in response to microbial pathogens [19,20]. IL-12 is a critical component of anti-bacterial host defence. It stimulates the production of interferon-gamma, a cytokine critical for the induction of Th1 cells. After intratracheal infection with Klebsiella pneumoniae, IL-12 is produced by alveolar macrophages. IL-12 neutralization impairs bacterial clearance from the lungs [21].

IP-10 (CXCL10) is a chemokine important for the innate immune response to bacterial infection by attracting CXCR3 cells (macrophages, dendritic cells, NK cells and activated T cells) to the site of inflammation [22]. Neutralization of IP-10 in mice who underwent intrapulmonary administration of Klebsiella pneumoniae resulted in reduced bacterial clearance from the lungs [23].

IL-6 is a pleiotropic cytokine. Rapid production of this cytokine contributes to host defence during infection [24]. The IL-6-driven hepatic synthesis of acute phase proteins has a critical role in the defence against Klebsiella pneumoniae and Streptococcus pneumoniae [24]. A few studies showed that circulating IL-6 could be a potential risk marker of SAP [8–10].

At this stage, clinical feasibility of our composite biomarker is questionable. The blood stimulation requires a laboratory dedicated to cell culture experiments. This barrier could be overcome by using commercially available tubes pre-loaded with cell culture media and an immune stimulant [25]. In this method, stimulation is performed in room air using a heating block. Minimal sample handling and a low amount of blood needed for stimulation might facilitate an application of this solution in the clinical practice.

Our study has several limitations. First, our results need confirmation in an independent and larger cohort. Second, we did not perform routinely microbiological studies. Thus, the etiology of pneumonia in our cohort remains unknown. Of note, cultures, especially these taken in early phase of SAP, are often negative or contaminated with oral flora [26]. The CDC criteria do not require the identification of a relevant pathogen [4]. Third, we did not repeat routinely a chest X-ray. The CDC criteria recommend that in patients with pre-existing cardiopulmonary disease, a chest X-ray should be repeated. Our approach reflects clinical practice in which second chest-X ray is performed if treatment of pneumonia is not satisfactory or there is a suspicious of other lung diseases. Fourth, in this study we were looking for a biomarker of early SAP (a biomarker of disease). The predictive value of our multimarker score as a risk marker for SAP needs to be determined. Fifth, the time from the onset of pneumonia symptoms to blood collection might modify a cytokine profile [27]. It remains unknown if our biomarker is useful for the diagnosis of pneumonia if a blood sample is taken in later phase of SAP.

In this study we focused only on cytokines and we did not take into account other inflammatory biomarkers. The results of one *meta*-analysis suggest modest predictive value of CRP for diagnosis of respiratory tract infections in stroke patients [28]. It would be interesting to investigate in future the discriminatory ability of a composite biomarker that includes selected ex vivo synthesized cytokines and circulating

Table 3
Discriminatory ability of studied cytokines.

	AUC	Cut-off value	Sensitivity	Specificity
IL-12	0.78 (0.70–0.87)	1.21 pg/ml.	0.80	0.71
IL-1β	0.73 (0.60–0.85)	999.74 pg/ml.	0.70	0.77
TNFα	0.73 (0.63–0.83)	1632.70 pg/mL	0.65	0.76
IP-10	0.79 (0.68–0.90)	149.27 pg/ml.	0.70	0.84
Plasma IL-6	0.85 (0.78–0.93)	6.86 pg/ml.	0.95	0.62
Multimarker score (IL-12 + IP-10 + plasma IL-6)*	0.90 (0.81–0.98)	2.39	0.89	0.88

* Cytokine values were standardized to a mean of 0 and standard deviation of 1.

markers of inflammation (for example, CRP, pentraxin-related protein 3, etc.) for SAP diagnosis.

In conclusion, our results suggest the potential utility of ex vivo synthesized cytokines in combination with plasma IL-6 for the diagnosis of SAP.

CRediT authorship contribution statement

Elżbieta Gradek-Kwinta: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft. **Mateusz Czyzycki:** Investigation, Writing - review & editing. **Kazimierz Weglarczyk:** Methodology, Investigation, Writing - review & editing. **Agnieszka Slowik:** Conceptualization, Writing - review & editing. **Maciej Siedlar:** Conceptualization, Writing - review & editing. **Tomasz Dziedzic:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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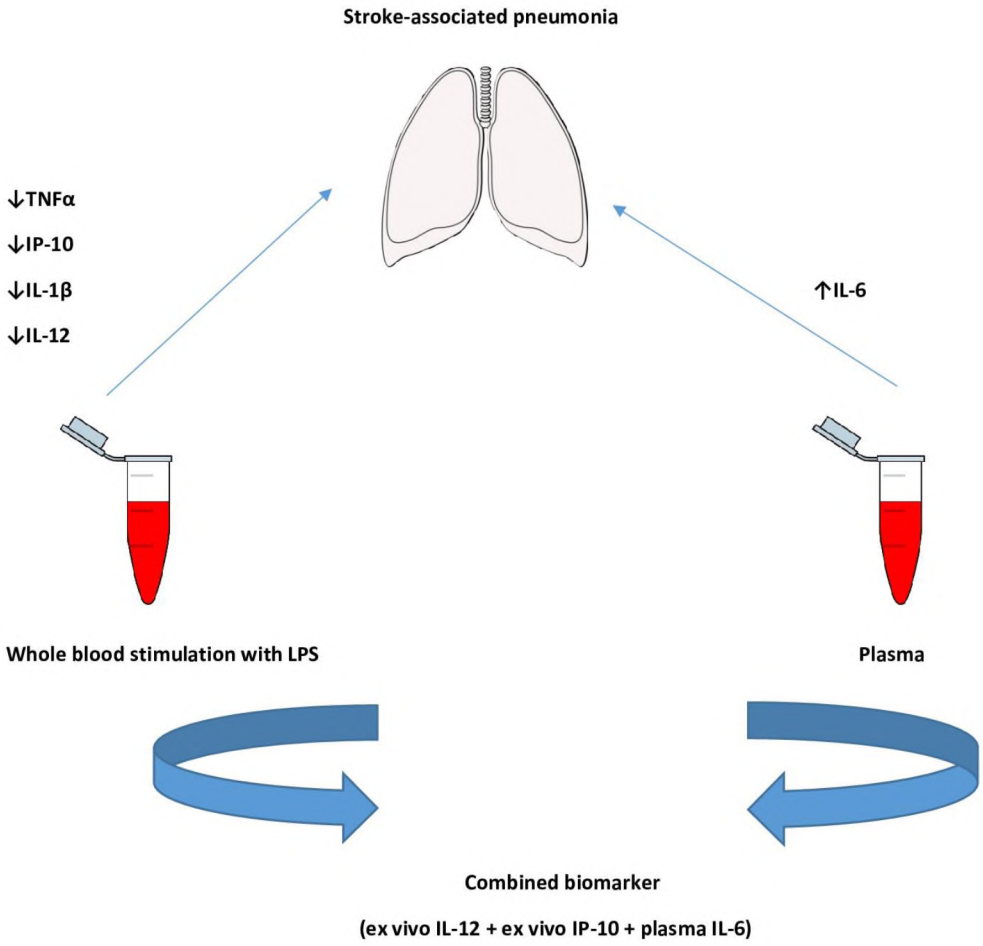
Stroke-associated pneumonia
↓TNFα
↓IP-10
↓IL-1β
↓IL-12
↑IL-6
Whole blood stimulation with LPS
Plasma
Combined biomarker
(ex vivo IL-12 + ex vivo IP-10 + plasma IL-6)

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Lipopolysaccharide binding protein and sCD14 as risk markers of stroke-associated pneumonia

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ABSTRACT

To determine the utility of lipopolysaccharide binding protein (LBP) and soluble CD14 (sCD14) as risk markers of stroke-associated pneumonia (SAP). We included 331 stroke patients. The plasma levels of LBP (median: 19.4 vs 15.3 μg/mL, $P < 0.01$) and sCD14 (median: 1.5 vs 1.4 μg/mL, $P = 0.04$) were elevated in SAP. In multivariate analysis, a higher level of LBP (OR: 1.09, 95%CI: 1.05–1.13), but not sCD14 (OR: 2.16, 0.94–4.97), was associated with SAP. The addition of LBP or sCD14 to the clinical model did not improve its discriminatory ability. Our results suggest the modest value of studied biomarkers for SAP prediction.

1. Introduction

Roughly 1 in 10 stroke patients experience pneumonia during the acute phase of stroke (Badve et al., 2019; Westendorp et al., 2011). The incidence of pneumonia is even higher in stroke patients hospitalized in intensive care units and approximates to 30% (Hannawi et al., 2013; Westendorp et al., 2011).

Stroke-associated pneumonia (SAP) is related to poor functional outcome, considerable mortality, and significant cost of medical care (Hannawi et al., 2013; Katzan et al., 2007, 2003).

The early diagnosis of SAP and identification of stroke patients who are at risk of a lower respiratory tract infection is important for the prevent of respiratory failure and sepsis. The early diagnosis of pneumonia complicating stroke remains challenging for several reasons (Mann et al., 1999). Clinical presentation of pneumonia in stroke patients may be non-specific. The Centres for Disease Control and Prevention (CDC) includes altered consciousness and fever in the diagnostic criteria for hospital-acquired pneumonia (Horan et al., 2008). These both signs might be caused directly by brain injury regardless of concomitant infections. Cough, one of the diagnostic symptoms of pneumonia, may be impaired in patients with hemispheric stroke (Ward et al., 2010). Even chest X-ray, an important component of diagnostic criteria, is of limited value because lung infiltrates might be not visible in the early stages of pneumonia. Furthermore, the presence of co-morbidities or mimickers such as cardiac failure, an acute exacerbation of chronic obstructive pulmonary disease, interstitial lung diseases or pulmonary embolism could make the diagnosis of SAP difficult in some patients (Shaddock, 2016). Finally, there is a lack of prospective studies validating diagnostic criteria for SAP. Taking into account these obstacles, there is an urgent need to develop novel biomarkers to help establishing the early diagnosis of SAP.

Lipopolysaccharide binding protein (LBP) and soluble clusters of differentiation 14 (sCD14) are acute-phase proteins that are important for endotoxin signalling (Bas et al., 2004; Schumann and Zweigner, 1999). LBP is synthesized by hepatic and gastrointestinal epithelial cells in the response to endotoxin (Schumann and Latz, 1999). LBP plays an important role in the innate immune response to both Gram-negative and Gram-positive infections (Zweigner et al., 2006). Soluble CD14 is secreted from the liver or derived by enzymatic cleavage of myeloid cells membrane-bound CD14 (Bazil and Strominger, 1991; Tapping and Tobias, 1999). LBP directly binds to endotoxin and facilitates its transport to CD14 receptor. CD14, a pattern recognition receptor, promotes LPS binding to toll-like receptors to activate production of pro-inflammatory cytokines. Soluble CD14 enables responses to lipopolysaccharide by cells that do not express CD14 (Frey et al., 1992).

The blood levels of LBP and sCD14 rise during ischemic stroke (Hoffmann et al., 2017; Klimiec et al., 2016). In the study that measured plasma LBP levels on days 1–4 after stroke, the highest LBP level was seen in patients with SAP on day 4 (Hoffmann et al., 2017). Elevated blood levels of LBP and sCD14 were associated with an increased risk of

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death and unfavourable short-term functional outcome (Klimiec et al., 2018; Mengel et al., 2019). Moreover, in ischemic stroke patients without infections, plasma LBP moderately correlated with the acute lesion volume (Hotter et al., 2019).

Several studies showed elevated LBP in the blood of patients with severe infections or sepsis due to Gram-negative or Gram-positive infections(Blairon et al., 2003; Gaïni et al., 2007; Opal et al., 1999). Zobel et al. found an increased serum level of LBP in patients with severe course of community-acquired pneumonia (Zobel et al., 2012). Also stroke patients with infections had an elevated level of circulating LBP (Hoffmann et al., 2017; Wartenberg et al., 2011; Worthmann et al., 2015). In elderly persons, higher sCD14 levels were associated with an increased risk of infection during hospitalization (Paillaud et al., 2018).

To the best of our knowledge, sCD14 has not been studied as a risk marker of SAP. In this study we aimed to determine the clinical utility of plasma LBP and sCD14 for prediction of SAP.

2. Material and methods

2.1. Patient selection and clinical assessment

We retrospectively analysed the data of patients who participated in the prospective study on the relationship between endotoxemia and stroke outcome (Klimiec et al., 2018). This study was conducted in the Department of Neurology, University Hospital, Krakow, Poland, between September 2014 and March 2016. The inclusion criteria for this study were: (1) ischemic stroke; (2) time from the onset of stroke symptoms to admission <24 h; and (3) pre-stroke modified Rankin Scale score 0–2 (independent of daily activities). We excluded patients with chronic inflammatory, autoimmune or cancerous diseases. Each patients gave an informed consent. The Bioethics Committee of Jagiellonian University approved the study protocol. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

We assessed pneumonia symptoms and signs during the first 7 days of hospitalization. A treating physician made the final diagnosis of SAP if acute respiratory symptoms, signs, and compatible infiltrates on a chest X-ray were present. The CDC criteria of pneumonia (Horan et al., 2008) served as guidelines, but the fulfilment of these criteria was not mandatory for a final diagnosis. In particular, in patients with pre-existing cardiopulmonary disease, the repeated chest X-ray showing new infiltrates was not required to fulfil the criteria of pneumonia.

We assessed the neurological deficit on admission using the National Institute of Health Stroke Scale (NIHSS) (Lyden et al., 1994). Higher scores indicate greater impairment and more severe stroke.

2.2. Laboratory assays

We took blood samples on day 1 (within 24 h after the stroke onset). We used commercially available ELISA kits from Hycult Biotech (Netherlands) and R&D Systems (Minneapolis, MN) to measure plasma LBP and sCD14, respectively. The detection limit was 4.4 ng/mL for LBP and 125 mg/mL for sCD14. The intra-assay coefficient of variability was below 5% for LBP and below 6% for sCD14. The inter-assay coefficient of variability was below 10% for both LBP and sCD14.

2.3. Statistical analysis

We used the χ^2 test to compare proportions and the Mann–Whitney U test to compare continuous variables between groups. We used logistic regression to determine the predictors of pneumonia. Variables with P value below 0.05 in the univariate analysis were included into multivariate models.

To evaluate the ability of models to discriminate between patients with pneumonia and patients without pneumonia, we calculated areas under receiver operator curves (AUC). An AUC of 1 indicates perfect discrimination and 0.5 no discrimination.

The calculations were performed using the program STATISTICA for Windows (version 12.5, Statsoft, Poland).

3. Results

We included 331 patients (median age: 71 years, interquartiles: 63–81; 47.4% women; median NIHSS score on admission: 6, interquartiles: 3–15). We diagnosed pneumonia in 43 patients (13.0%). Twenty-seven patients with SAP (62.8%) displayed symptoms and/or signs of a lower respiratory tract infection within 48 h after admission.

The baseline characteristic of patients with pneumonia and those without pneumonia are shown in Table 1.

Patients with pneumonia were older, more often suffered from atrial fibrillation, and had more severe neurological deficit on admission.

Patients with pneumonia had a higher level of plasma LBP and sCD14. The ratio of LBP to sCD4 was also higher in patients with pneumonia compared with patients without pneumonia. LPS activity did not differ between groups.

In patients without infections, both LBP and sCD14 levels correlated with NIHSS score on admission ($R = 0.26$, $P < 0.01$ and $R = 0.16$, $P = 0.02$, respectively).

In the univariate logistic regression analysis, the odds ratios for SAP were: 1.09 (95%CI: 1.05–1.13, $P < 0.01$) for LBP, 2.76 (95%CI: 1.31–5.82, $P < 0.01$) for sCD14, and 1.19 (95%CI: 1.10–1.29, $P < 0.01$) for the ratio of LBP to sCD14. Other predictors of pneumonia were: age (OR: 1.6, 95%CI: 1.03–1.09, $P < 0.01$), NIHSS score on admission (OR: 1.13, 95%CI: 1.08–1.18, $P < 0.01$), and atrial fibrillation (OR: 2.17, 95% CI: 1.08–4.34, $P = 0.03$).

In the multivariate logistic regression analysis adjusted for age, NIHSS score, and atrial fibrillation, a higher level of LBP (OR: 1.09, 95% CI: 1.05–1.13, $P < 0.01$) and a higher ratio of LBP to sCD14 (OR: 1.18, 95%CI: 1.08–1.29, $P < 0.01$) were associated with SAP. Soluble CD14 was not an independent predictor of SAP in the multivariate model (OR: 2.16, 0.94–4.97, $P = 0.07$).

AUC values as well as sensitivity and specificity values for studied biomarkers are shown in Table 2.

The AUC for clinical model of SAP that included age, NIHSS score, and atrial fibrillation was 0.80 (95%: 0.72–0.86). The addition of LBP to the clinical model did not significantly improve discriminatory ability of

Table 1
The characteristics of patients with pneumonia and patients without pneumonia.

	Patients with pneumonia (N = 43)	Patients without pneumonia (N = 288)	P value
Age, median (IQs)	81 (69–86)	70 (62–80)	**<0.01**
Female, n (%)	22 (51.2)	135 (46.9)	0.60
Hypertension, n (%)	32 (74.4)	209 (72.6)	0.80
Diabetes mellitus, n (%)	13 (30.2)	76 (26.4)	0.59
Atrial fibrillation, n (%)	15 (34.9)	57 (19.8)	**0.02**
Ischemic heart disease, n (%)	3 (7.0)	27 (9.4)	0.60
Previous stroke, n (%)	9 (21.4)	42 (14.6)	0.27
NIHSS score on admission, median (IQs)	16 (7–20)	5 (2.5–13)	**<0.01**
Intravenous thrombolysis, n (%)	14 (51.2)	135 (46.9)	0.60
Mechanical thrombectomy, n (%)	3 (7.0)	17 (5.9)	0.78
LBP (μg/mL), median (IQs)	19.4 (14.6–29.6)	15.3 (12.6–19.4)	**<0.01**
sCD14 (μg/mL), median (IQs)	1.5 (1.3–1.8)	1.4 (1.2–1.7)	**0.04**
LBP/sCD14 ratio, median (IQs)	13.3 (10.1–16.6)	11.2 (9.0–13.2)	**<0.01**
LPS activity (EU/mL), median (IQs)	0.52 (0.37–0.64)	0.56 (0.44–0.67)	0.33

IQs: interquartiles.

Table 2
Discriminatory abilities of studied biomarkers.

	AUC	Cut-off value	Sensitivity	Specificity
LBP	0.67 (95CI: 0.58–0.76)	18.5 μg/mL	0.58	0.70
sCD14	0.60 (95%CI: 0.52–0.68)	1.27 μg/mL	0.98	0.30
LBP/sCD14 ratio	0.65 (95CI: 0.55–0.74)	12.8	0.56	0.73

this model (AUC: 0.84, 95%CI: 0.78–0.90, P = 0.11). Similarly, neither the addition of sCD14 (AUC: 0.81. 95%CI: 0.78–0.84, P = 0.15) nor the ratio of LBP to sCD14 (AUC: 0.82, 95%CI: 0.76–0.89, P = 0.24) to the clinical model led to improvement of predictive ability of the model.

4. Discussion

There are three main findings of our study. First, plasma levels of LBP and sCD14 were higher in patients with SAP compared with patients without SAP. Second, in the multivariate analysis adjusted for the important clinical predictors of pneumonia such as age and stroke severity, a higher plasma level of LBP, but not sCD14, was associated with SAP. Third, the addition of LBP or sCD14 to the clinical model did not improve predictive properties of this model for SAP.

In our study, both LBP and sCD14 had modest accuracy for prediction SAP. The AUC of these biomarkers was lower than the AUC of the clinical model. Moreover, the incorporation of these biomarkers to the clinical model did not result in better discrimination between patients with SAP and patients without SAP.

Our results are in line with the results of two meta-analyses that showed that circulating LBP seemed to have a moderate to low diagnostic accuracy for the diagnosis of sepsis and could not be recommended for the daily clinical practice (Chen et al., 2016; Liu et al., 2016).

A plasma level of LBP and sCD14 rises during ischemic stroke (Klimiec et al., 2016). Since LBP and sCD14 are the acute-phase proteins (Bas et al., 2004; Schumann and Zweigner, 1999), their blood concentration depends on the extent of the systemic inflammatory response to both infectious and non-infectious stimuli. It could make that these biomarkers do not aid in discriminating an infection from inflammation.

We cannot exclude the possibility that the predictive value of LBP and sCD14 might be better in the selected groups of stroke patients (e.g. those with Gram-negative infections). CRP is a good example of biomarker that has a limited value for the diagnosis of SAP, but could be useful in specific clinical situations. Although CRP has modest predictive value for the diagnosis of respiratory tract infections in stroke patients (Bustamante et al., 2017), it could increase diagnostic accuracy of SAP in patients with severe stroke (Warusevitane et al., 2016) or afebrile patients (Kalra et al., 2019).

Our findings corroborate the results of other studies showing the limited value of inflammatory and stress markers for predicting SAP. Although several blood-derived markers such as procalcitonin, proadrenomedullin, proatrionatriuretic peptide, copeptin and proendothelin were elevated in patients with SAP, these markers only slightly improved prediction of SAP over clinical parameters (Hotter et al., 2021, 2020).

Our study has several limitations. First, the rate of pneumonia in our study was higher than reported in previous systematic reviews (≈10%) (Badve et al., 2019; Westendorp et al., 2011). The higher frequency of pneumonia in our cohort could be due to diagnostic approach used by us. We based the diagnosis of SAP on clinical judgement of a treating physician rather than on strict diagnostic criteria. It could result in overdiagnosis of pneumonia, especially in patients with severe stroke. Of note, the frequency of pneumonia in our patients was similar to the rate of SAP reported by one of systemic reviews which was aimed to identify existing diagnostic approaches to SAP (14.3%) (Mann et al., 1999). Second, since the majority of patients with SAP had symptoms of infection within 48 h after admission, we are not able to differentiate whether the investigated biomarkers might predict a risk of SAP (a risk marker) or detect an early infection (a marker of disease). Third, we did not assess dysphagia, an eminent predictor of SAP (Eltringham et al., 2018). Information about dysphagia is not necessary to make a diagnosis of SAP, but could be important for selecting a subgroup of patients with aspiration pneumonia and improve the predictive value of a clinical model. Moreover, the airway clearance ability, an important pathophysiological factor for pneumonia, has not been assessed. Finally, we measured plasma levels of LBP and sCD14 only once. We cannot exclude the possibility that serial measurements of these biomarkers could be more useful for selecting patients with SAP.

In conclusion, our results suggest the modest value of LBP and sCD14 for prediction of SAP. These proteins could not be recommended as biomarkers of SAP for the clinical practice.

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Declaration of interest

None.

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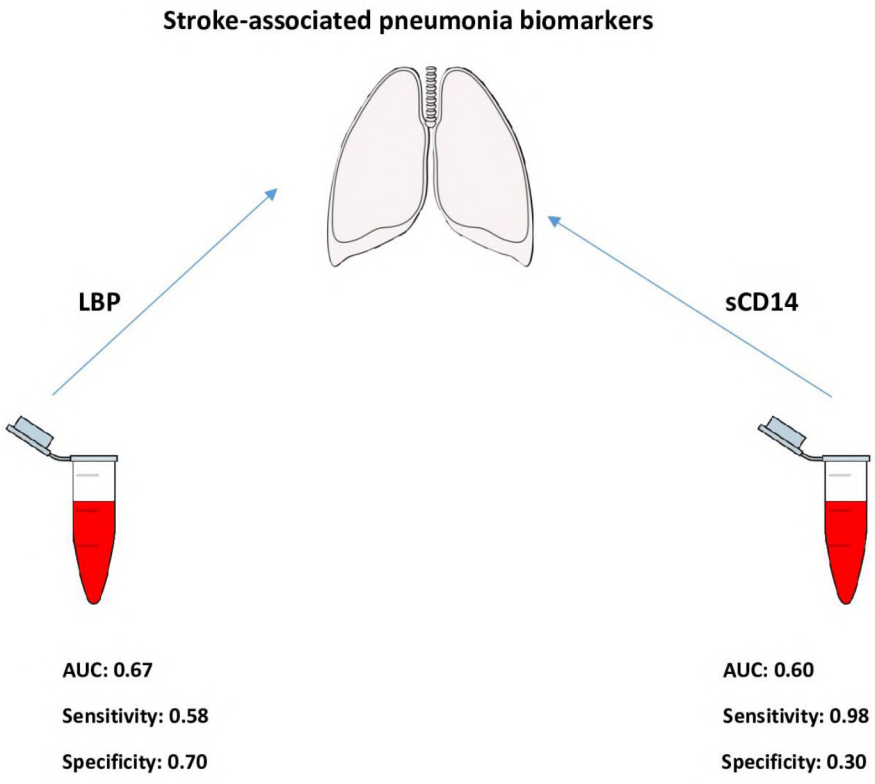
Stroke-associated pneumonia biomarkers
LBP
sCD14
AUC: 0.67
Sensitivity: 0.58
Specificity: 0.70
AUC: 0.60
Sensitivity: 0.98
Specificity: 0.30

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ORIGINAL ARTICLE

The use of anticholinergic medication is associated with an increased risk of stroke-associated pneumonia

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Abstract

Background Pneumonia is a frequent medical complication after stroke. A few studies showed that the use of anticholinergic medication is associated with a higher risk of community acquired pneumonia in the elderly. We aimed to determine if there is any association between anticholinergic medication used before stroke and stroke-associated pneumonia (SAP).

Methods We analysed prospectively collected data of 675 patients with acute stroke (mean age 71.4 ± 13.3; 53.1% female). We used the Anticholinergic Drug Scale to assess anticholinergic exposure during a month preceding stroke onset.

Results We diagnosed SAP in 14.7% of patients. The use of anticholinergic medication was associated with an elevated risk of SAP (OR 2.56, 95% CI 1.59–4.11, $P < 0.01$) in univariate analysis. This association remained significant in multivariable analysis adjusted for age, stroke severity, atrial fibrillation, previous myocardial infarction and respiratory tract diseases (OR 2.06, 95% CI 1.01–4.22, $P = 0.04$).

Conclusions The use of anticholinergic medication before stroke is associated with an increased risk of SAP.

Keywords Pneumonia · Stroke · Anticholinergic · Drugs

Introduction

Stroke-associated pneumonia (SAP) occurs in about 12–14% of patients [1, 2]. SAP is associated with an increased risk of poor outcome and death [3, 4].

Numerous clinical factors such as older age, dysphagia, male sex, severe stroke, decreased conscious level, coronary artery disease, congestive cardiac failure, atrial fibrillation, pre-admission dependency, current smoking, heavy alcohol consumption and chronic obstructive pulmonary disease may predispose individuals to SAP [5, 6]. Better understanding of predisposing factors is important for prevention and prediction of SAP.

Anticholinergic medication is widely prescribed for older people for various medical conditions including pain, incontinence, depression or insomnia [7]. A few studies showed that the use of anticholinergic medication is associated with a higher risk of community acquired pneumonia in the elderly [8–10]. The aim of our study was to determine if the use of anticholinergic medication is associated with a risk of SAP.

Materials and methods

We analysed the data that were collected during the PRospective Observational POLIsh Study on post-stroke delirium (PROPOLIS). PROPOLIS was a prospective, single center study conducted in Department of Neurology, University Hospital, Krakow, Poland [11]. Inclusion criteria to the PROPOLIS were: (1) acute stroke (ischemic stroke, transient ischemic attack (TIA) or intracerebral hemorrhage); (2) age ≥ 18 years; (3) admission to the hospital within 48 h from symptoms onset; (4) informed consent of a patient or his/legal legal guardian; (5) Polish as a native language. The exclusion criteria were: coma, alcohol withdrawal syndrome, cerebral venous thrombosis, vasculitis and diseases with life expectancy ≤ 1 year. The Bioethics Committee of Jagiellonian University approved the study's protocol. Each patient or his/her legal guardian gave informed consent.

The final diagnosis of SAP was made by a treating physician if acute respiratory symptoms, signs, and compatible infiltrates on a chest X-ray were present. The CDC criteria

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of pneumonia [12] served as guidelines, but the fulfilment of these criteria was not mandatory for a final diagnosis. In particular, in patients with pre-existing cardiopulmonary disease, the repeated chest X-ray showing new infiltrates was not required to fulfil the criteria of pneumonia.

We used the Anticholinergic Drug Scale (ADS) [13] to assess anticholinergic exposure during a month preceding stroke onset. ADS is a well-established scale which correlates with serum anticholinergic activity. It classifies drugs into four different levels: no anticholinergic effect (level 0), weak anticholinergic effect (level 1), moderate anticholinergic effect (level 2) and strong anticholinergic effect (level 3). We used the list of anticholinergic medications proposed by Naples et al. [14] (Electronic Supplementary Material (ESM) Appendix 1). We calculated anticholinergic burden by summing up individual anticholinergic scores.

We evaluated neurological deficit on admission using the National Institute of Health Stroke Scale (NIHSS) [15].

We used the respiratory subscale of the Cumulative Illness Rating Scale (CIRS) [16] to assess respiratory comorbidities. The respiratory subscale of CIRS includes the following categories: no problem (score 0); recurrent episodes of acute bronchitis/currently treated asthma with prn inhalers/cigarette smoker > 10 but < 20 pack years (score 1); X-ray evidence of COPD/requires daily theophylline or inhalers/treated for pneumonia two or more times in the past 5 years/smoked 20–40 pack years (score 2); limited ambulation secondary to limited respiratory capacity/requires oral steroids for lung disease/smoked > 40 pack years (score 3); requires supplemental oxygen/at least one episode of respiratory failure requiring ventilation/any lung cancer (score 4).

We used the χ^2 test to compare proportions and the Mann–Whitney's test to compare continuous variables between groups. Continuous variables are shown as means with standard deviations. We used logistic regression to determine independent predictors of SAP. Relevant variables that met a threshold of 0.01 in univariate analysis were included a multivariable analysis. The identification of the most strongly associated variables with SAP was confirmed by the use of backward elimination model with a retention value of $P = 0.05$. Analyses were performed with STATISTICA for Windows software (version 13.3, TIBCO Software Inc., Poland).

Results

The PROPOLIS cohort included 750 patients (mean age 71.7 ± 13.1; mean NIHSS score on admission: 8.5 ± 7.3; 53.1% female). Information about pre-stroke anticholinergic medication was available for 657 patients (mean age 71.4 ± 13.3; mean NIHSS score on admission: 8.2 ± 7.2; 53.1% female) and these patients were included into analysis.

SAP was diagnosed in 14.7% of patients. The baseline characteristics of patients with SAP and without SAP are shown in Table 1.

Compared with patients without SAP, patients with SAP were older, more often suffered from atrial fibrillation, previous myocardial infarction and respiratory diseases and had greater neurological deficit on admission. Patients with SAP had higher total scores of ADS.

In the univariate analysis, older age (OR 1.05, 95% CI 1.03–1.07, $P < 0.01$), higher NIHSS score (OR 1.13, 95% CI 1.10–1.17, $P < 0.01$), atrial fibrillation (OR 2.60, 95% CI 1.65–4.12, $P < 0.01$), previous myocardial infarction (OR 2.38, 95% CI 1.40–4.03, $P < 0.01$), a CIRS respiratory

Table 1 Baseline characteristics of patients with and without pneumonia

	Patients with pneumonia ($N = 97$)	Patients without pneumonia ($N = 560$)	P value
Age, mean (SD)	77.2 (10.8)	70.4 (13.5)	< 0.01
Female, *n* (%)	52 (53.6)	297 (53.0)	0.92
Ischemic stroke/TIA, *n* (%)	87 (89.7)	525 (93.7)	0.14
Intracerebral hemorrhage, n (%)	10 (10.3)	35 (6.3)	
NIHSS score on admission, mean (SD)	14.1 (7.0)	7.2 (6.8)	< 0.01
Hypertension, *n* (%)	74 (76.3)	386 (68.9)	0.14
Diabetes mellitus, *n* (%)	33 (34.0)	146 (26.1)	0.11
Atrial fibrillation, *n* (%)	38 (39.2)	111 (19.8)	< 0.01
Myocardial infarction, *n* (%)	24 (24.7)	68 (12.1)	< 0.01
CIRS-respiratory, mean (SD)	2.1 (1.3)	0.75 (0.9)	< 0.01
CIRS-respiratory > 2, *n* (%)	61 (62.9)	14 (2.5)	< 0.01
Total ADS score, mean (SD)	0.5 (1.0)	0.2 (0.7)	< 0.01
ADS score > 0, *n* (%)	33 (34.0)	94 (16.8)	< 0.01

ADS Anticholinergic Drug Scale, *CIRS* Cumulative Illness Rating Scale, *NIHSS* National Institute of Health Stroke Scale

subscale score > 2 (OR 66.08, 95% CI 33.71–129.52, $P < 0.01$) and a ADS score > 0 (OR 2.56, 95% CI 1.59–4.11, $P < 0.01$) were independent predictors of SAP.

In the multivariable analysis, independent predictors of SAP remained a NIHSS score (OR 1.12, 95% CI 1.08–1.17, $P < 0.01$), atrial fibrillation (OR 2.07, 95% CI 1.04–4.15, $P = 0.04$), a CIRS respiratory subscale score > 2 (OR 66.55, 95% CI 31.46–140.78, $P < 0.01$) and a ADS score > 0 (OR 2.06, 95% CI 1.01–4.22, $P = 0.04$).

There was a significant association between a total ADS score and risk of SAP in univariate (OR 1.44, 95% CI 1.14–1.82, $P < 0.01$), but not in multivariate analysis (OR 1.34, 95% CI 0.97–1.84, $P = 0.07$).

Discussion

In our study, the use of anticholinergic medication was associated with an increased risk of SAP. This association was independent from age, stroke severity, atrial fibrillation, previous myocardial infarction and respiratory tract diseases.

A few population-based, case–control studies demonstrated the relationship between anticholinergic drugs and community acquired pneumonia in older people [8–10]. In one of them, overall use of anticholinergic medication was associated with a 65% higher risk of pneumonia in persons aged 65 or older [9]. An association between anticholinergic exposure and an increased risk of pneumonia was also observed in patients with Alzheimer's disease [17]. Moreover, anticholinergic load during hospital stay was related to aspiration pneumonia in geriatric patients [18].

Published studies yielded inconsistent results regarding the relationship between potency of anticholinergic drugs and risk of pneumonia. In the study of Paul et al., high-potency anticholinergic medication had similar association with pneumonia risk as low-potency medication [8]. This may suggest that existing scales used to measure anticholinergic potency of drugs are not biologically relevant. Alternatively, even low potency anticholinergic drugs might convey risk for pneumonia. In other studies, the use of drugs with lower anticholinergic effects, but not those with higher effects, was related to pneumonia [9, 17]. In our study, an association between a total ADS score and SAP was significant in univariate, but not in multivariate analysis.

Anticholinergic medication could be linked to a risk of pneumonia in several ways [9, 19]. Dryness of mouth triggered by anticholinergic drugs may lead to dysphagia [20]. Other anticholinergic effects such as reduced antimicrobial activity of saliva, low levels of mucosal secretion and impaired mucociliary transport can predispose to bacterial growth in the respiratory tract. In addition, lowered esophageal sphincter pressure and sedation might facilitate aspiration.

Cholinergic transmission plays an important role in regulation of both innate and adaptive immunity [21, 22]. The cholinergic anti-inflammatory pathway constrains innate immune responses. This mechanism is dependent on the alpha7 subunit of the nicotinic acetylcholine receptor (α7nAchR), which inhibits NF-kappaB nuclear translocation and suppresses cytokine release by monocytes and macrophages [21, 23]. The α7nAchR is also expressed by alveolar macrophages and lung epithelial cells [24, 25]. Immunosuppression mediated by the cholinergic anti-inflammatory pathway could exert either beneficial or negative effect on lung. In experimental models of sterile lung inflammation, for example ventilator-induced lung injury, stimulation of α7nAchR pathway attenuated acute lung damage [26]. In contrast, activation of this pathway was deleterious in experimental bacterial pneumonia [27]. Immunosuppression induced by cholinergic anti-inflammatory pathway may inhibit first-line antimicrobial response and increase susceptibility to infections. In animal models of cerebral ischemia, activation of α7nAchR pathway resulted in more severe lung injury [25, 28]. Unfortunately, ADS and other anticholinergic drug scales do not capture nicotinic effects of drugs. Thus, they are not appropriate to investigate the potential immunomodulatory effects of anticholinergic medications.

There are several limitations of our study. First, the diagnosis of SAP was based on clinical judgement of a treating physician rather than strict diagnostic criteria what could lead to over-diagnosis of pneumonia. Second, we are not able to rule out possibility that the observed association reflects residual confounding related to comorbidities. For example, we did not adjust our model for dysphagia because this sign was not routinely examined in patients participating in the PROPOLIS study. However, taking into account the relationship between anticholinergic medication and problems with swallowing, dysphagia should be considered as a mediating variable rather than a confounding variable in statistical analysis [20]. Third, we did not gather in our database information about individual medication. Consequently, we are not able to analyze which anticholinergic drugs were particularly associated with SAP. We also did not consider drug doses and the duration of drug administration.

In conclusions, our results suggest that the use of anticholinergic medication before stroke is associated with an increased risk of SAP. Clinicians should be aware of side-effects of these drugs and monitor their use in acute stroke patients.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40520-022-02123-x.

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Data availability The datasets analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethics approval Approval was obtained from the Bioethic Committeee of Jagiellonian University. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Consent to participate Informed consent was obtained from participants or their legal guardians.

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Supplementary Material.

Appendix 1. Medications for the Anticholinergic Drug Scale.

Level 1

Amantadine

Aminophylline

Azathioprine

Azelastine nasal

Barberry

Bromocriptine

Captopril

Chlordiazepoxide

Chloroquine

Chlorthalidone

Citalopram

Clonazepam

Clorazepate

Cycloserine

Cyclosporine

Dexamethasone

Digitoxin

Ephedrine

Ergotamine

Estazolam

Escitalopram

Famotidine

Fentanyl (topical)

Fluphenazine

Fluoxetine

Flurazepam

Fluvoxamine

Gentamicin

Hydralazine

Hydrocortisone

Isosorbide

Lithium

Loratadine

Lorazepam

Methadone

Methylprednisolone

Mirtazapine

Nalbuphine

Nizatidine

Oxycodone

Perphenazine

Phenelzine

Phenobarbital

Prednisolone

Prednisone

Procainamide

Prochlorperazine

Quinidine

Sertraline

Temazepam

Theophylline

Tiagabine

Tobramycin

Tramadol

Tranylcypromine

Triamcinolone

Triamterene

Triazolam

Valproic acid

Level 2

Cimetidine

Cyclobenzaprine

Disopyramide

Loxapine

Methotrimeprazine

Paroxetine

Pimozide

Quetiapine

Ranitidine

Trifluoperazine

Triflupromazine

Level 3

Amitriptyline

Amoxapine

Atropine

Azatadine

Benztropine

Brompheniramine

Carbinoxamine

Chlorpheniramine

Chlorpromazine

Chlorprothixene

Clemastine

Clidinium

Clomipramine

Clozapine

Cyproheptadine

Darifenacin

Desipramine

Dexbrompheniramine

Dexchlorpheniramine

Dicyclomine

Dimenhydrinate

Diphenhydramine

Doxepin

Doxylamine

Fesoterodine

Flavoxate

Glycopyrrolate

Homatropine

Hydroxyzine

Hyoscyamine

Imipramine

Meclizine

Methscopolamine

Nortriptyline

Olanzapine

Orphenadrine

Oxybutynin

Phenindamine

Pheniramine

Phenyltoloxamine

Promethazine

Propantheline

Protriptyline

Pyrilamine

Scopolamine

Solifenacin

Thioridazine

Tolterodine

Trihexyphenidyl

Trimethobenzamide

Trimipramine

Triprolidine

Trospium

IX. STRESZCZENIE

Celem pierwszej pracy było określenie profilu syntetyzowanych ex vivo cytokin u pacjentów z zapaleniem płuc w przebiegu udaru mózgu (ang. stroke-associated pnuemonia, SAP) oraz ocena przydatności klinicznej pojedynczych cytokin i ich kombinacji jako biomarkerów SAP. Do badania włączono 279 pacjentów z udarem niedokrwienny mózgu (mediana wieku: 69 lat; 41.6% kobiet). Próbki krwi pobrane w 3 dobie od momentu udaru mózgu stymulowano ex vivo za pomocą lipopolisacharydu (LPS), a następnie mierzono stężenie uwalnianych do nadsącza cytokin (TNFα, IP-10, IL-1β, IL-6, IL-8, IL-10, IL-12p70). Ponadto, oznaczono w osoczu stężenie interleukiny-6 (IL-6) jako markera systemowej reakcji zapalnej. W ciągu pierwszych 5 dni od momentu udaru, SAP wystąpiło u 7.2% pacjentów. Pacjenci z SAP byli starsi, częściej chorowali na migotanie przedsionków i chorobę niedokrwienną serca oraz mieli większy deficyt neurologiczny przy przyjęciu. U pacjentów z SAP stwierdzono zmniejszone wydzielanie TNFα, IL-1β, IL-12 i IP-10 w odpowiedzi na stymulację krwi za pomocą LPS oraz podwyższone stężenie IL-6 w osoczu w porównaniu do pacjentów bez SAP. Wskaźnik uwzgledniający stężenie produkowanych ex vivo IL-12 i IP-10 oraz osoczowej IL-6 wykazywał bardzo dobre własności dyskryminacyjne w odniesieniu do SAP (pole pod krzywą [area under curve, AUC]: 0.90; czułość: 0.89, swoistość: 0.88). Zdolność dyskryminacyjna tego wskaźnika była lepsza niż pojedynczych cytokin. Wyniki badania sugerują potencjalną przydatność syntetyzowanych ex vivo cytokin jako biomarkera SAP.

Celem drugiej pracy było określenie przydatności klinicznej białka wiążącego lipopolisacharyd (lipopolysaccharide binding protein, LBP) i rozpuszczalnego CD14 (sCD14) jako markerów ryzyka SAP. Do badania włączono 331 pacjentów z udarem niedokrwiennym mózgu (mediana wieku: 71 lat; 47.4% kobiet). Próbki krwi do badania pobrano w ciągu pierwszych 24 godzin od momentu wystąpienia objawów udaru. SAP zdiagnozowano u 13% pacjentów. Stężenia LBP (mediana: 19.4 vs 15.3 μg/ml, P< 0.01) i sCD14 (mediana: 1.5 vs 1.4 μg/ml, P=0.04) w osoczu było wyższe u pacjentów z SAP w porównaniu do pacjentów bez SAP. W analizie jednoczynnikowej regresji logistycznej, wyższe stężenie LBP (OR: 1.09, 95%CI: 1.05 – 1.13, P<0.01) i sCD14 (OR: 2.76, 95%CI: 1.31 – 5.82, P<0.01) związane było z podwyższonym ryzykiem SAP. W analizie wieloczynnikowej uwzględniającej wiek, stopień deficytu neurologicznego oraz migotanie przedsionków, wyższe stężenie LBP (OR: 1.09, 95%CI: 1.05–1.13, P<0.01), ale nie sCD14 (OR: 2.16, 0.94–4.97, P=0.07), było czynnikiem predykcyjnym SAP.

AUC dla modelu klinicznego utworzonego z takich zmiennych, jak wiek, ilość punktów w skali NIHSS (National Institute of Health Stroke Scale) oraz migotanie przedsionków wynosiło 0.80. Zdolności dyskryminacyjne LBP (AUC: 0.67) i sCD14 (AUC: 0.60) były gorsze niż modelu klinicznego. Ponadto, dodanie LBP (AUC: 0.84, P=0.11) lub sCD14 (AUC: 0.81, P=0.15) do modelu klinicznego nie poprawiało jego zdolności predykcyjnych. Wyniki badania sugerują, że ani LBP, ani sCD14 nie są przydatne w codziennej praktyce jako biomarkery predykcyjne SAP ze względu na ograniczone zdolności dyskryminacyjne.

Celem trzeciej pracy było ustalenie, czy istnieje związek pomiędzy zażywaniem przed udarem leków antycholinergicznych a wystąpieniem SAP. Przeanalizowano prospektywnie zebrane dane 675 pacjentów z ostrym udarem mózgu (mediana wieku 71.4; 53.1% kobiet). Do oceny ekspozycji na leki antycholinergiczne w okresie 1 miesiąca poprzedzającego udar mózgu wykorzystano skalę ADS (Anticholinergic Drug Scale). ADS to dobrze ugruntowana skala, która koreluje z aktywnością antycholinergiczną surowicy. SAP zdiagnozowano u 14.7% pacjentów. W analizie jednoczynnikowej, stosowanie leków antycholinergicznych przed udarem mózgu wiązało się z podwyższonym ryzykiem SAP (OR: 2.56; 95% CI: 1.59–4.11; P<0.01). Związek ten pozostał istotny w analizie wieloczynnikowej skorygowanej o wiek, ciężkość udaru, migotanie przedsionków, przebyty zawał mięśnia sercowego i choroby układu oddechowego (OR: 2.06, 95% CI: 1.01–4.22, P=0.04). Wyniki badania sugerują, że stosowanie leków antycholinergicznych przed udarem wiąże się ze zwiększonym ryzykiem SAP.

X. SUMMARY

The aim of the first study was to determine a profile of ex vivo released cytokines in patients with stroke-associated pneumonia (SAP) and to assess the clinical utility of individual cytokines and their combination as a biomarker of SAP. Two hundred seventy-nine patients with ischemic stroke were included into this study (median age: 69 years; 41.6% female). Blood samples were collected on the third day after the onset of stroke and stimulated ex vivo with lipopolysaccharide (LPS). LPS-induced cytokine concentrations (TNFα, IP-10, IL-1β, IL-6, IL-8, IL-10 and IL-12p70) were measured in supernatants. In addition, interleukin-6 (IL-6), a marker of systemic inflammation, was measured in plasma. During the first 5 days after stroke pneumonia occurred in 7.2% of patients. Patients with pneumonia were older, more often suffered from atrial fibrillation and ischemic heart disease, and had more severe neurological deficit on admission. Compared to patients without SAP, patients with SAP had lower ex vivo release of TNFα, IL-1β, IL-12, IP-10 and a higher level of circulating IL-6. The multimarker score composed of ex vivo synthesized IL-12, IP-10, and plasma IL-6 displayed very good discriminatory properties for SAP (area under curve [AUC]: 0.90) with sensitivity of 0.89 and specificity of 0.88. Discriminatory abilities of the multimarkers score was better than individual cytokines. The results of the study suggest the potential utility of ex vivo synthesized cytokines as a biomarker of SAP.

The aim of the second study was to determine the clinical utility of lipopolysaccharide binding protein (LBP) and soluble CD14 (sCD14) as risk markers of SAP. Three hundred thirty-one patients with ischemic stroke were included into study (median age: 71 years; 47.4% female). Blood samples were taken within 24 hours after stroke onset. SAP was diagnosed in 13% of patients. Patients with SAP had higher plasma levels of LBP (median: 19.4 vs 15.3 μg/mL, $P < 0.01$) and sCD14 (median: 1.5 vs 1.4 μg/mL, $P = 0.04$) compared to patients without SAP. In univariate logistic regression analysis, higher LBP and sCD14 levels were associated with an increased risk of SAP (OR: 1.09, 95%CI: 1.05 – 1.13, $P<0.01$ and OR: 2.76, 95%CI: 1.31 – 5.82, $P<0.01$, respectively). In multivariate analysis adjusted for age, National Institute of Health Stroke Scale (NIHSS) score, and atrial fibrillation, a higher level of LBP (OR: 1.09, 95%CI: 1.05–1.13, $P<0.01$), but not sCD14 (OR: 2.16, 0.94–4.97, $P=0.07$), was associated with SAP.

The AUC for the clinical model of SAP that included age, NIHSS and atrial fibrillation was 0.80. Discriminatory ability of both LBP and sCD14 were inferior to the clinical model (AUC: 0.67 and 0.60, respectively). Moreover, addition of LBP (AUC: 0.84, P=0.11) or sCD14 (AUC: 0.81, P=0.15) to the clinical model did not improve its discriminatory ability. Our results suggest that due to the limited predictive value, LBP and sCD14 could not be recommended as biomarkers of SAP for the clinical practice.

The aim of the third study was to determine if there is any association between anticholinergic medication used before stroke and SAP. Prospectively collected data of 675 patients with acute stroke (mean age: 71.4±13.3; 53.1% women) were analyzed. The Anticholinergic Drug Scale (ADS) was used to assess anticholinergic exposure during a month preceding stroke onset. ADS is a well-established scale which correlates with serum anticholinergic activity. SAP was diagnosed in 14.7% of patients. The use of anticholinergic medication before stroke was associated with an elevated risk of SAP (OR: 2.56, 95% CI: 1.59–4.11, P<0.01) in univariate analysis. This association remained significant in multivariable analysis adjusted for age, stroke severity, atrial fibrillation, previous myocardial infarction and respiratory tract diseases (OR: 2.06, 95% CI: 1.01–4.22, P=0.04). The results of the study suggest that the use of anticholinergic medication before stroke is associated with an increased risk of SAP.

XI. PODSUMOWANIE

1. Biomarker uwzględniający stężenia IL-12 i IP-10 produkowanych ex vivo po stymulacji pełnej krwi za pomocą endotoksyny oraz stężenie IL-6 w osoczu wykazuje bardzo dobre zdolności dyskryminacyjne i może być potencjalnie przydatny do diagnostyki SAP.

2. Podwyższone stężenie LBP i sCD14 we krwi w pierwszej dobie udaru niedokrwiennego mózgu jest związane ze zwiększonym ryzykiem SAP. Jednakże ze względu na niską wartość dyskryminacyjną, białka te nie są pomocne jako potencjalne markery predykcyjne SAP.

3. Zażywanie przed udarem leków o działaniu anty-cholinergicznym zwiększa ryzyko SAP.

XII. OŚWIADCZENIA WSPÓŁAUTORÓW

Kraków, dnia 13.12.2022 roku

Prof. dr hab. Tomasz Dziedzic

OŚWIADCZENIE

Jako współautor pracy: „Ex vivo synthesized cytokines as a biomarker of stroke-associated pneumonia " Clinica Chimica Acta (2020) 510: 260-263 oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji polegał na: opracowaniu koncepcji badania, koordynacji badania, analizie i interpretacji wyników, przygotowaniu manuskryptu publikacji.

Jednocześnie wyrażam zgodę na przedłożenie ww. pracy przez lek. Elżbietę Gradek-Kwinta jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

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Czyżycki

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Kraków, dnia 13.12.2022 roku

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Dr n. med. Elżbieta Klimiec-Moskal

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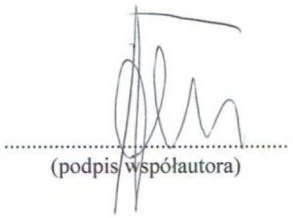
Jednocześnie wyrażam zgodę na przedłożenie ww. pracy przez lek. Elżbietę Gradek-Kwinta jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek. Elżbiety Gradek-Kwinta polegający na: opracowywaniu koncepcji badania, analizie i interpretacji wyników, przygotowaniu manuskryptu publikacji.



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(podpis współautora)