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Immunoadsorption *versus* plasma exchange *versus* combination for treatment of myasthenic deterioration

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Abstract

Objectives: The goal of this study was to analyze safety and assess the efficacy of standard plasma exchange (PE) compared with immunoadsorption (IA) alone, or an alternating combination of both in deteriorating myasthenia gravis (MG).

Methods: A total of 72 patients with MG who had received PE procedures for treatment of severe deterioration were retrospectively analyzed. They received either five cycles of PE (1–1.5 plasma volumes), or five cycles of IA in line with plasma separation, or a sequential alternating procedure of one cycle of PE followed by two cycles of IA, which was repeated once or more if needed.

Results: A total of 19 patients received PE, 24 patients IA, and 29 the alternating combination therapy. All groups were equally distributed by sex and mean MG score before treatment. The number of treatment cycles and days on therapy did not differ between the groups. Mean MG scores at discharge were 3.0 (PE), 1.8 (IA) and 1.6 (combination) ($p = 0.028$ for combination *versus* PE). Inpatient time was 30.7 days (PE), 22.3 days (IA) and 20.0 days in combination therapy ($p < 0.05$ for combination *versus* PE). Side effects such as allergic reactions or hypocoagulability were significantly more frequent in the PE group (37% in PE *versus* 4% in IA and 3.6% in the alternating combination, $p < 0.05$).

Conclusion: Semiselective IA in combination with PE, and to a lesser extent IA alone, was associated with a shorter hospital stay and more pronounced reduction of the MG score than PE.

Keywords: immunoadsorption, myasthenia gravis, myasthenic crisis, plasma exchange, therapy

Introduction

Myasthenia gravis (MG) is a prototypic autoimmune disease with autoantibodies directed against acetylcholine receptor (AChR) [Lindstrom *et al.* 1976; Toyka *et al.* 1975; Howard *et al.* 1987; Lefvert *et al.* 1978; Vincent and Newsom-Davis, 1978] or, much less commonly, against muscle-specific tyrosine kinase (MuSK) [Hoch *et al.* 2001]. Recently, in up to 50% of double-seronegative patients, antibodies against low-density lipoprotein receptor related protein 4 (LRP4), which was identified as the agrin receptor, were detected [Higuchi *et al.* 2011; Pevzner *et al.* 2012]. Myasthenic crisis is a life-threatening complication of MG, with generalized weakness, swallowing difficulties and respiratory insufficiency. Intensive care treatment is mandatory. In myasthenic crisis, plasma exchange (PE) and intravenous immunoglobulin (IVIg) were shown to be of almost equal efficacy as shown in a comparative study by Gajdos and coworkers [Gajdos *et al.* 1997], but significantly less effective than PE or immunoadsorption (IA) regarding clinical outcome parameters in the study by Liu and coworkers [Liu *et al.* 2010], Yet, PE not only eliminates pathogenic autoantibodies [Sato *et al.* 1988], cytokines and complement but also many other proteins such as fibrinogen [Rawer *et al.* 1983]. Due to loss of plasma proteins during

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PE a substitution with albumin (or other plasma-replacing solutions) is necessary. Semiselective IA was introduced in the therapy of myasthenic crisis in 1985 [Heininger *et al.* 1985, 1987].

Thereafter, the positive effects of PE and IA in myasthenic crisis have been extensively studied including studies comparing the efficacy of IA *versus* PE [Köhler *et al.* 2011] or of IA or PE to IVIg [Liu *et al.* 2010; Gajdos *et al.* 1997]. Also the efficacy of long-term IA treatment for refractory late onset MG has been established in a small patient sample [Haas *et al.* 2002].

IA allows for a more selective adsorption of (auto) antibodies such as anti-AChR autoantibodies. The selectivity for pathogenic AChR antibodies depends on the adsorbents used. In the last decades many efforts to improve the selectivity of IA have been made. To enhance selectivity of IA, different adsorbents were developed (peptides representing amino acids 183–200 of the torpedo or human α-subunit [Takamori and Maruta, 2001] or individual recombinant parts of the extracellular domain (ECD) of human AChR subunits expressed by *Escherichia coli* or yeast [Zisimopoulou *et al.* 2008]. Lagoumintzis and coworkers recently published a further approach to enhance safety and selectivity of the IA procedure by using denaturated *E. coli* expressed ECDs as ligands for the Sepharose matrix and testing wholeblood apheresis [Lagoumintzis *et al.* 2014]. They found no complement activation and no evidence for a transfer of pyrogens from the ECD columns to the treated MG plasmas. This is important since, in contrast to standard PE, the eluted plasma can be reinfused in IA. In IA, immunoglobulins, immune complexes and also coagulation factors, are adsorbed in a smaller portion than in PE, and adsorption of nonpathogenic and protective antibodies is widely avoided. However, as in the PE procedure, fibrinogen is eliminated in a relevant proportion; this implicates a preferred day over day procedure as in standard PE. In terms of adverse events and safety we postulated that IA, when administered in an alternating combination with PE might be superior to PE given alone. This was the basis for offering patients a modified treatment protocol.

We now retrospectively examined 72 patients with AChR-antibody positive MG and severe deterioration of MG who had been treated by plasmapheresis therapy according to a standardized protocol over a time period of 12 years. The primary goal of this study was to analyze safety aspects of IA alone or in combination with standard PE and to assess efficacy of PE alone *versus* IA or an alternating combination of both. The overall efficacy appeared better with IA or a combination approach than with PE alone.

Patients

All MG patients who were treated for deterioration of myasthenic symptoms in the Department of Neurology of the University of Würzburg, Germany and underwent PE, PE and IA or IA alone during the period from July 1989 to December 2002 were screened for this retrospective evaluation. During the respective time period the different protocols (PE, PE and IA or IA alone) were administered in the different patients according to theoretical reflections representing the state of clinical science at this time. The data were retrospectively analyzed by a doctoral thesis student with ethics approval by the University of Würzburg for retrospective evaluation of patient data.

A total of 72 AChR-antibody positive MG patients with generalized MG Osserman grade IIa to IV were identified to have received PE, IA or a combination of both according to a predefined standardized protocol. Age distribution was not significantly different between the treatment groups ($p = 0.42$; Kruskal–Wallis test) with a mean age of 67.4 years (standard deviation, SD 16.1 years) in the male patients and 49.2 years (SD 22.9 years) in the female patients. Also gender distribution was not significantly different between the treatment groups ($p = 0.32$; Chi-square test) with a female to male ratio of 8:11 in the PE group, 18:11 in the IA and PE group, and 11:13 in the IA group. Therefore, a possible influence of age or gender on the results could be excluded. These patients received either five times PE or IA only, or a sequential alternating procedure of one cycle of PE followed by two cycles of IA, which was repeated at least once. Treatment modality was selected upon the discretion of the primary consultant, assuming that there was no high-grade evidence for superiority of a specific exchange procedure. If improvement was unsatisfactory, further treatment cycles were added.

In patients who had more than one exacerbation and received more than one course of PE treatment, only one event of myasthenic deterioration or crisis was evaluated. In these cases the most precisely documented crisis was evaluated for this study.

Table 1. Modified myasthenia gravis score according to Besinger and coworkers [Besinger *et al*. 1983][a].

	No weakness (0)	Mild weakness (1)	Moderate weakness (2)	Marked weakness (3)
Arm outstretched time[b]	>180 s	60–180 s	10–60 s	<10 s
Leg outstretched time[c]	>45 s	30–45 s	5–30 s	<5 s
Head holding time[d]	>90 s	30–90 s	5–30 s	<5 s
Vital capacity[e]	>4.0 l (m)	2.5–4 l (m)	1.5–2.5 l (m)	<1.5 l (m)
	>3.0 l (w)	2.0–3.0 l (w)	1.2–2 l (w)	<1.2 l (w)
FEV_1	>90%	60–90%	40–60%	<40%
				Need for artificial ventilation
Chewing/swallowing	Normal	Fatiguing (with solid food)	Soft foods only	Gastrostomy needed
Facial expression	Normal	Lid closure weak	Incomplete lid closing	No facial expression
Diplopia	>60 s	10–60 s	>0–10 s	Spontaneous diplopia
Ptosis	>60 s	10–60 s	>0–10 s	Spontaneous ptosis

[a]The original score has been adapted and extended for clinical trials by the Myasthenia Gravis Foundation of America (MGFA).
[b]Dominant arm, outstretched horizontal during sitting; in semi prone, severely ill patients, lift arm by about 30–45° (the outstretched times are only approximate measures).
[c]Supine, dominant leg, lifted 45°.
[d]Supine, head lifted 45°.
[e]Vital capacity measured while seated. Originally, vital capacity has been assessed as the standard bedside procedure. Complete testing of pulmonary function is recommended at the initial examination to check for non-MG related respiratory disorders.
FEV_1, forced expiratory volume at one second; m, men; w, women.

All patients had undergone standardized strength testing by using a quantitative clinical score according to Besinger and coworkers [Besinger *et al.* 1983] and continuous monitoring. All patients had given their informed consent for retrospective evaluation of clinical and laboratory data according to the Helsinki declaration and with permission of the local ethics committee.

We analyzed the patient groups for age, sex, Besinger and coworkers MG score at baseline and at the end of treatment, baseline antibody titers on admission. In addition to adverse events we retrospectively analyzed numbers of PEs or IAs needed for stabilization, and number of days in hospital.

Methods

PE was performed using automatic cell separators (either COBE Spectra, COBE BCT, Lakewood CA, USA, or AS104, Fresenius, Friedberg, Germany) separating blood cells and plasma by centrifugation. Blood cells were directly reinfused, whilst plasma was substituted by commercially available human albumin-containing solutions. Each intervention had a duration of about 1.5–2 hours with a plasma flow of 20–35 ml/min and an average of 1.5 plasma volumes (2000–4000 ml) exchanged per session.

IA was performed using Immunosorba TR-350 (L) (ASAHI Medical Co., Ltd., Tokyo, Japan). Each IA was performed within about 2 hours with a plasma flow of 20 ml at maximum and an average filtrated plasma volume of 2000–2500 ml inserted in the return line. In this setting, replacement with blood products such as plasma or human albumin was not needed because the autologous plasma was given back to the patient together with the cellular components after passing the adsorption column.

Clinical evaluation

For evaluation of myasthenic signs on admission, after each PE therapy and at discharge, we used the Osserman MG score and a slightly modified quantitative MG score according to Besinger and coworkers [Besinger *et al.* 1983] (Table 1).

We also analyzed the length of stay in hospital (LOS), and the numbers of treatment sessions and duration of treatment in each treatment group.

Fibrinogen

Fibrinogen values were measured according to the method of Clauss [Clauss 1957] using a Behring Coagulation System (BCS) machine (Dade Behring, Marburg, Germany) and multifibrin U (Dade Behring, Marburg, Germany).

Table 2. Gender distribution in the different treatment groups.

Therapy group	Male		Female		*p* value
	n	%	*n*	%	
PE	8	42%	11	58%	
PE and IA combination	18	62%	11	38%	0.32
IA	11	46%	13	54%	
Total	**37**	**51%**	**35**	**49%**	

Comparison of percentages of female and male patients in each treatment group using the Chi-square test.
IA, immunoadsorption; PE, plasma exchange.

Table 3. Myasthenia gravis score at time of admission.

Therapy group	Patients sum of MG score points on admission			
	n	Median	Mean	SD
PE	19	11	11.6	3.8
PE and IA combination	29	10	9.7	3.6
IA	24	9	9.5	4.1

Modified myasthenia gravis score on admission according to Besinger and coworkers for each treatment group: Median and mean value of points derived by testing of ocular, bulbar, respiratory functions and endurance in arm, leg and neck extension with SD. The total score is calculated by dividing the sum score by the number of items tested.
IA, immunoadsorption; MG, myasthenia gravis; PE, plasma exchange; SD, standard deviation.

Statistical tests

For statistical evaluation we used the Chi-square test for pairwise comparisons, the nonparametric Mann–Whitney test to compare results between two different patient groups, a Kruskal–Wallis test to compare results between more than two groups and the Rank correlation analysis according to Kendal.

Results

Demographic data

Table 2 gives an overview of the clinical characteristics in the three treatment groups. Our retrospective analysis included only those patients with complete datasets. We found no significant gender or age differences between the groups (Chi-square-test and Kruskal–Wallis test).

Severity and clinical course of myasthenia gravis

On admission, gender and age distribution and the sum of points in the MG score were not significantly different between female and male patients (data not shown). A total of 19 patients received PE, 24 patients IA, and 29 the combination therapy. All groups showed almost identical and not significantly different MG scores before PE therapy ($p = 0.61$; ranked variance analysis Kruskal –Wallis test) (Table 3).

Mean MG score reduction was 8 points (range 3–16 points). Mean MG score values at discharge were 3.0 ± 2.4 (SD) points in the PE group, 1.8 ± 1.7 in the IA group, and 1.6 ± 1.7 in the group that received PE and IA in combination ($p = 0.028$ *versus* PE; Mann–Whitney test) (Table 4). Values of the IA group and the PE group were not significantly different. The mean number of treatment cycles and days on therapy did not differ between groups.

Length of hospital stay

Mean LOS was about 24 days for the entire patient cohort. When comparing the treatment groups, as shown in Table 3, LOS of the combination therapy group was significantly shorter than in the PE group ($p = 0.048$, Mann–Whitney test). Older patients had a longer LOS than

Table 4. Comparison of outcome parameters in the three treatment groups.

Outcome	*n*	mean	SD	*p* value
Score at discharge				
PE	18	3.0	2.4	**0.028***
PE and IA	29	1.6	2.1	
IA	24	1.8	1.8	Not significant
Number of apheresis sessions				
PE	19	4.6	2.1	0.4
PE and IA	29	4.9	1.96	
IA	24	4.8	1.9	Not significant
Days on apheresis				
PE	19	10.4	6.3	0.83
PE and IA	29	9.9	5.1	
IA	24	11.3	7.1	Not significant
Length of hospital stay				
PE	19	30.7	21.7	**0.048***
PE and IA	29	20.0	10.2	
IA	24	22.3	10.6	Not significant

Myasthenia gravis (MG) score at discharge was significantly better and length of hospital stay (LOS) was significantly shorter in the group that received IA and PE as compared to the group which was treated by PE only. There were no significant differences with regard to the outcome parameters in the IA group and the groups that were treated with IA and PE or with PE only (Mann–Whitney test). IA alone was not superior to PE alone and not significantly less effective than the combination of PE and IA.
IA, immunoadsorption; MG, myasthenia gravis; PE, plasma exchange; SD, standard deviation.

younger patients (tau 0.29, $p < 0.0003$; Rank correlation analysis according to Kendall).

Numbers of treatment sessions and duration of treatment

The number of treatment sessions correlated with the MG score on admission and was not significantly different between the three treatment groups as shown in Table 4. About half of the patients received five PE sessions or less. Mean duration of treatment for all patients was about 11 days and was not significantly different between the three treatment groups.

Fibrinogen

AChR values could not be evaluated since they were not systematically determined before and after extracorporeal treatment sessions. Fibrinogen values were always obtained for safety reasons to assess the bleeding risk due to low levels of coagulation factors (see below). Fibrinogen values before the treatment 1–10 in each treatment group showed a rapid decline from the first to the fifth treatment and successive stabilization and moderate increase despite continuation of the treatment.

Side effects

Allergic reactions, hypocoagulability, and bronchorespiratory infections were significantly more frequent in the PE group than in the other two groups (36.9% PE *versus* 4.2% IA *versus* 3.6% combination, $p < 0.05$, Chi-square test).

One patient in the PE group needed fibrinogen substitution. One patient in the PE group and two patients in the IA group were switched to immunoglobulin therapy because of pneumonia without sepsis. One patient developed a skin rash during PE which was classified as a possible allergic reaction to albumin. An 80-year-old patient with preexisting extrasystolia Lown III suffered three occurrences of asystolia for over five seconds with need for a short cardiopulmonary resuscitation, once during IA. As such events did not reappear after lowering the pyridostigmine dosage, bradycardia was not

classified as a complication of IA but as a drug side effect.

Discussion

Before the introduction of intensive care medicine, PE procedures and IVIg in the treatment of myasthenic crisis, prognosis of severe myasthenic deterioration was poor [Osterhuis *et al.* 1989] but has improved to a mortality rate of less than 5% now [Hohlfeld *et al.* 2003].

Removal of pathogenic autoantibodies and proinflammatory cytokines are the rationale of PE procedures also in other autoimmune diseases such as CIDP [Dalakas and Medscape, 2011]. On theoretical grounds, IA with tryptophane-polyvinyl-alcohol columns has a greater potential to eliminate autoantibodies and largely avoids the negative impact on reducing polyclonal immunoglobulin levels and also partly on coagulation factors when compared with standard PE, thus termed a semiselective procedure [Heininger *et al.* 1985, 1987; Lagoumintzis *et al.* 2014]. We here analyzed the safety and efficacy of PE and IA as well as an alternating combination of both. The alternating combination of PE and IA was significantly more and more rapidly effective in improving signs of myasthenic deterioration as shown by significant stronger reduction of the MG score, and associated with a shorter LOS and treatment duration as compared with treatment with PE. We suppose that the enhanced efficacy of the combined therapy may be related to the synergistic effect of the combination of selective antibody elimination by IA and elimination of small humoral pathogenic factors (cytokines, chemokines, complement) by PE. In the IA group the improvement of the MG score was almost comparable with that achieved by IA/PE combination and length of hospital was also markedly shorter than that in the PE group (22.3 *versus* 30.7 days) but both parameters were not statistically different from those in the PE group. In a previous comparative study fourteen days after start of treatment equal effects of PE and IA were shown [Köhler *et al.* 2011], yet in a quite smaller number of patients than in our study with only 10 patients in the PE group, 9 patients in the IA group and only 3–5 treatments (3.5 in the PE and 3.4 in the IA group) during a period of only 7 days. Due to the smaller number of patients studied, the shorter treatment duration, and the predefined treatment volume of 1.5 l this study is not directly comparable to our analysis. However, the impression that IA alone was seemingly more effective than PE in our analysis, needs further confirmation. Side effects such as hypocoagulability and bronchorespiratory infections were significantly more frequent in the PE group than in the other two groups, which is not confirmed by others [Köhler *et al.* 2011]. The selection of the best documented crisis could have introduced a bias, i.e. could have favored the selection of the worst crises in which the severity of symptoms or complications required a more detailed documentation, but if so the selection of the more severe myasthenic deterioration episodes would far more strengthen the relevance of our results.

We are aware that the usage of IA as medical procedure is still limited in some countries of the world, especially in Northern America. Yet in view of a growing number of antibody specific neuroimmunological syndromes, e.g. onconeuronal antibodies, we consider it helpful to attract the attention of the neurological community to the usage of IA. The additional costs for IA are more than balanced by the reduced LOS time as well as the reduced need of plasma substituting solutions and handling of side effects. In particular, IA could be improved by further modifications of antigen used as shown by recent efforts to enhance selectivity and safety of the method [Lagoumintzis *et al.* 2014]. Although the validity of our study is limited by the retrospective nature of the analysis, we would like to emphasize that the single-center character of our trial with standardized approaches and scoring still make the dataset better comparable.

A larger prospective trial is needed to eliminate potential bias inherent to a retrospective and nonrandomized observational study.

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