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Autoimmune myasthenia gravis : the impact of heterogeneous patterns of muscle weakness on outcome measures and diagnosis

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PART I

Heterogeneity and fluctuations
in muscle weakness





Chapter 2

Heterogeneity and Shifts in Distribution of Muscle Weakness in Myasthenia Gravis

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ABSTRACT

Introduction

The distribution of muscle weakness in myasthenia gravis (MG) patients with acetylcholine receptor (AChR) antibodies is highly variable. As muscle groups respond differently to therapeutic interventions, it is important to acknowledge this variability. We analysed the distribution of muscle weakness in AChR MG patients over time and the relation with treatment.

Methods

We systematically investigated the distribution of muscle weakness in 225 AChR MG patients using questionnaires. Symptoms were inquired for three consecutive periods: baseline, time of maximum severity and last follow-up. On the basis of combinations of muscle weakness, seven phenotypes were defined: 'ocular' (O), 'bulbar' (B), 'neck/limbs/respiratory' (NLR), or a combination (O+B, O+NLR, B+NLR and O+B+NLR).

Results

Throughout the disease course MG was restricted to ocular weakness in 5%, whereas 7% never had any form of ocular weakness. At last follow-up, ocular or bulbar weakness had resolved more frequently than NLR weakness (40%, 38% and 25%; $p = 0.003$, respectively). Patients with O, B or OB phenotype at baseline had a higher age at onset and were more frequently male than patients with NLR, ONLR, BNLR or OBNLR phenotype (52.7 ± 17.5 vs. 44.0 ± 18.9 ; $p = 0.007$ and 64% vs. 37%; $p = 0.002$, respectively).

Discussion

MG patients have a large heterogeneity in the distribution of muscle weakness and frequently shift between phenotypes. The phenotypic variations found in AChR MG suggest that also other factors aside from the AChR antibody mediated immune response are of importance in determining the disease expression in MG.

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disease, mediated by antibodies to a number of well characterized autoantigens.¹ However, current knowledge on the involved autoantigens does not provide conclusive answers for the large heterogeneity that is observed in distribution of muscle weakness and the shifts in this distribution within individual MG patients. The most frequently involved autoantigen is the acetylcholine receptor (AChR), against which antibodies are present in about 85% of MG patients.² Although the exposure of the target antigens to circulating serum antibodies should be similar for all muscles, different patterns of muscle weakness are observed in individual patients. Acknowledging different phenotypes in MG might be of importance in treatment choices and clinical trials as some studies show that ocular or generalized weakness responds distinctly to different therapies.³⁻⁵ Moreover, the sensitivity of outcome scales for changes in muscle weakness depends on the muscles involved.^{6,7} In this study, we therefore systematically analysed the distribution of muscle weakness in AChR MG patients and shifts in this distribution within patients.

METHODS

Patients

We included a prospective cohort of MG patients in the Netherlands and Belgium that participated in the Dutch-Belgian Myasthenia Registry between 2016 and 2018 (website address: <https://www.lumc.nl/org/neurologie/research/myasthenie-register/>). The diagnosis of AChR MG was based on a combination of clinically confirmed fluctuating muscle weakness and the presence of serum autoantibodies to AChR.⁸ This study was approved by the Medical Ethics Boards of the Leiden University Medical Center. All patients provided informed, written consent prior to study participation.

Clinical data

To allow the systematic inclusion of a large number of patients we used questionnaires to investigate the localization of muscle weakness. We asked for the presence of the following forms of myasthenic weakness: ptosis, diplopia, dysarthria, chewing weakness, swallowing weakness, facial weakness, neck weakness, respiratory weakness, hand weakness, arm weakness and leg weakness. These questions are available as a supplement 1. The presence of symptoms was inquired for three consecutive periods: baseline (initial weakness during the first six months from the onset of MG), time of maximum severity (defined as the period in which patients experienced the highest burden of disease) and last follow-up (last three months). In addition, the use of pyridostigmine, prednisone and other immunosuppressants (azathioprine, mycophenolate mofetil, rituximab, cyclosporine, methotrexate or

cyclophosphamide) in the last three months was inquired. All patients gave permission to access the clinical reports and results of additional electrophysiological and laboratory investigations of their treating physicians. This information was used to validate the information provided by the patient.

Phenotypes

Muscle weakness was categorized into three groups: ocular (ptosis or diplopia), bulbar (dysarthria, chewing weakness, swallowing weakness or facial weakness) and neck/limbs/respiratory (neck weakness, respiratory weakness, hand weakness, arm weakness or leg weakness; abbreviated 'NLR'). The combination of muscles included in these groups were based on prior clinical subdivision of muscle weakness in MG and the allotype to which these muscles belong.⁹⁻¹² Based on the presence of muscle weakness in one or more of these groups, seven phenotypes were formulated. 'ocular' (O), 'bulbar' (B) or 'neck/limbs/respiratory' (NLR) are phenotypes in which weakness of only one group is present. Alongside these three phenotypes with 'isolated' weakness, four phenotypes entail combinations of these forms of muscle weakness: O+B (OB), O+NLR (ONLR), B+NLR (BNLR) and O+B+NLR (OBNLR). We classified all patients in phenotypes using two different classifications: a 'point in time' phenotype and a 'cumulative' phenotype. The 'point in time' phenotype of each patient was based on the distribution of muscle weakness at baseline, time of maximum severity or last follow-up. The cumulative phenotype was based on the involvement of all affected muscle categories at any time up until that point in time.

As an example, a patient with only ocular weakness throughout the disease course is classified as an O phenotype in both phenotype classifications. A patient starting with ocular weakness, who later on develops bulbar and NLR weakness will be classified as O at the start of disease, but as OBNLR in the cumulative phenotype classification, even if the three forms of weakness are present at different points in time.

Statistical Analysis

We used a chi-squared test to assess differences in baseline characteristics and differences in the frequency of phenotypes at different points in time. We used a unpaired t-test to analyze differences in mean age at onset. *P*-values < 0.05 were considered significant. Statistical analyses were performed using SPSS version 23 (IBM Corp., Armonk, NY) and GraphPad Prism version 7.00 (GraphPad Software Inc., San Diego, CA).

RESULTS

We included 225 AChR MG patients. Source data was used to check antibody status and age at onset. 168 (75%) of patients correctly indicated AChR as their antibody status in the

questionnaire, the rest of the patients indicated that they did not know their antibody status. Baseline characteristics, symptoms at baseline, at maximum severity and at last follow-up are shown in table 1.

Table 1. Baseline characteristics of 225 patients with AChR MG included in this study

Demographic characteristics			
Age, y	59.4 ± 15.1		
Age at onset, y	45.6 ± 19.0		
Gender (% male)	94 (42)		
Disease duration	13.7 ± 13.6		
Clinical characteristics			
<i>Medication use</i>	Baseline	Max. Severity	Last FU
Pyridostigmine			166 (74)
Prednisone			110 (49)
Other immunosuppressants			105 (47)
<i>Symptoms</i>			
Ptosis	175 (78)	166 (74)	98 (44)
Diplopia	162 (72)	160 (71)	82 (36)
Dysarthria	144 (64)	152 (68)	75 (33)
Chewing weakness	139 (62)	151 (67)	64 (28)
Swallowing weakness	142 (63)	152 (68)	77 (34)
Facial weakness	141 (63)	143 (64)	71 (32)
Neck weakness	108 (48)	132 (59)	71 (32)
Respiratory weakness	94 (42)	124 (55)	88 (39)
Hand weakness	127 (56)	142 (63)	93 (41)
Arm weakness	130 (58)	162 (72)	94 (42)
Leg weakness	128 (57)	151 (67)	114 (51)

Baseline characteristics, medication use at last follow-up, symptoms at baseline, at maximum severity and at last follow-up are shown. Data are presented as number of patients (%) for categorical variables and as mean ± SD for continuous variables.

Abbreviations: Baseline = initial weakness during the first six months from the onset of MG, Max. Severity = time of maximum severity, Last FU = last three months

At baseline, 86% of patients reported ocular weakness, 76% bulbar weakness and 81% NLR weakness. Ocular or bulbar weakness had resolved more frequently at last follow-up than NLR weakness (40%, 38% and 25%; $p = 0.003$, respectively). Clinical remission occurred more frequently in patients without NLR weakness at baseline (O, B or OB phenotypes) than in patients with NLR weakness (NLR, ONLR, BNLR or OBNLR) (33% and 13%; $p = 0.002$, respectively). The former group (O, B or OB at baseline) had a higher age at onset and had a higher proportion of male patients than the latter group (NLR, ONLR, BNLR or OBNLR at baseline) (52.7 ± 17.5 vs. 44.0 ± 18.9 ; $p = 0.007$ and 64% vs. 37%; p

= 0.002, respectively). The same differences were seen for cumulative phenotypes at last follow-up: patients without NLR weakness throughout the disease course, in whom the weakness never developed beyond the O, B or OB phenotype, had a higher age at onset and had a higher proportion of male patients than patients with NLR weakness at some point in the disease course (cumulative NLR, ONLR, BNLR or OBNLR at last follow-up) (55.1 ± 17.6 vs. 44.6 ± 18.9 ; $p = 0.016$ and 67% vs. 39% ; $p = 0.020$, respectively). Characteristics of MG patients subdivided by phenotype are shown in figure 1 and table 2.

Table 2. Characteristics of MG patients subdivided by phenotype

A. Baseline phenotypes							
Baseline phenotypes	O N=25	B N=4	OB N=13	NLR N=9	ONLR N=19	BNLR N=18	OBNLR N=137
Age, y	63.7 ± 12.8	53.0 ± 16.1	64.9 ± 10.4	55.6 ± 13.7	59.2 ± 15.9	57.4 ± 13.7	58.7 ± 15.9
Age at onset, y	51.5 ± 20.2	42.3 ± 17.5	58.2 ± 8.7	42.7 ± 15.4	49.0 ± 19.5	42.9 ± 17.9	43.5 ± 19.3
Gender (% male)	17 (68)	2 (50)	8 (62)	3 (33)	6 (32)	6 (33)	52 (38)
Disease duration	12.2 ± 15.4	10.8 ± 2.2	6.7 ± 5.8	12.9 ± 7.3	10.3 ± 8.8	14.6 ± 12.2	15.2 ± 14.8
<i>Medication use</i>							
Pyridostigmine	15 (60)	4 (100)	9 (69)	6 (67)	16 (84)	10 (56)	106 (77)
Prednisone	16 (64)	1 (25)	6 (46)	2 (22)	11 (58)	7 (39)	67 (49)
Other immunosuppressants	6 (24)	4 (100)	5 (39)	1 (11)	5 (26)	13 (72)	71 (52)
B. Cumulative phenotypes at last FU							
Cumulative phenotypes (Last FU)	O N=12	B N=1	OB N=8	NLR N=1	ONLR N=17	BNLR N=13	OBNLR N=173
Age, y	64.4 ± 13.6	61.0 ± 0	64.3 ± 9.5	70.0 ± 0	60.1 ± 16.5	55.1 ± 16.2	58.9 ± 15.2
Age at onset, y	52.4 ± 22.3	51.0 ± 0	59.5 ± 8.3	60.0 ± 0	51.6 ± 19.1	43.6 ± 16.1	43.9 ± 19.0
Gender (% male)	9 (75)	0 (0)	5 (63)	1 (100)	6 (35)	4 (31)	69 (40)
Disease duration	12.0 ± 19.2	10 ± 0	4.8 ± 5.2	10.0 ± 0	8.5 ± 8.2	11.5 ± 7.4	15.0 ± 14.1
<i>Medication use</i>							
Pyridostigmine	7 (58)	1 (100)	6 (75)	0 (0)	15 (88)	6 (46)	131 (76)
Prednisone	5 (42)	1 (100)	3 (38)	1 (100)	9 (53)	4 (31)	87 (50)
Other immunosuppressants	1 (8)	1 (100)	2 (25)	0 (0)	3 (18)	10 (77)	88 (51)

Characteristics of MG patients subdivided by phenotype are shown. Table A shows data for phenotypes at baseline. Table B shows data for cumulative phenotypes at last follow-up. 'cumulative phenotypes' represent all the weakness the patients had experienced up until that point in time (i.e. last follow-up). Data are presented as number of patients (%) for categorical variables and as mean \pm SD for continuous variables.

Abbreviations: O = ocular, B = bulbar, OB = oculobulbar, NLR = neck/limbs/respiratory, ONLR = O+NLR, BNLR = B+NLR, OBNLR = O + B + NL, Baseline = initial weakness during the first six months from the onset of MG, Max. Severity = time of maximum severity, Last FU = last three months

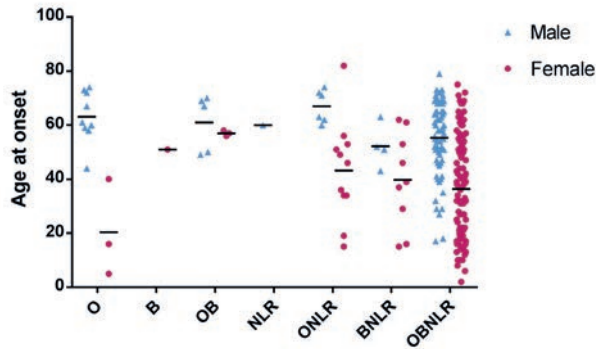


Figure 1. Age at onset and gender subdivided by cumulative phenotypes

Dot plots showing the age at onset and gender of MG patients subdivided by phenotype. The mean is shown by lines.

Abbreviations: O = ocular, B = bulbar, OB = oculobulbar, NLR = neck/limbs/respiratory, ONLR = O+NLR, BNL = B+NLR, OBNL = O + B + NLR, Baseline = initial weakness during the first six months from the onset of MG, Max. Severity = time of maximum severity, Last FU = last three months

At baseline, 25 of patients had a pure ocular phenotype. Twelve of these patients (48%) remained purely ocular throughout their disease course. The appearance of NLR weakness (secondary generalization) was reported by 12 of these patients (48%), and one patient developed an oculobulbar cumulative phenotype. Non-ocular phenotypes (B, NLR, BNL) were reported by 31 patients (14%) at baseline. Of these patients, 15 (48%) never had any ocular weakness throughout their disease course. Figure 2 shows the frequency of “point-in-time” phenotypes and transitions between these phenotypes. Figure 3 gives an overview of the “cumulative” phenotypes over time.

DISCUSSION

In a cohort of 225 AChR MG patients, we found a high heterogeneity in the distribution of muscle weakness and a frequent occurrence of shifts between phenotypes in individual patients. Ocular or bulbar weakness disappeared more frequently than NLR weakness, suggesting that the latter form of weakness responds less well to therapy or that oculobulbar muscles are more capable of adapting to the chronic exposure to autoantibodies. Clinical remission occurred more often in patients that did not have any form of NLR weakness in the first 6 months of disease. This suggests that the initial phenotype in AChR MG patients is of prognostic value.

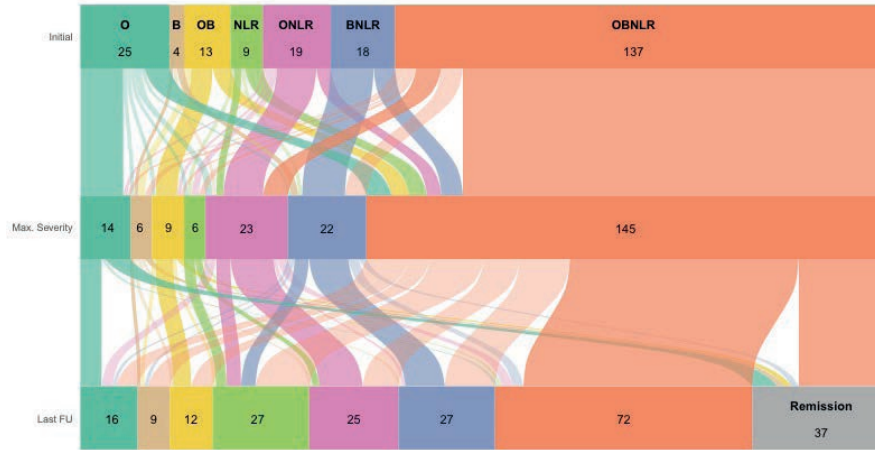


Figure 2. Point-in-time phenotypes and shifts over time

The frequency of point-in-time phenotypes within AChR MG and transitions between these phenotypes are shown. The two most frequent transitions for each phenotype are shown more prominently (darker colour). The frequencies of the phenotypes are shown in their respective boxes. At last follow-up, an additional category is added: remission (the absence of any form of myasthenic weakness).

Abbreviations: O = ocular, B = bulbar, OB = oculobulbar, NLR = neck/limbs/respiratory, ONLR = O+NLR, BNLR = B+NLR, OBNLR = O + B + NLR, Baseline = initial weakness during the first six months from the onset of MG, Max. Severity = time of maximum severity, Last FU = last three months



Figure 3. Cumulative phenotypes and shifts over time

The frequency of cumulative phenotypes within AChR MG and transitions between these phenotypes are shown. ‘cumulative phenotypes’ represent all the weakness the patients had experienced up until that point in time. The two most frequent transitions for each phenotype are shown more prominently (darker colour). The frequencies of the phenotypes are shown in their respective boxes. At Max. Severity and Last FU only one patient had phenotype B and only one patient had phenotype NLR.

Abbreviations: O = ocular, B = bulbar, OB = oculobulbar, NLR = neck/limbs/respiratory, ONLR = O+NLR, BNLR = B+NLR, OBNLR = O + B + NLR, Baseline = initial weakness during the first six months from the onset of MG, Max. Severity = time of maximum severity, Last FU = last three months

The appearance of NLR weakness in priorly ocular MG patients, occurred in 48% of our AChR MG patients. Earlier reported rates of secondary generalization vary greatly. In a Chinese cohort only 26% of patients with ocular MG developed generalized MG during a 13-year follow-up period.¹³ On the other hand, Grob et al. found that generalized weakness emerged in 80% of initially ocular MG patients and in 88% generalization occurred within one year from disease onset.¹⁴ Other studies reported secondary generalization rates between of these extremes: Schlezinger et al. (56%), Wirtz et al. (58%), Li et al. (61%), Oosterhuis (69%).^{9, 15-17} One explanation underlying this variation may be caused by differences in the use of the term 'generalization'. For example, Wirtz et al. defined 'generalized weakness' as "ocular or bulbar plus limb muscle weakness", but in the definition of Li et al. the presence of limb muscle weakness was not necessary and bulbar weakness alone was sufficient ("symptoms other than ocular symptoms, such as dysarthria, dysphagia, dyspnea or weakness of the jaw, neck or arms and legs").^{9, 17} For this reason we avoided the term 'generalized weakness' in our phenotype classification and opted to describe different muscle regions separately.

We do expect that the phenotypes with NLR weakness (NLR, ONLR, BNLR, OBNLR) would mostly be recognized as 'generalized MG'. These four 'generalized' phenotypes all occurred in significant frequencies and were observed throughout the disease course. We did find a relatively low secondary generalization rate in patients without NLR weakness at baseline. This is likely explained by the broader period which we considered to be the baseline phenotype. Oosterhuis, for example, looked at initial signs within three months from onset, as opposed to six months from onset in our study.¹⁶

A more precise classification based on muscle weakness patterns may be useful to gain a more detailed understanding of the effect of treatment. Currently, trials generally regard both patients with NLR weakness and patients with OBNLR symptoms as belonging to the same subgroup ('generalized'). However, this broad classification could affect the outcome in clinical trials as the latter group will have the possibility to respond to 'oculobulbar' as well as 'generalized' items in outcome measures whereas the former will only respond to 'generalized' items.

Interestingly, in 12 patients (5%) MG was restricted to ocular weakness, whereas 15 other patients (7%) never had any form of ocular weakness throughout their disease course. These phenotypic extremes suggest that other factors, such as characteristics of individual muscles and their resistance against the antibody mediated attack, are of importance in determining the disease expression in MG. The O phenotype group with pure ocular MG consisted mainly of male patients with an age at onset higher than 60 and the 3 female patients had a much lower age at onset (mean around 20). This distribution of age at onset and gender was similar to that found in a previous study.¹⁸ The B and OB phenotype group was characterised by late age at onset. Some case reports also suggest that MG presenting with bulbar weakness is mainly found in late-onset patients.^{19, 20} In the phenotype groups with NLR

weakness (NLR, ONLR, BNLR and OBNLR), male patients had a mean age at onset around 60 whereas female patients had a mean age at onset around 40. This distribution was comparable to earlier studies on ‘generalized’ MG.⁸ The phenotype groups without any ocular or oculobulbar weakness (BNLR and NLR, respectively) were mainly constituted by female patients with early-onset of disease. The predominance of female patients was also found in an earlier study on limb-girdle MG (comparable to our NLR phenotype).²¹ It is likely that the final phenotype in MG patients is the result of a complex interplay between a set of immunological parameters, among which the fine specificity of the AChR antibodies, the capacity for complement activation, the frequency of AChR blocking antibodies and HLA allotype on one hand, and a set of the muscle characteristics on the other hand, including regenerative capacity, complement resistance, or innervation patterns.²²⁻³³

Limitations of this study include recall bias as patients were asked to report symptoms from an early stage of disease and the categorization of muscles in groups that may be considered somewhat arbitrary (O, B and NLR). The purpose of this categorization, however, was not to introduce a new classification system for MG, but to elucidate the heterogeneity of muscle weakness and shifts during the disease course in a standardized way. Our categorization, which was based on prior clinical subdivisions and on the allotype to which these muscles belong⁹⁻¹², served this purpose well. Furthermore, including one type of muscle weakness in another category (e.g. including neck weakness in the B phenotype instead of the NLR phenotype) would not affect the main conclusions of this paper.

In this study, we found a high heterogeneity in the distribution of muscle weakness and a frequent occurrence of shifts between phenotypes in individual patients. The phenotypic extremes found in AChR MG suggest that other factors than solely AChR antibodies are of importance in determining the disease expression in MG. Exploring these factors may help in developing a ‘personalized medicine’ approach in MG by taking patient phenotypes into account in future clinical trials and in day-to-day treatment decisions.

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Supplemental material

SUPPLEMENT 1

Questionnaire Dutch-Belgian Myasthenia Registry

Abbreviated version translated from the original Dutch questionnaire

1. Which symptoms of myasthenia did you experience at the beginning of the disease? (within the first 6 months of disease)

- Weak or falling eyelids (droopy eyelids)
- Double vision
- Hand weakness (difficulty opening jars, squeezing or grabbing)
- Arm weakness (difficulty combing hair, washing or taking something out of a high cabinet)
- Leg weakness (difficulty walking stairs or walking long distances)
- Neck weakness (difficulty keeping your head straight up)
- Facial weakness (difficulty to whistle or to smile)
- Difficulty with speaking (bad articulation, unclear speech)
- Difficulty with chewing (difficulty to chew food)
- Difficulty with swallowing (difficulty to swallow food and fluids)
- Difficulty with breathing (deep breathing during minimal exercise)

2. Which symptoms of myasthenia did you experience when the disease was at its worst?

- Weak or falling eyelids (droopy eyelids)
- Double vision
- Hand weakness (difficulty opening jars, squeezing or grabbing)
- Arm weakness (difficulty combing hair, washing or taking something out of a high cabinet)
- Leg weakness (difficulty walking stairs or walking long distances)
- Neck weakness (difficulty keeping your head straight up)
- Facial weakness (difficulty to whistle or to smile)
- Difficulty with speaking (bad articulation, unclear speech)
- Difficulty with chewing (difficulty to chew food)
- Difficulty with swallowing (difficulty to swallow food and fluids)
- Difficulty with breathing (deep breathing during minimal exercise)

3. Which symptoms of myasthenia have you experienced in the last three months?

- Weak or falling eyelids (droopy eyelids)
- Double vision
- Hand weakness (difficulty opening jars, squeezing or grabbing)
- Arm weakness (difficulty combing hair, washing or taking something out of a high cabinet)
- Leg weakness (difficulty walking stairs or walking long distances)
- Neck weakness (difficulty keeping your head straight up)
- Facial weakness (difficulty to whistle or to smile)
- Difficulty with speaking (bad articulation, unclear speech)
- Difficulty with chewing (difficulty to chew food)
- Difficulty with swallowing (difficulty to swallow food and fluids)
- Difficulty with breathing (deep breathing during minimal exercise)

4. Which of the following medications or therapies did you use or undergo in the last three months?

- Pyridostigmine (Mestinon)
- Prednisolone or prednisone
- Azathioprine (Imuran)
- Mycophenolate mofetil (Cellcept)
- Rituximab (MabThera)
- Cyclosporine (Neoral, Sandimmune)
- Methotrexate (Metoject, Emthexate)
- Cyclophosphamide (Endoxan)

