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## Effectiveness of plasmapheresis in ANCA clearance in patients with ANCA-associated vasculitides

Skuteczność plazmaferez w eliminacji przeciwciał przeciwko cytoplazmie neutrofilów u pacjentów z zapaleniami naczyń związanymi z ANCA

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**Dodatkowe słowa kluczowe:**  
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**Introduction:** Plasmapheresis is a therapeutic method based on removal of high molecular weight particles from blood. It is used in a variety of clinical entities in which pathogenic role of such particles has been proven e.g. ANCA-associated vasculitides (granulomatosis with polyangiitis [GPA] and microscopic polyangiitis [MPA]). Efficacy of plasmapheresis in ANCA antibodies removal and its impact on disease activity has not been adequately investigated so far. Influence of antibodies levels on disease activity also remains unknown.

**Objectives:** Analysis of plasmapheresis effect on serum ANCA levels in patients with ANCA-associated vasculitides.

**Patients and methods:** Seven patients with diagnoses of ANCA-associated vasculitides were enrolled in the study between November 2015 and April 2016. All of them underwent plasmapheresis procedures. Serum ANCA levels were measured before and after plasmaphereses using fluoroimmunoenzymatic assay (FEIA). Disease activity was assessed using Birmingham Activity Vasculitis Score; BVAS ver. 3 before first plasma exchange procedure.

**Results:** Patients in disease exacerbation (BVAS 5-10) with positive ANCA antibodies were enrolled in the study: 2 patients with GPA and 5 patients with MPA. Number of performed plasmapheresis procedures ranged from 5 to 7 (median=7). Decrease of serum antibodies concentration was observed in all patients (mean decrease of 88.3% ( $\pm 10.1\%$ )) with statistically significant difference in mean antibodies concentration before and after plasmapheresis procedures (113.0 vs 15.4;  $p=0.014$ ).

**Conclusions:** Plasmapheresis is an effective method of ANCA antibodies removal in patients in disease exacerbation.

**Wprowadzenie:** Plazmafereza jest metodą leczniczą polegającą na usuwaniu z krwi związków o dużej masie cząsteczkowej. Jest ona stosowana w licznych jednostkach chorobowych, w których patogenie udowodniono ich rolę jak np. zapalenia naczyń związane z przeciwciałami ANCA (ziarniniakowość z zapaleniem naczyń oraz mikroskopowe zapalenie naczyń). Skuteczność plazmaferezy w usuwaniu przeciwciał ANCA i jej wpływu na aktywność choroby nie została dotychczas wystarczająco zbadana, nieznany jest również wpływ poziomu przeciwciał na aktywność choroby.

**Cele:** Analiza wpływu plazmaferezy na poziom przeciwciał ANCA u pacjentów z zapaleniami naczyń związanymi z przeciwciałami ANCA.

**Pacjenci i metody:** Siedmiu pacjentów z rozpoznaniem zapalenia naczyń związanego z przeciwciałami ANCA zostało zakwalifikowanych do badania pomiędzy listopadem 2015 a kwietniem 2016. Wszystkich poddano zabiegom plazmaferezy. Poziom przeciwciał przeciwko cytoplazmie neutrofilów został zmierzony metodą fluoroimmunoenzymatyczną (FEIA) przed i po zabiegach wymiany osocza. Aktywność choroby została oceniona przy użyciu skali Birmingham Activity Vasculitis Score (BVAS) ver. 3 przed pierwszym zabiegiem.

**Wyniki:** Do badania zakwalifikowano chorych w zaostrzeniu choroby (BVAS 5-10) i z obecnymi przeciwciałami ANCA: 2 pacjentów z ziarniniakowością z zapaleniem naczyń oraz 5 pacjentów z mikroskopowym zapaleniem naczyń. Liczba wykonanych zabiegów wynosiła pomiędzy 5 a 7 (mediana=7). Spadek poziomu przeciwciał zaobserwowano u wszystkich pacjentów (średnio o 88,3% ( $\pm 10,1\%$ )) i wykazano istotną statystycznie różnicę w poziomach przeciwciał przed i po zabiegach plazmaferezy (113,0 vs 15,4;  $p=0,014$ ).

**Wnioski:** Plazmafereza jest skuteczną metodą usuwania przeciwciał ANCA u pacjentów w zaostrzeniu choroby.

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## Introduction

Plasmapheresis (PLEX) is a mechanical method of removal of high molecular weight substances (e.g. antibodies) from blood. It is particularly useful in diseases associated with pathogenic proteins. It has been used as a therapeutic method for over 40 years in multiple clinical entities e.g. glomerulonephritis and cryoglobulinemia [1], and although invasive it is considered to be relatively safe [2,3]. Plasmapheresis has been studied as a potential therapeutic option in ANCA-associated vasculitides (AAV) for 30 years but due to lack of high quality data from large randomized clinical trials there is still insufficient evidence to recommend it as the first line treatment in aforementioned diseases [4].

AAV belong to small vessel vasculitides and comprise granulomatosis with polyangiitis (GPA; formerly known as Wegener's granulomatosis), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA; formerly Churg-Strauss) [5]. Pathogenic role of ANCA (anti-neutrophil cytoplasmic antibodies) has been proven both in vitro and in vivo studies although the evidence is much stronger for MPO-ANCA than for PR3-ANCA [6]. Despite pathophysiological rationale for use of plasmapheresis in AAV clinical evidence of its effectiveness is limited, especially with respect to less severe disease and long term outcomes. Its role in ANCA clearance is not well studied either.

In current study we aimed to determine the effect of plasma exchange on ANCA clearance in patients with newly diagnosed or relapsing AAV.

## Patients and methods

Consecutive patients with AAV, who underwent plasma exchange between November 2015 and April 2016 were enrolled in this observational study. Indications for PE included diagnosis of new or relapsing GPA or MPA, as per Chapel-Hill conference consensus definitions, ANCA positivity (proteinase 3-ANCA or myeloperoxidase-ANCA) and high disease activity with progressive renal disease (active urine sediment and eGFR <50 ml/min/1.73 m<sup>2</sup> calculated with Cockcroft-Gault formula) or pulmonary hemorrhage (diffuse pulmonary infiltrates on the chest x-ray or CT scan that could not be explained otherwise than by alveolar hemorrhage due to active vasculitis).

Plasmapheresis therapy (7 cycles, if possible within 10 days) consisted of plasma

exchanges of at least 60ml/kg based on patient's actual weight. 5% albumin + fresh frozen plasma (FFP) were used as replacement fluid and filter separation technique was used as method of plasmapheresis. In one case we had to reduce the number of plasma exchanges to 5, because of poor compliance of a patient during procedures

Baseline disease activity was measured using Birmingham Activity Vasculitis Score (BVAS) ver. 3.

Statistical analysis was performed using StatSoft, Inc. (2012). STATISTICA (data analysis software system), version 11.0 www.statsoft.com. Data are presented as the mean ± SD or median with interquartile range, when applicable. The statistical analysis was performed with Student's t-test for paired variables. P values less than 0.05 were considered statistically significant.

## Results

Seven patients with AAV (2 patients with GPA, 5 patients with MPA) were treated with plasmapheresis. Median age was 55.71±14 years, time from diagnosis 0 (0-11) months. Detailed characteristics of the group is shown in table I.

Baseline BVAS score was 7 (range 5 to 10). Median serum creatinine level on admission was 361 µmol/L (101-522 µmol/L). Organs involved in the course of the disease were: oral and nasal cavity (2 patients; 28.6%), lungs (6 patients; 85.7%), kidneys (7 patients; 100%). Diffuse alveolar hemorrhage occurred in 4 patients (57.1%) resulting in respiratory failure in one patient (14.3%). Immunosuppressive therapy before plasmapheresis was administered to all patients and included: continuous steroid therapy (5 patients; 71.4%), steroid pulses (6 patients; 85.7%), intravenous cyclophosphamide (6 patients; 85.7%), rituximab (1 patient; 14.3%). There was no association between baseline ANCA antibodies and corresponding BVAS score.

Number of plasmapheresis procedures ranged from 5 to 7 (median=7). ANCA levels decreased in all patients after plasmapheresis treatment with mean antibodies concentration decrease by 88.3% (±10.1%). This fall in ANCA titre concentration was statistically significant 113.0 IU/ml vs 15.4 IU/ml; p=0.014 (Fig. 1). C-reactive protein and creatinine levels decreased in all patients except one (85.7%) with a median of 22.3 mg/L (range: 0.6-54.5; quartiles: 13.4-28.6). Decrease in serum creatinine levels was observed in 6 patients (85.7%) with median

of 80.0 µmol/L (range: 26.0-276.0 µmol/L; quartiles, IQR: 31.0-240.0 µmol/L). Renal replacement therapy (RRT) was initiated in 3 study patients (42.9%) - in two patients RRT was started before plasmapheresis, in the third case simultaneously with first plasma exchange.

## Discussion

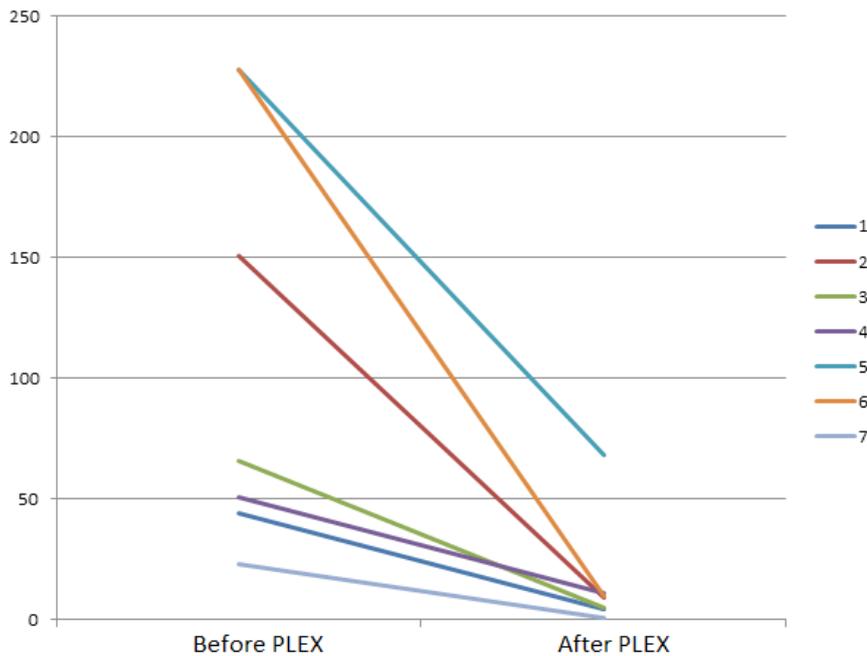
There is insufficient evidence to support a recommendation of plasma exchange as a first line therapy in AAV patients, especially in less severe cases. There is some data showing that plasma exchange may be used as adjuvant therapy for patients with progressive disease despite induction therapy with high-dose glucocorticoids and cyclophosphamide or rituximab [7]. The only randomized trial - MEPEX (Methyl Prednisolone versus Plasma Exchange in vasculitis) study which recruited patients with AAV and severe renal involvement (serum creatinine > 500 µmol/L (5.8 mg/dL) or dialysis requirement) showed 24% reduction in dialysis dependency at 12 months in patients initially treated with plasmapheresis compared to standard therapy. Moreover, there was no significant difference in mortality between both groups [8]. Subsequent meta-analyses showed reduction of dialysis dependence risk but no difference in mortality [9,10]. The currently ongoing trial: plasma exchange and glucocorticoid dosing in the treatment of antineutrophil cytoplasm antibody associated vasculitis (PEXIVAS NCT00987389) hopefully will bring more answers whether plasmapheresis should be used in patients with less severe renal involvement or/and alveolar hemorrhage [11]. In current study we aimed to evaluate whether plasma exchange treatment is effective in ANCA antibodies clearance in patients with new diagnosis or severe relapse of MPA or GPA. Serum anti-MPO or anti-PR3 antibodies measured with FEIA method significantly decreased in all patients after course of PLEX. Furthermore, reduction in ANCA levels was matched by clinical improvement as shown by decrease in CRP and creatinine levels. Our study demonstrates that plasmapheresis is an effective method of ANCA removal. However, to date there is no good evidence that level of ANCA antibodies correlates with disease activity or risk of relapse. In a study conducted by Jayne et al. ANCA levels were measured in patients with AAV on a monthly basis. Authors proved that increase in ANCA antibodies was followed by relapse in about half of the cases [12].

Table I

Clinical characteristics of patients with ANCA-associated vasculitides treated with plasmapheresis.

Charakterystyka kliniczna pacjentów z zapaleniami naczyń związanymi z ANCA leczonych zabiegami plazmaferez.

Sex	Age	Disease	Disease status	Time from diagnosis (months)	BVAS before PLEX	Number of PLEX	Antibodies before PLEX (IU/ml)	Antibodies after PLEX (IU/ml)
M	47	GPA	Relapse	11	7	7	44	4.2
F	77	MPA	New	1	5	5	151	9.1
F	60	MPA	New	0	5	7	66	5
M	51	MPA	Relapse	11	7	7	51	11
M	57	GPA	New	0	10	7	228	68
M	65	MPA	New	0	7	7	228	10
M	33	MPA	New	0	9	7	23	0.7



**Figure 1**  
Changes in ANCA antibodies concentrations (IU/ml) before and after plasmapheresis procedures.  
Zmiany w stężeniach przeciwciał ANCA (IU/ml) przed i po zabiegach plazmaferez.

Interestingly, according to literature, there is a conflicting data about MPO-ANCA and PR3-ANCA antibodies in terms of their utility as relapse markers and disease activity assessment tools. Data about MPO-antibodies came from French study group which proved that in patients with antimyeloperoxidase-associated vasculitis, anti-MPO levels were useful and relevant surrogate marker of disease activity during follow-up monitoring. Moreover, Terrier et al. proved that increase in antibodies levels and their reappearance was an independent marker of disease relapse. Finally, they showed that there was time dependence between increase in antibodies level and relapse of the disease [13]. There is no much data on the PR-3 antibodies and the course of the disease. In one prospective study Finkelman et al. despite, the authors showed a weak association of PR-3 antibodies with disease activity they concluded, that PR-3 antibodies can not be used as a reliable tool to predict neither disease relapse nor its remission [14]. On the other hand data from retrospective studies suggest that presence of PR-3

antibodies can be an independent predictor of relapse [15,16]

To our best knowledge our study is the first one to evaluate the ANCA clearance directly after PE in AAV patients. Decrease in the ANCA provides a possible rationale for PE usage in these entities.

The major limitation of the study is the small cohort of the patients, which can be partially explained due to rarity of the disease. Other limitations of the study include: observational character of the study, no control group to compare patients treated without plasmapheresis and their decline in ANCA serum levels.

#### Conclusion

Plasmapheresis is an effective method of ANCA removal associated with clinical improvement in patients with new diagnosis or relapse of AAV. Data from large scale randomised control trials are required to assess its clinical efficacy in comparison to standard therapy.

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