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Immunological alterations in intracranial aneurysm: a prospective study on selected biomarker profiles in blood collected during endovascular neurointervention

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ABSTRACT

Introduction. Previous studies showed that the concentrations of selected chemokines are locally elevated in samples collected from the lumen of intracranial aneurysms (IA). Our objective was to determine whether the observed differences in analyte concentrations were influenced by the origin of the blood samples (i.e. cerebral versus peripheral), thus providing insight into the localised nature of these alterations and their significance in IA pathogenesis.

Material and methods. This prospective study included 24 patients with IA who underwent endovascular embolisation. Concentrations of selected analytes were analysed in blood samples from the IA lumen, feeding artery, and aorta. The analytes included MPO, Lipocalin-2/NGAL, sICAM-1, sVCAM-1, and serum amyloid A.

Results. Higher median plasma concentrations of MPO, lipocalin-2/NGAL, sVCAM-1, and SAA were found in samples obtained from the IA lumen and the feeding artery compared to the aorta. The concentration of sICAM-1 was significantly higher in the IA compared to the aorta, but did not differ between the proximal artery and the aorta. No significant differences in any analyte concentration were observed between the IA and the proximal artery.

Conclusions. These findings suggest that the IA and the proximal vessel share similarities in the local immunological environment, which is different from that observed in the aorta. Further studies are needed to fully understand and elucidate these observations.

Keywords: intracranial aneurysm, immunology, biomarker profile, endovascular treatment, local microenvironment

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Introduction

Intracranial aneurysms (IAs) represent a relatively prevalent condition, with nationwide data indicating that unruptured IA affects c.2–5% of the general population without comorbidities [1]. Moreover, the prevalence of IA is notably higher within specific populations, such as family members of affected patients [2]. With the growing availability

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of non-invasive imaging modalities such as computed tomography (CT) and magnetic resonance imaging (MRI), IAs are increasingly being discovered incidentally [3]. IAs can remain asymptomatic but may eventually rupture, causing subarachnoid haemorrhage (SAH) with high mortality and neurological deficits. Personalised risk assessment is crucial for selecting the best approach i.e. intervention or conservative management [4, 5]. Therapeutic intervention in an IA is always a matter of balancing the benefits and risks of treatment. Due to the unclear natural history, selection of the optimal management strategy, particularly for small IAs, remains controversial [6–8].

Treatment of IAs currently depends on case-by-case risk stratifications for rupture. However, the accuracy of these risk stratifications is not always reliable, highlighting the need for new tools to better assess the risk. One of the strategies to overcome this problem is the analysis of biomarker variability in blood samples derived from IA patients [9]. In addition to established rupture risk factors such as size or location, immunological factors are increasingly associated with the risk of IA development and rupture [10–13]. A number of markers have been identified that may help to indicate that an IA is particularly prone to rupture [14–17]. A study analysing blood drawn directly from the lumen of the IA showed an altered profile of selected biomarkers compared to peripheral blood samples [17]. However, that analysis had several limitations, including a small number of patients enrolled, and a limited profile of the analysed biomarkers. Moreover, no direct analysis was performed to determine the differences between blood collected from the IA and samples from the feeding artery. Therefore, it remains unclear whether the observed differences in cytokine expression are influenced by the origin of the blood samples, emphasising the need for additional studies in this regard.

One promising marker of IA is myeloperoxidase (MPO), because it is an enzyme associated with degenerative remodelling within the IA wall [18–20]. Studies have shown that local MPO levels increase inside IA, and that these changes can contribute to IA progression and rupture in animal studies [18]. Moreover, MPO may serve as a biomarker linked to evaluating the risk of intracranial aneurysm rupture and imaging aneurysm instability [19]. Its relevance extends to its association with immune responses, particularly the infiltration of specific types of inflammatory cells, while MPO primarily serves as a marker for leukocytes, specifically neutrophils [21].

Lipocalin-1/NGAL and serum amyloid A (SAA) are other factors involved in the pathogenesis of the aneurysms. SAA is a protein that correlates with IA wall degeneration, rupture, and increased inflammatory cell infiltration, suggesting its potential role in the pathobiological processes that precede IA rupture [22, 23]. Its association with other inflammatory biomarkers indicates SAA's involvement in the development of a more susceptible state for IA rupture, highlighting its significance in understanding this disease [22].

Lipocalin-1/NGAL, or neutrophil gelatinase-associated lipocalin, is emerging as a promising biomarker within the context of IA. Elevated NGAL levels have been detected both in resected aneurysms and in plasma from IA patients [24]. Given its association with key proteins released from neutrophils, and its involvement in extracellular matrix degradation and neutrophil activation, NGAL's elevation underlines its involvement in the inflammatory cascade within aneurysms' pathogenesis, suggesting its relevance as a predictive marker for disease severity and risk of rupture [25, 26].

Furthermore, soluble vascular cell adhesion molecule-1 (sVCAM-1) and soluble intercellular adhesion molecule-1 (sICAM-1) are other potential IA biomarkers due to their role in leukocyte adhesion and inflammation [27, 28]. They facilitate the inflammatory response by enabling immune cell adherence and transmigration through the vascular endothelium into the tissues, with particular recognition of their role in regulating leukocyte recruitment to sites of inflammation. Additionally, they have been shown to participate in the pathogenesis of aneurysms and their complications, including SAH [29, 30].

The aim of the present study was to investigate how changes in the local IA microenvironment can help us gain a better understanding of IA pathobiology, thus aiding the development of new risk stratification tools. We analysed the profile of selected inflammatory biomarkers in the intrasaccular blood of IA. To provide a comprehensive comparison, we compared this to blood samples taken from the feeding artery, located just proximal to the IA site, as well as samples from the aorta. The aim was to determine whether differences in biomarker expression could be detected locally within the IA sac, or whether they were the result of a generalised process. By assessing the biomarker profiles within the IA sac and comparing them to other sites, we sought insight into the localised nature of these alterations.

Material and methods

The study protocol has been approved by the Institutional Ethical Board of the Medical University of Warsaw (approval no. KB/2/2020) and was conducted in accordance with the principles outlined in the Helsinki Declaration.

Patient enrollment protocol

This single-centre, prospective study enrolled consecutive patients with saccular IA who were treated endovascularly between 2020 and 2021. The presence of IA was confirmed using contrast-enhanced CT, MRI or digital subtraction angiography (DSA). The study was open to all patients who were eligible for IA endovascular embolisation. This included subjects who were: 1) qualified for elective endovascular treatment of an unruptured IA using coils, regardless of the presence of clinical

symptoms; or 2) qualified for urgent endovascular treatment of a ruptured IA using coils. Only patients with a confirmed good cognitive condition, enabling them to provide informed consent autonomously, were considered for inclusion in our study cohort. Patients considering embolisation were informed as to its benefits and risks as per standard care. After their acceptance, we explained the research study, its objectives, procedures, and how it would not impact upon their treatment. We obtained explicit consent for research separately from embolisation consent. The following exclusion criteria were used: 1) patients who refused to participate or were unable to give informed consent; 2) the presence of chronic diseases e.g. asthma, chronic obstructive pulmonary disease, heart failure or connective tissue diseases; 3) patients on glucocorticosteroids or other immunosuppressants; 4) the presence of a dissecting aneurysm; 5) the presence of another intracranial vascular pathology e.g. vascular malformation or dural arteriovenous fistula; 6) no indication for coil placement during DSA at the beginning of the procedure; 7) aneurysm with diameter < 3 mm; 8) giant IA (diameter > 25 mm); 9) fusiform IA; 10) blood blister-like IA; and 11) giant serpentine aneurysm.

The final study cohort included 24 patients referred for IA embolisation. There were five individuals (21%) with symptomatic ruptured IA leading to SAH. None of them were incidentally diagnosed through imaging techniques. Each subject gave informed consent based on a clear understanding of the risks associated with treatment. All patients underwent standard evaluation prior to endovascular surgery, and participation in the study did not affect their choice of treatment strategy. To be eligible for elective IA repair, patients had to meet specific qualification criteria including a normal chest X-ray and selected laboratory findings within the norm (including C-reactive protein levels, creatinine, liver function tests, and morphology). In addition, all patients had negative screening results for the hepatitis B virus, the hepatitis C virus, and the human immunodeficiency virus.

For urgent ruptured IA repair, the qualification was the same, but a deviation from the norm was not an absolute contraindication for participation.

Clinical data collection

Data was obtained from each patient's medical records. In particular, the following variables were included: demographic data (i.e. age, sex, height, weight, comorbidities, and smoking) and relevant risk factors for IA formation and rupture (hypertension, prior history of SAH, and size and location of aneurysm).

Protocol for sample collections

All embolisation procedures were performed under general anaesthesia using common femoral artery vascular access. DSA of cerebral arteries were performed to confirm the presence of an IA. Following DSA and visualisation of the IA, an initial blood sample of c.3 ml was obtained from the descending aorta using a diagnostic catheter. Care was taken to account for the catheter dead space and to discard the initially aspirated blood to minimise contamination of the sample with residual contrast or other unwanted material. Subsequently, the vascular microcatheter was advanced into the vessel feeding the IA. Before reaching the IA lumen, the catheter was stopped c.2 cm proximal to the IA neck. At this point, a second arterial blood sample, with a volume of c.1 ml, was collected through the microcatheter. Subsequently, the microcatheter was advanced and placed inside the IA lumen. A third arterial blood sample (1 mL) was collected just prior to coil deployment. In addition to sample collection, each patient underwent standard management procedures and their therapeutic decision was independent of participation in the study.

Each sample from the feeding artery and IA lumen was collected via a microcatheter (Excelsior SL-10 Microcatheter, Stryker Neurovascular) using repeated aspiration with the 1 mL twist-off syringe inserted into the catheter hub. The catheter dead space was estimated to be 0.5 mL. To minimise contamination of the target sample, the initial volume of blood from each location was collected and removed with a separate syringe. All blood samples were collected in ethylenediaminetetraacetic acid (EDTA)-coated sample tubes. Immediately after collection, each sample was centrifuged at 15,000 rpm for 10 minutes, and the supernatant plasma was frozen and stored at −80°C until further analysis.

Biochemical measurements

The plasma concentrations of selected substances in IA and intracranial artery samples were quantified by a MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead kit (Merck Millipore) using Luminex-based immunoassay (Luminex xMAP Technology) according to the manufacturer's instructions. All samples were tested in duplicate, and results were expressed in pg/ml. Concentrations of the following markers were determined: MPO, Lipocalin-2/NGAL, SAA, sVCAM-1 and sICAM-1 The set of analysed substances was selected based on the data and conclusions obtained from previous IA studies.

Statistical analysis

Statistical significance was determined with a threshold of $p \leq 0.05$. Statistical calculations and graph generation were performed using Python software version 3.11.1 (Python Software Foundation) with the use of the following libraries: pandas, numpy, scipy, and matplotlib. Kruskal–Wallis test was used to compare the medians of three samples taken from each patient. Moreover, a pairwise Mann–Whitney U test was used to examine specific group differences.

Results

Of the 24 patients included in our study, 19 (79%) were female and five (21%) were male, with a median age of 56.5 years. The median maximum diameter of the IA dome was 8 mm

(range 3–24). Of the 24 IAs analysed, five of them (21%) were found to be ruptured. A total of 11 patients (46%) had more than one IA. No patient had evidence of a mural thrombus inside the IA. Table 1 sets out detailed patient characteristics. Within the entire IA cohort, only one patient exhibited an abnormal laboratory finding. This individual, experiencing SAH, presented with slightly elevated neutrophil levels (WBC 12.5 × G/L) during the qualification for the coiling procedure. Apart from this instance, all other laboratory parameters, including CRP, creatinine, ALT, WBC, neutrophils (with four missing values in the database), RBC, and PLT, were within normal ranges.

The median plasma concentrations of MPO, lipocalin-2/NGAL, sVCAM-1 and SSA were found to be significantly higher in samples obtained from the IA lumen as well as from the feeding artery compared to plasma obtained from the aorta, as shown in Figure 1 and Table 2. The concentration of sICAM-1 was significantly higher in the IA compared to the aorta, but did not differ between the proximal artery and the aorta. Notably, there were no significant differences in the concentration of any investigated analytes between the IA and the proximal artery. Table 2 sets out the concentrations of selected analytes in different samples.

Subgroup analysis showed a significant difference in the concentration of MPO between the samples collected from the proximal artery located in the anterior circulation compared to the posterior circulation (pooled basilar artery and vertebral arteries), with concentrations of 139.47 ng/mL and 164.18 ng/mL, respectively ($p = 0.03$). A similar trend was observed in the samples collected from the lumen of the IA, where the MPO concentration was 149.32 ng/mL for the anterior circulation and 178.15 ng/mL for the posterior circulation. However, the p-value ($p = 0.08$) did not reach the level of statistical significance, suggesting a trend toward higher MPO concentration in the posterior circulation, but not reaching a conclusive result. There were no significant differences in the concentration of MPO in samples from the aorta between those two analysed subgroups. Apart from that, after adjusting for baseline characteristics, there were no significant differences observed in the concentration of other analytes between the IA lumen, proximal vessel, or aorta. Similarly, no significant variations were found between individual sampling sites in relation to each other (data not shown).

Table 1. Patient characteristics

Characteristic	Number (%) or median (range)
Number of patients	24
Age	56.5 (27–78)
Female	19 (79)
Ruptured IA with SAH	5 (21)
Median IA diameter (mm)	8 (3–24)
IA location	
AcoA	9 (38%)
ICA	7 (29%)
BA	5 (21%)
VA	2 (8%)
ACA	1 (4%)
IA in anterior vascular territory	18 (75%)
Hypertension	15 (63)
Smoker	15 (63)

Discussion

The primary goal of this study was to examine whether immunological changes in the IA microenvironment can be detected locally using endovascular techniques. We performed an analysis of the blood samples collected directly from the lumen of the IA during intravascular embolisation. These samples were then compared to material collected from the feeding artery located proximal to the IA as well as from the aorta. The goal was to determine whether changes in the concentrations of inflammatory proteins could be detected locally, and whether their levels varied depending on factors such as the origin of the blood (i.e. larger versus medium-sized artery).

Our main finding was that samples obtained from both the IA lumen and the feeding artery had significantly higher concentrations of most of the selected biomarkers compared to those obtained from the aorta. In contrast, there were no significant differences in the concentration of any of the analytes studied between the IA and the proximal artery.

The exact pathophysiology of IA remains uncertain, but it is believed that a complex interplay of multiple factors, including immunological alterations, play a significant role [9, 31]. While previous studies have primarily focused on exploring potential biomarkers of IA in peripheral blood, the use of spot analyte analysis has the potential to offer greater prognostic insights into the disease [32]. To date, only a limited number of studies have examined samples obtained directly from the IA lumen, due to the challenge of obtaining this unique material. Collection of such samples is only possible using endovascular access, which adds to the difficulty of obtaining this type of material.

Previous studies have shown that samples collected from the IA lumen have elevated concentrations of certain biomarkers. This suggests that the levels of chemokines and proinflammatory cytokines are increased in aneurysmal pathological arterial walls, and that there may be molecules that can serve as clinically relevant indicators. One of the promising markers of IA is MPO, which is associated with degenerative remodelling and other pathological changes in the aneurysmal wall leading to its weakening [18–20]. Preliminary results from

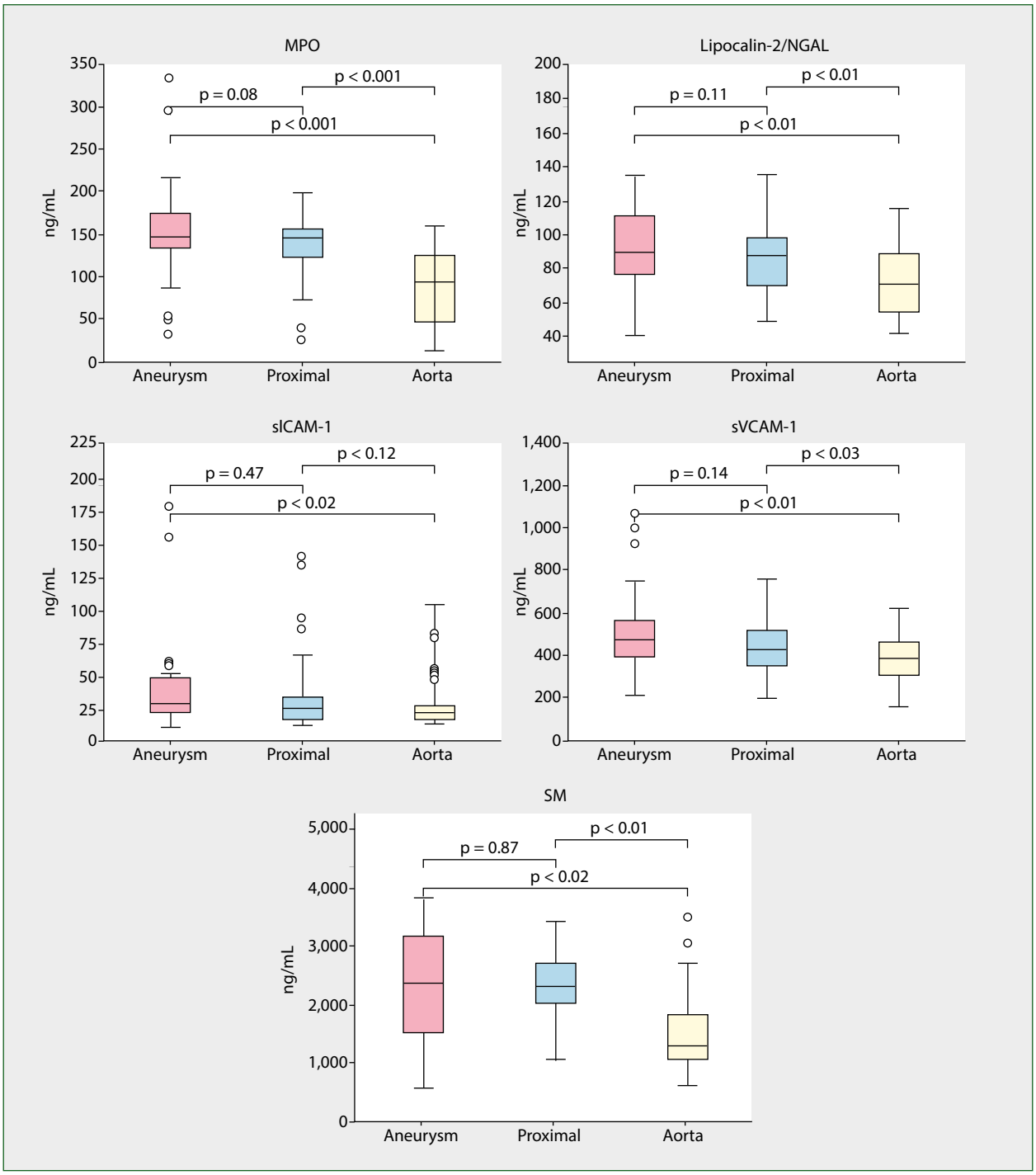

Figure 1. Box-plots showing concentrations of examined analytes in different samples

Table 2. Plasma concentrations of examined analytes. Concentrations of all analytes are expressed in ng/mL

Analyte	IA lumen	Proximal artery	Aorta	p-value
MPO	153.06 (32.07–333.67) ± 57.80	144.215 (26.79–197.88) ± 41.37	94.215 (11.61–158.39) ± 46.78	< 0.01
Lipocalin-2/NGAL	90.26 (40.38–134.73) ± 29.59	87.47 (49.69–135.83) ± 23.13	70.34 (41.95–115.82) ± 21.77	< 0.01
sICAM-1	28.02 (12.31–180.57) ± 33.08	27.12 (14.0–141.4) ± 26.49	22.67 (13.96–83.69) ± 15.49	0.06
sVCAM-1	485.67 (223.32–1600.94) ± 279.70	440.97 (217.93–772.74) ± 122.93	397.995 (176.81–633.61) ± 110.58	< 0.01
SSA	2,378.87 (550.3–3837.89) ± 1,021.92	2,326.9 (1,025.13–3410.53) ± 652.14	1,262.99 (618.77–3512.09) ± 854.29	0.01

human histopathological studies of resected IA tissues have demonstrated a correlation between MPO activity and the risk of IA rupture [20]. Those authors highlighted the need to develop less invasive techniques to obtain sufficient material, and suggested that future studies should confirm these findings in a larger study group. Another study suggested that MPO levels increase locally and that these changes significantly contribute to IA progression and rupture in murine model studies [18]. That analysis showed a significant increase in MPO concentrations in plasma collected from the lumen of the IA compared to that collected from the femoral artery. Specifically, the MPO concentrations were found to be 2.7-fold higher in the IA. We observed a similar phenomenon, as the concentration of MPO in the sample from the IA was approximately 1.7-fold higher than that of the aorta, but did not differ between the IA and the proximal artery.

Localised increases in analytes within the IA lumen have also been investigated in other studies. One potential IA marker that can be detected locally are extracellular matrix degradation products [33, 34]. Studies have shown that the process of extracellular matrix fragmentation by proteolytic enzymes leads to the release of aneurysmal wall fragments and their instability, thus increasing the risk of rupture [34, 35]. Soluble elastin fragments (sELAF) are potential markers of IA rupture risk. Nakagawa et al. showed that sELAF concentrations are elevated in aneurysmal sacs prone to rupture [16]. However, that study did not compare sELAF levels between intrasaccular and peripheral blood.

In another study, the authors investigated the gene expression patterns within the blood collected from the lumen of the IA and compared them to samples taken from the proximal vessel — a comparable methodology to ours [36]. That study revealed significant differential expression of nine genes in the intraluminal blood compared to the feeding vessel. The authors hypothesised that gene expression changes occur within the IA tissue, leading to altered gene expression patterns in circulating blood cells. The comparison of gene expression within each IA to that within the respective proximal parent vessel served as an internal control. However, this may not fully account for potential systemic differences between different blood compartments. The gene expression patterns observed in that study could have been additionally influenced by the presence of comorbidities or other confounding factors such as variations in the sample collection protocol, which were not fully accounted for in the analysis. In that study, the sample collection protocols for the IA and proximal vessels varied, while the use of different catheters in combination with various guidewires may have introduced variability into the collected blood samples. Of note, the differences in sample volume (10 mL from the parent vessel versus 3 mL intraluminally) could also contribute to variations in gene expression analysis. In contrast, in our study the protocols for sample collection between the IA and the proximal vessel did not vary. Moreover, we observed that collecting material from the lumen of the IA using a microcatheter can be technically challenging. Using a small syringe (1 mL or less) is generally an easier way to collect dense liquids such as blood through a microcatheter that serves as a tiny capillary. We found that using syringes larger than 1 ml is not feasible in some instances. Additionally, we limited our cohort to patients with aneurysm domes larger than 3 mm due to our initial unsuccessful attempts with smaller IA.

Finally, Chalouhi et al. [17] compared a number of selected inflammatory markers between blood from the IA and the systemic circulation. That study showed significant differences in the concentrations of selected analytes between these two sample sources, in line with our findings. The authors hypothesised that the locally increased concentration of these substances could be a consequence of the activity of immune cells in an aneurysmal microenvironment. However, that study was limited by its sample size which consisted of 16 patients, and it was unclear whether the observed changes in cytokine profiles might be due to the origin of the blood (systemic versus intracerebral arteries) rather to changes in the aneurysmal microenvironment itself.

Our study took a novel approach by comparing the analytes between the IA and the feeding artery. This provides unique insights into the biomarker expression between these two locations. For the first time, our study showed no significant differences in the analyte profile between the IA and the feeding artery, although the significance of this finding is unclear and raises an important point for discussion. Moreover, the significantly higher concentration of most of the selected biomarkers in blood collected from both the lumen of the IA and the proximal artery compared to material taken from the aorta raises further questions.

There are several potential explanations for these observations. Firstly, the local inflammatory response must be considered. IAs are known to be associated with immune alterations and inflammation [37]. The higher biomarker levels observed in the IA lumen and feeding arteries may indicate an ongoing local inflammatory response not only within the IA dome but also in its immediate vicinity. This could be due to non-specific phenomena such as oxidative stress, immune cell activation, or the locally enhanced production of inflammatory mediators.

Secondly, haemodynamic alterations within the IA and its immediate vicinity are known to be one of the causes of the IA formation and progression [38, 39]. Factors such as turbulent bloodflow and altered shear forces may possibly contribute to locally elevated biomarker levels [40]. Previous studies have indicated that these haemodynamic alterations can lead to endothelial dysfunction, vascular remodelling, and the production of inflammatory proteins, resulting in elevated biomarker concentrations[41].

Moreover, higher biomarker levels in feeding arteries compared to the aorta may be due to different vessel calibres and blood origins (systemic versus cerebral). The intracranial arteries and the aorta represent two different vascular

environments, each with specific features and characteristics [42, 43]. The significant differences observed in our study between the arteries of the anterior and posterior cerebral vasculature support this explanation.

These findings provide additional evidence for differences in the inflammatory profile within different vascular territories, underlining the importance of considering the specific location of the vessels when assessing the pathogenesis of IA. Endothelial and immune cell populations in these different areas may have inherent variations in their immune response, cytokine production, or susceptibility to immunological stimuli [44, 45]. Therefore, differences in the cytokine concentration observed between blood samples from the aorta and intracranial arteries may be due to the heterogeneity of these vascular areas rather than specific changes within the IA microenvironment. Similarly, the lack of significant MPO differences in aorta samples may indicate more generalised MPO expression across these arterial segments, possibly due to systemic influences or variations in MPO contributions from cell types other than immune cells, such as red blood cells. This raises questions regarding the specificity of MPO in the context of inflammatory alterations within intracranial aneurysms. Larger cohort studies and detailed investigations into local microenvironmental factors and cellular interactions could elucidate these trends and provide deeper insights into the nuanced mechanisms underlying MPO expression in different arterial territories. It is worth noting that we did not observe any complications during the collection of samples throughout the entire study. Similarly, no adverse events have been reported to date in more than 100 patients who have undergone aneurysmal blood sampling, as documented in the available reports [16–18]. Considering the present experiments as well as the existing literature, the potential risk of damaging the IA dome is expected to be minimal.

The main drawback of the proposed collection method is the extension of the total duration of the endovascular procedure by approximately five minutes compared to the standard procedure.

Limitations

Differences in sampling methods may lead to variations in the concentration of the selected analytes. Blood from the aorta was collected using a 5-French catheter during the initial DSA, which allowed for rapid collection of the entire material within a few seconds. In contrast, the material from the IA and the feeding artery was collected using a microcatheter with an internal diameter of less than 1 mm. Such differences in sampling techniques between different sites (IA and proximal artery versus aorta) might potentially contribute to the observed differences in analyte concentration in the present study compared to the study by Chalouhi et al. [17]. To address this limitation, future studies should use an identical technique for blood collection at all measurement sites.

Furthermore, it is important to acknowledge the potential presence of pre-laboratory errors. Currently available methods for biomarker analysis are too complicated and time-consuming to serve as real-time tools. Currently, there is no method for intraoperative biomarker assessment in the setting of endovascular treatment of IA. However, the introduction of novel diagnostic methods that enable real-time substrate analysis may serve as a potential solution. The future development of probe-like sensors for real-time analysis will be of great interest to the endovascular surgeon.

In addition, the limited sample size and single-centre study design with strict eligibility criteria both affect the generalisability of our findings, as they may not be representative of external populations. Moreover, it is important to acknowledge that the current study was not sufficiently powered to detect small differences in analyte concentration within subgroups. To improve the statistical power and generalisability of the results, it would be beneficial to increase the sample size and perform multicentre analyses.

Finally, this study has focused on selected biomarkers as a specific aspect of the IA microenvironment, and may have overlooked other relevant factors included in the complex immunological disturbances within IA. Notably, the majority of the analysed biomarkers predominantly belong to one type of immune cell, i.e. neutrophils. We did not analyse specific inflammatory events related to individual immune cell types. A comprehensive examination of each stage of the inflammatory cascade involving diverse immune cells — evaluating both pro- and anti-inflammatory immunophenotypes in local and peripheral environments — could significantly advance our understanding of these mechanisms and facilitate more precise preventive strategies.

However, despite this limitation, our study of immunological changes within the IA microenvironment provides valuable insights into the underlying pathophysiology. Future studies may integrate a more comprehensive approach, considering various contributory factors beyond selected analytes. This would help to explain the complex interplay between immunological changes and other relevant variables, ultimately leading to improved strategies for the management of IA.

Conclusions

This study showed that blood samples from the IA lumen and the feeding artery had higher concentrations of most of the selected analytes compared to samples collected from the aorta. There were no significant differences between the IA and the proximal artery.

These results suggest that the IA and the proximal vessel may share similarities in the local immunological microenvironment, similarities that distinguish them from the profile observed in the systemic circulation. This finding should be treated with

caution, considering the limited panel of analytes included, which are primarily associated with one cell population — neutrophils. Further research is needed to clarify these findings.

Article information

Data availability statement: *The datasets analysed during the current study are available from the corresponding author upon reasonable request.*

Ethics statement: *The study protocol has been approved by the Institutional Ethical Board of the Medical University of Warsaw (approval no. KB/2/2020). The study was conducted in accordance with the principles outlined in the Helsinki Declaration.*

Authors' contributions: *Conceptualisation: KB, JŻ; methodology: KB, JŻ, TR; formal analysis: KB, JŻ; investigation: all authors; resources: KB; data curation: KB; writing – original draft preparation: KB; writing – review & editing: all authors; biochemical analysis: DS; visualisation: KB; supervision: JŻ, TR, MJ; funding acquisition: KB*

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Conflicts of interest: *The authors declare no conflict of interest.*

Supplementary material: *None.*

References

1. Vlak MHm, Algra A, Brandenburg R, et al. Prevalence of unruptured intracranial aneurysms, with emphasis on sex, age, comorbidity, country, and time period: a systematic review and meta-analysis. Lancet Neurol. 2011; 10(7): 626–636, doi: 10.1016/S1474-4422(11)70109-0, indexed in Pubmed: 21641282.
2. Frösen J, Tulamo R, Paetau A, et al. Saccular intracranial aneurysm: pathology and mechanisms. Acta Neuropathol. 2012; 123(6): 773–786, doi: 10.1007/s00401-011-0939-3, indexed in Pubmed: 22249619.
3. Keedy A. An overview of intracranial aneurysms. Mcgill J Med. 2006; 9(2): 141–146, indexed in Pubmed: 18523626.
4. Signorelli F, Sela S, Gesualdo L, et al. Hemodynamic stress, inflammation, and intracranial aneurysm development and rupture: a systematic review. World Neurosurg. 2018; 115: 234–244, doi: 10.1016/j.wneu.2018.04.143, indexed in Pubmed: 29709752.
5. Thompson BG, Brown RD, Amin-Hanjani S, et al. American Heart Association Stroke Council, Council on Cardiovascular and Stroke Nursing, and Council on Epidemiology and Prevention, American Heart Association, American Stroke Association. Guidelines for the management of patients with unruptured intracranial aneurysms: a guideline for healthcare professionals from the american heart association/american stroke association. Stroke. 2015; 46(8): 2368–2400, doi: 10.1161/STR.0000000000000070, indexed in Pubmed: 26089327.
6. Jeong HW, Seo JH, Kim ST, et al. Clinical practice guideline for the management of intracranial aneurysms. Neurointervention. 2014; 9(2): 63–71, doi: 10.5469/neuroint.2014.9.2.63, indexed in Pubmed: 25426300.
7. Zanaty M, Daou B, Chalouhi N, et al. Evidence that a subset of aneurysms less than 7 mm warrant treatment. J Am Heart Assoc. 2016; 5(8), doi: 10.1161/JAHA.116.003936, indexed in Pubmed: 27509908.
8. Hackenberg KAM, Hänggi D, Etminan N. Unruptured intracranial aneurysms. Stroke. 2018; 49(9): 2268–2275, doi: 10.1161/STROKEAHA.118.021030, indexed in Pubmed: 30355003.
9. Hussain S, Barbarite E, Chaudhry NS, et al. Search for biomarkers of intracranial aneurysms: a systematic review. World Neurosurg. 2015; 84(5): 1473–1483, doi: 10.1016/j.wneu.2015.06.034, indexed in Pubmed: 26117089.
10. Ostergaard JR. Risk factors in intracranial saccular aneurysms. Aspects on the formation and rupture of aneurysms, and development of cerebral vasospasm. Acta Neurol Scand. 1989; 80(2): 81–98, doi: 10.1111/j.1600-0404.1989.tb03847.x, indexed in Pubmed: 2683556.
11. Ryba M, Jarzabek-Chorzelska M, Chorzelski T, et al. Is vascular angiopathy following intracranial aneurysm rupture immunologically mediated? Acta Neurochir (Wien). 1992; 117(1-2): 34–37, doi: 10.1007/BF01400632, indexed in Pubmed: 1387503.
12. Fennell VS, Kalani MY, Atwal G, et al. Biology of saccular cerebral aneurysms: a review of current understanding and future directions. Front Surg. 2016; 3: 43, doi: 10.3389/fsurg.2016.00043, indexed in Pubmed: 27504449.
13. Liu Z, Ajimu K, Yalikun N, et al. Potential therapeutic strategies for intracranial aneurysms targeting aneurysm pathogenesis. Front Neurosci. 2019; 13: 1238, doi: 10.3389/fnins.2019.01238, indexed in Pubmed: 31849575.
14. Jiang H, Wang M, Ye J, et al. Serum levels of complement-c1q/tumor necrosis factor-related protein-3 decreased in patients with acute aortic dissection. Am J Cardiol. 2018; 122(7): 1244–1248, doi: 10.1016/j.amjcard.2018.06.024, indexed in Pubmed: 30072126.
15. Tulamo R, Frösen J, Junnikkala S, et al. Complement activation associates with saccular cerebral artery aneurysm wall degeneration and rupture. Neurosurgery. 2006; 59(5): 1069–1076; discussion 1076, doi: 10.1227/01.NEU.0000245598.84698.26, indexed in Pubmed: 17016232.
16. Nakagawa D, Zanaty M, Hudson J, et al. Plasma soluble human elastin fragments as an intra-aneurysmal localized biomarker for ruptured intracranial aneurysm. J Am Heart Assoc. 2018; 7(17): e010051, doi: 10.1161/JAHA.118.010051, indexed in Pubmed: 30371156.
17. Chalouhi N, Points L, Pierce GL, et al. Localized increase of chemokines in the lumen of human cerebral aneurysms. Stroke. 2013; 44(9): 2594–2597, doi: 10.1161/STROKEAHA.113.002361, indexed in Pubmed: 23887838.
18. Chu Yi, Wilson K, Gu He, et al. Myeloperoxidase is increased in human cerebral aneurysms and increases formation and rupture of cerebral aneurysms in mice. Stroke. 2015; 46(6): 1651–1656, doi: 10.1161/STROKEAHA.114.008589, indexed in Pubmed: 25922506.
19. Gounis MJ, Vedantham S, Weaver JP, et al. Myeloperoxidase in human intracranial aneurysms: preliminary evidence. Stroke. 2014; 45(5): 1474–1477, doi: 10.1161/STROKEAHA.114.004956, indexed in Pubmed: 24713525.
20. Ollikainen E, Tulamo R, Lehti S, et al. Myeloperoxidase associates with degenerative remodeling and rupture of the saccular intracranial aneurysm wall. J Neuropathol Exp Neurol. 2018; 77(6): 461–468, doi: 10.1093/jnen/nly028, indexed in Pubmed: 29718300.
21. Zhong W, Su W, Li T, et al. Aneurysm wall enhancement in unruptured intracranial aneurysms: a histopathological evaluation. J Am Heart Assoc. 2021; 10(2): e018633, doi: 10.1161/JAHA.120.018633, indexed in Pubmed: 33410330.

22. Huuska N, Netti E, Tulamo R, et al. Serum amyloid a is present in human saccular intracranial aneurysm walls and associates with aneurysm rupture. J Neuropathol Exp Neurol. 2021; 80(10): 966–974, doi: 10.1093/jnen/nlab086, indexed in Pubmed: 34534311.
23. Sun Z, Li Y, Chang Fu, et al. Utility of serum amyloid A as a potential prognostic biomarker of aneurysmal subarachnoid hemorrhage. Front Neurol. 2022; 13, doi: 10.3389/fneur.2022.1099391, indexed in Pubmed: 36712452.
24. Serra R, Volpentesta G, Gallelli L, et al. Metalloproteinase-9 and neutrophil gelatinase-associated lipocalin plasma and tissue levels evaluation in middle cerebral artery aneurysms. Br J Neurosurg. 2014 [Epub ahead of print], doi: 10.3109/02688697.2014.913777, indexed in Pubmed: 24799278.
25. Poppenberg KE, Tutino VM, Li Lu, et al. Classification models using circulating neutrophil transcripts can detect unruptured intracranial aneurysm. J Transl Med. 2020; 18(1): 392, doi: 10.1186/s12967-020-02550-2, indexed in Pubmed: 33059716.
26. Groeneveld M, Struik J, Musters R, et al. High levels of lipocalin-2 (NGAL) in blood of patients with a ruptured abdominal aortic aneurysm. European Journal of Vascular and Endovascular Surgery. 2019; 58(6): e56–e57, doi: 10.1016/j.ejvs.2019.06.576.
27. Rothoerl RD, Schebesch KM, Kubitza M, et al. ICAM-1 and VCAM-1 expression following aneurysmal subarachnoid hemorrhage and their possible role in the pathophysiology of subsequent ischemic deficits. Cerebrovasc Dis. 2006; 22(2-3): 143–149, doi: 10.1159/000093243, indexed in Pubmed: 16691023.
28. Bui TM, Wiesolek HL, Sumagin R. ICAM-1: A master regulator of cellular responses in inflammation, injury resolution, and tumorigenesis. J Leukoc Biol. 2020; 108(3): 787–799, doi: 10.1002/JLB.2MR0220-549R, indexed in Pubmed: 32182390.
29. Polin RS, Bavbek M, Shaffrey ME, et al. Detection of soluble E-selectin, ICAM-1, VCAM-1, and L-selectin in the cerebrospinal fluid of patients after subarachnoid hemorrhage. J Neurosurg. 1998; 89(4): 559–567, doi: 10.3171/jns.1998.89.4.0559, indexed in Pubmed: 9761049.
30. Fan J, Li X, Zhong L, et al. MCP-1, ICAM-1 and VCAM-1 are present in early aneurysmal dilatation in experimental rats. Folia Histochem Cytobiol. 2010; 48(3): 455–461, doi: 10.2478/v10042-010-0042-y, indexed in Pubmed: 21071353.
31. Kamińska J, Tylicka M, Dymicka-Piekarska V, et al. Canonical NF-κB signaling pathway and GRO-α/CXCR2 axis are activated in unruptured intracranial aneurysm patients. Sci Rep. 2022; 12(1): 21375, doi: 10.1038/s41598-022-25855-2, indexed in Pubmed: 36494512.
32. Ahn SH, Burkett A, Paz A, et al. Systemic inflammatory markers of persistent cerebral edema after aneurysmal subarachnoid hemorrhage. J Neuroinflammation. 2022; 19(1): 199, doi: 10.1186/s12974-022-02564-1, indexed in Pubmed: 35927663.
33. Molacek J, Treska V, Zeithaml J, et al. Blood biomarker panel recommended for personalized prediction, prognosis, and prevention of complications associated with abdominal aortic aneurysm. EPMA J. 2019; 10(2): 125–135, doi: 10.1007/s13167-019-00173-2, indexed in Pubmed: 31258818.
34. Bacigaluppi S, Piccinelli M, Antiga L, et al. Factors affecting formation and rupture of intracranial saccular aneurysms. Neurosurg Rev. 2014; 37(1): 1–14, doi: 10.1007/s10143-013-0501-y, indexed in Pubmed: 24306170.
35. Memon AA, Zarrouk M, Ågren-Witteschus S, et al. Identification of novel diagnostic and prognostic biomarkers for abdominal aortic aneurysm. Eur J Prev Cardiol. 2020; 27(2): 132–142, doi: 10.1177/2047487319873062, indexed in Pubmed: 31466471.
36. Tutino VM, Lu Y, Ishii D, et al. Aberrant whole blood gene expression in the lumen of human intracranial aneurysms. Diagnostics (Basel). 2021; 11(8), doi: 10.3390/diagnostics11081442, indexed in Pubmed: 34441376.
37. Jung KH. New pathophysiological considerations on cerebral aneurysms. Neurointervention. 2018; 13(2): 73–83, doi: 10.5469/neuroint.2018.01011, indexed in Pubmed: 30196677.
38. Tang H, Wang Q, Xu F, et al. Underlying mechanism of hemodynamics and intracranial aneurysm. Chin Neurosurg J. 2021; 7(1): 44, doi: 10.1186/s41016-021-00260-2, indexed in Pubmed: 34847937.
39. Rayz VL, Cohen-Gadol AA. Hemodynamics of cerebral aneurysms: connecting medical imaging and biomechanical analysis. Annu Rev Biomed Eng. 2020; 22: 231–256, doi: 10.1146/annurev-bioeng-092419-061429, indexed in Pubmed: 32212833.
40. Turjman AS, Turjman F, Edelman ER. Role of fluid dynamics and inflammation in intracranial aneurysm formation. Circulation. 2014; 129(3): 373–382, doi: 10.1161/CIRCULATIONAHA.113.001444, indexed in Pubmed: 24446407.
41. Lombarski L, Kunert P, Tarka S, et al. Impact of inflammation-related degenerative changes on the wall strength of unruptured intracranial aneurysms: a pilot study. Folia Neuropathol. 2022; 60(4): 403–413, doi: 10.5114/fn.2022.122342, indexed in Pubmed: 36734382.
42. Lu Y, Sun Yu, Xu K, et al. Aorta in pathologies may function as an immune organ by upregulating secretomes for immune and vascular cell activation, differentiation and trans-differentiation-early secretomes may serve as drivers for trained immunity. Front Immunol. 2022; 13, doi: 10.3389/fimmu.2022.858256, indexed in Pubmed: 35320939.
43. Mastorakos P, McGavern D. The anatomy and immunology of vasculature in the central nervous system. Sci Immunol. 2019; 4(37), doi: 10.1126/sciimmunol.aav0492, indexed in Pubmed: 31300479.
44. Gifre-Renom L, Daems M, Luttun A, et al. Organ-Specific endothelial cell differentiation and impact of microenvironmental cues on endothelial heterogeneity. Int J Mol Sci. 2022; 23(3), doi: 10.3390/ijms23031477, indexed in Pubmed: 35163400.
45. Shao Y, Saredy J, Yang WY, et al. Vascular endothelial cells and innate immunity. Arterioscler Thromb Vasc Biol. 2020; 40(6): e138–e152, doi: 10.1161/ATVBAHA.120.314330, indexed in Pubmed: 32459541.