

## ORIGINAL ARTICLE

# Treatment of myasthenia gravis patients with calcineurin inhibitors in Japan: A retrospective analysis of outcomes

Kimiaki Utsugisawa,<sup>1</sup> Yuriko Nagane,<sup>1</sup> Tomihiro Imai,<sup>2</sup> Masakatsu Motomura,<sup>3</sup> Masayuki Masuda,<sup>4</sup> Shingo Konno<sup>5</sup> and Shigeaki Suzuki<sup>6</sup>

<sup>1</sup>Department of Neurology, Hanamaki General Hospital, Hanamaki, Japan, <sup>2</sup>Sapporo Medical University School of Health Sciences, Sapporo, Japan, <sup>3</sup>First Department of Internal Medicine, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan, <sup>4</sup>Department of Neurology, Tokyo Medical University, Tokyo, Japan, <sup>5</sup>Department of Neurology, Toho University Medical Center Oh-hashii Hospital, Tokyo, Japan, <sup>6</sup>Department of Neurology, Keio University School of Medicine, Tokyo, Japan

## Keywords

calcineurin inhibitors; cyclosporine; myasthenia; quality of life; tacrolimus

## Correspondence

Kimiaki Utsugisawa, MD, PhD, Department of Neurology, Hanamaki General Hospital, 4-28 Kajoh-chou, Hanamaki 025-0075, Japan.  
Tel: +81-198-23-3311  
Fax: +81-198-24-8163  
Email: kutsugi@s4.dion.ne.jp

Received: 27 October 2014; revised: 24 November 2014; accepted: 10 December 2014.

## Abstract

**Objectives** Calcineurin inhibitors (CNI) are approved for the treatment of myasthenia gravis (MG) in Japan. However, the extent to which CNI have been effective remains unclear. Here we report data regarding CNI use and outcomes of MG.

**Methods** We evaluated 640 consecutive MG patients by a multicenter survey. Patients not receiving any immune treatment were excluded, and cross-sectional and retrospective data of 515 patients receiving immune treatment with ( $n = 312$ ) or without ( $n = 203$ ) CNI were analyzed.

**Results** Compared with patients treated without CNI, those treated with CNI had a higher frequency of MG Foundation of America Class III–V and higher severity disease at the worst clinical condition, and also had current higher severity, worse quality of life and higher daily doses of prednisolone, despite taking equivalent prednisolone dosages during the course of treatment. Achieving a treatment target was less frequent in the group treated with CNI. Onset age was not different between the two groups. Duration before CNI use after starting corticosteroids was  $4.4 \pm 6.3$  years. Among those treated with CNI, late-onset MG patients achieved a more favorable current condition than did those with early-onset and thymoma-associated MG, whereas there was no such difference without CNI treatment.

**Conclusions** CNI were given to severely ill MG patients with no attempt to select those more likely to respond, and failed to exert a strong impact on MG therapy. CNI should be given aggressively to patients with factors known to enhance susceptibility to these drugs, such as higher age at onset and early-stage disease.

## Introduction

Myasthenia gravis (MG) is an autoimmune disease mediated by antibodies against molecules in the neuromuscular junction, impairing neuromuscular transmission.<sup>1</sup> Pathogenic autoantibodies in MG are generally anti-acetylcholine receptor antibodies (AChR-Ab) that are detected in 80% of patients, infrequently anti-muscle-specific receptor tyrosine kinase antibodies (MuSK-Ab) in 2–3% or fairly infrequently anti-low-density lipoprotein receptor-related protein 4 antibodies.<sup>1–5</sup> Production of AChR-Ab in

B cells depends on AChR-specific T cells.<sup>6,7</sup> Calcineurin inhibitors (CNI), such as cyclosporine and tacrolimus, bind to different target molecules (cyclophilin and FK-binding protein, respectively), but they inhibit T-cell activation in the same way, through calcineurin inhibition.<sup>8–10</sup> A considerable number of reports have shown CNI efficacy against MG.<sup>11–17</sup>

Oral corticosteroids are still the most common agent for long-term immunosuppressive treatment of MG.<sup>2</sup> As MG is a severe disease with a high mortality rate, the spread of oral corticosteroid use has led to a non-lethal disease course for MG patients.<sup>18</sup>

However, many patients need to continue taking oral corticosteroids for many years and are thus burdened with associated side-effects, as the rate of MG patients achieving full remission and no longer requiring immune treatment is very low.<sup>4,19–22</sup> The frequency of patients achieving a treatment target of favorable health-related quality of life (HRQOL) is <50%.<sup>4,19</sup> It is clear that treatment primarily with oral corticosteroids does not actually ensure a favorable outcome.<sup>21</sup>

In Japan, CNI are approved for the treatment of MG under the national health insurance system, and are considered to be steroid-sparing and symptom-reducing agents for MG. However, the extent to which CNI exert a positive effect on improving symptoms and HRQOL of MG remains unclear. We have carried out a multicenter survey (Japan MG registry) obtaining detailed clinical parameters from >600 MG patients in order to clarify the condition of these patients including HRQOL.<sup>3,4,19–21</sup> In the present report, we delineate actual CNI use and patient status by analyzing this cross-sectional and retrospective dataset.

## Methods

### Patients

The present study was carried out at 11 neurological centers in Japan (Japan MG Registry Group).<sup>3,4,21</sup> We evaluated adult patients with established MG between April and July 2012. To avoid potential bias, we enrolled consecutive patients at various stages of illness over a short duration (4 months). The present study required detailed information of each patient, and 36 patients without full information or not providing written informed consent were excluded from the total of 676 patients seen during the study period.<sup>3,4,21</sup> Given that the purpose of this study was to elucidate impacts of CNI on the immune treatment of MG, we further excluded 125 patients who did not receive any immune treatments during the course of their disease. Ultimately, 515 MG patients provided written informed consent and were subjected to analysis. Clinical background data for the patients treated with ( $n = 312$ , 61%) or without ( $n = 203$ , 39%) CNI are shown in Tables 1 and 2.

The study protocols were approved by the ethics committees of each participating institution. Written informed consent was obtained from all patients participating in the study.

Diagnosis of MG was based on clinical findings (fluctuating symptoms with easy fatigability and recovery after rest) with amelioration of symptoms

**Table 1** Comparison of patients receiving immune treatments with and without calcineurin inhibitors regarding characteristics and baseline severity

	Type of treatments	
	Without CNI ( $n = 203$ )	With CNI ( $n = 312$ )
Age (years)	58.5 ± 16.8	57.0 ± 16.4
Age at onset (years)	46.8 ± 19.5	47.4 ± 17.7
Time since onset (years)	11.8 ± 10.7	9.8 ± 9.0
Treatment with PSL (%)	100	90.4
Duration between onset and starting immune treatments (years)	2.7 ± 5.6	2.2 ± 5.1
Duration before CNI use after starting immune treatments (years)	–	4.4 ± 6.3
Early-onset MG (%)	44.3	41.0
Late-onset MG (%)	37.4	32.4
Thymoma-associated MG (%)	18.2	26.6 <sup>#</sup>
Thymectomy (%)	54.2	57.7
MGFA clinical classification (%)		
Class I	23.2	9.3 <sup>##</sup>
Class II	46.8	36.5 <sup>#</sup>
Class III	18.7	31.4 <sup>##</sup>
Class IV	4.4	8.7
Class V	6.9	14.1 <sup>##</sup>
QMG at worst condition	12.1 ± 5.9 ( $n = 163$ )	15.7 ± 7.1 ( $n = 250$ )*
AChR-Ab-positivity (%)	81.8 (166/203)	80.4 (251/312)
MuSK-Ab-positivity (%)	3.0	1.9

\* $P < 0.0001$  (Mann–Whitney  $U$ -test); <sup>#</sup> $P < 0.05$ , <sup>##</sup> $P < 0.01$  ( $\chi^2$ -test) compared with patients without calcineurin inhibitors (CNI). AChR-Ab, anti-acetylcholine receptor antibodies; MG, myasthenia gravis; MGFA, Myasthenia Gravis Foundation of America; MuSK-Ab, anti-muscle-specific receptor tyrosine kinase antibodies; PSL, prednisolone; QMG, Myasthenia Gravis Foundation of America quantitative myasthenia gravis score.

after intravenous administration of anticholinesterase, decremental muscle response to a train of low-frequency repetitive nerve stimuli of 3 Hz, or the presence of AChR-Ab or MuSK-Ab. Single-fiber electromyography was not carried out routinely.

### Clinical factors

Clinical factors shown in Tables 1 and 2 were evaluated for each patient and subjected to analysis. Severity was determined according to the MG Foundation of America (MGFA) quantitative MG score (QMG).<sup>23</sup> For 413 of the 515 patients, QMG at the worst clinical condition was obtained from medical records. All patients completed the Japanese version of the 15-item MG-specific QOL scale (MG-QOL15-J).<sup>19</sup> Current clinical status after treatment was categorized according to the MGFA post-intervention status.<sup>23</sup> Among categories of MGFA post-intervention status, minimal manifestations (MM) or better

**Table 2** Comparison of patients receiving immune treatments with and without calcineurin inhibitors regarding treatment course and current status

	Type of treatments	
	Without CNI (n = 203)	With CNI (n = 312)
Current QMG	5.7 ± 4.4	8.1 ± 5.6***
Current MG-QOL 15-J	11.0 ± 10.5	16.6 ± 14.0***
Current dose of PSL (mg/day)	4.9 ± 6.2	5.8 ± 5.9*
Maximum dose of PSL (mg/day)	28.1 ± 19.6	26.1 ± 19.3
Total dose of PSL during the last 1 year (mg)	1581.4 ± 1666.4	2145.5 ± 2079.90**
Duration of PSL ≥20 mg/day (years)	0.8 ± 1.6	1.1 ± 3.1
PP and/or IVIg (%)	21.2	52.6###
Achieved 5 mg-MM or better (%)	44.8	32.4##

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.0001$  (Mann–Whitney  $U$ -test); ## $P < 0.01$ , ### $P < 0.0001$  ( $\chi^2$ -test) compared with patients without calcineurin inhibitors (CNI). IVIg, intravenous immunoglobulin; MG, myasthenia gravis; MG-QOL15-J, Japanese version of the 15-item myasthenia gravis-specific quality of life scale; MM, minimal manifestations; PP, plasmapheresis; PSL, prednisolone; QMG, Myasthenia Gravis Foundation of America quantitative myasthenia gravis score; QOL, quality of life.

status (i.e. pharmacological remission [PR] and complete stable remission [CSR]) were considered a favorably improved status, and achieving MM or better status with oral prednisolone (PSL)  $\leq 5$  mg/day (MM or better-5 mg) was identified as a treatment target.<sup>4,19,21</sup> Use of this category grouping into MM or better and the cut-off of PSL dose at 5 mg/day were repeatedly confirmed by analyses of clinical data from the Japan MG Registry and the East Japan MG study.<sup>3,4,19–21</sup> Thus, the frequency of patients achieving MM or better-5 mg was shown in the present study. The worst condition for each patient was classified according to the MGFA classification.<sup>23</sup> Current dose, maximum dose, total dose during the last year of PSL and the period taking  $\geq 20$  mg of PSL were recorded. The duration between onset and starting immune treatments (with PSL in 92.2%, with CNI in 7.8% of the patients) and duration up to CNI use after starting immune treatments were obtained from medical records. MG was classified into three subtypes as follows: early-onset MG (age at onset  $\leq 49$  years), late-onset MG (age at onset  $\geq 50$  years) or thymoma-associated MG.<sup>2,3</sup>

Serum AChR-Ab titers were estimated by radioimmunoassay using  $^{125}\text{I}$ - $\alpha$ -bungarotoxin, and levels  $\geq 0.5$  M were regarded as positive. MuSK-Ab was measured using a commercially available radioimmunoassay (RSR, Cardiff, UK).

## Statistical analysis

Differences between two groups were evaluated using the Mann–Whitney  $U$ -test for continuous variables and the  $\chi^2$ -test for categorical variables. All continuous data are expressed as the mean  $\pm$  standard deviation. Statistical analyses were carried out using UNISTAT version 5.6 (Unistat, London, UK) statistical software.

## Results

### Comparison of patients receiving immune treatments with and without CNI regarding baseline severity, treatment course and current status

#### *CNI were given to severely ill MG patients in Japan*

Baseline severity and other characteristics of both patient groups are shown in Table 1. The patient group treated with CNI had a lower frequency of MGFA clinical classifications class I and II, a higher frequency of class III–V and higher QMG at the worst clinical condition, compared with the group not treated with CNI (Table 1). CNI were apparently given to patients with severe MG symptoms. The frequency of thymoma-associated MG was higher in the group taking CNI (Table 1), which probably reflects the fact that thymoma-associated MG often involves severe symptoms.<sup>3</sup> It is known that MG patients at the early stages of disease respond well to CNI.<sup>24</sup> However, in the present participants, the mean duration before CNI use after starting immune treatment was  $4.4 \pm 6.3$  years, suggesting that CNI were not often used for early-stage patients. Age, age at onset, time since onset, duration between onset and starting immune treatments, frequency of early-onset and late-onset MG, and frequency of AChR- or MuSK-Ab positivity were not different between the two groups. Patients having a higher age at onset are more susceptible to CNI; however, CNI were not often used for patients with higher age at onset.<sup>24</sup>

No significant difference in baseline severity and other characteristics was noted between patients treated with cyclosporine (107/312, 34.3%) and those treated with tacrolimus (205/312, 65.7%).

#### *Patients treated with CNI remained in an unfavorable condition*

The treatment course and current status of both patient groups are shown in Table 2. The patient group treated with CNI currently had higher QMG, higher MG-QOL15-J (i.e. worse HRQOL), and a

higher daily dose and 1-year total dose of PSL, despite equivalent levels of maximum PSL dose and duration of PSL  $\geq 20$  mg/day, compared with the group not treated with CNI (Table 2). The frequency of patients who achieved MM or better-5 mg (a treatment target) was not high in either group, and was lower in the group treated with CNI (32.4% vs 44.8%; Table 2).

No significant difference in the treatment course and current status was noted between patients treated with cyclosporine and those treated with tacrolimus, which is consistent with a previous report that the effects of the two drugs on MG were almost identical.<sup>24</sup>

Patients with CNI remain in an unfavorable condition, possibly because of a strong bias favoring CNI use for severe and intractable MG. Regrettably, no positive correlation of CNI use to good outcome (e.g. achieving MM or better-5 mg, or lower current QMG or MG-QOL15-J) was detected.

#### Comparisons of treatment course and current status among three MG subtypes

*In the patient group receiving immune treatments with CNI, late-onset MG achieved a more favorable current condition than did the other two MG subtypes*

When analyzing the patient group receiving immune treatments with CNI, both current QMG and MG-QOL15-J were lower (i.e. lower severity and better HRQOL) for late-onset MG patients than for early-

onset and thymoma-associated MG patients (Table 3). The frequency of patients who achieved MM or better-5 mg was higher among late-onset MG patients than those having the other two subtypes (Table 3). Maximum dose, current dose and 1-year total dose of PSL, and duration of PSL  $\geq 20$  mg/day were also lower for late-onset MG than others (Table 3).

*In the patient group receiving immune treatments without CNI, there were no differences in current severity, HRQOL and the rate of MM or better-5 mg among the three MG subtypes*

In the patients not treated with CNI, current QMG, current MG-QOL15-J and frequency of patients achieving MM or better-5 mg were not different among the three MG subtypes (Table 4), in contrast to the patients treated with CNI (Table 3). Maximum dose, current dose and 1-year total dose of PSL, and duration of PSL  $\geq 20$  mg/day were lower for late-onset MG patients than the others (Table 4), which is consistent with the results of patients treated with CNI (Table 3).

#### Discussion

There is no doubt that CNI are generally effective for MG, but responders to the drugs comprise <70% of MG patients, at least at the dosage approved under the Japanese national health insurance system.<sup>11–17,24</sup> There are some characteristics of responder MG patients to CNI therapy, such as higher age at onset,

**Table 3** Among patients treated with calcineurin inhibitors, comparison of early-onset, late-onset and thymoma-associated myasthenia gravis groups regarding treatment course and current status

	Type of patients		
	Early-onset MG (n = 128)	Late-onset MG (n = 101)	Thymoma-associated MG (n = 83)
Time since onset (years)	12.8 $\pm$ 11.4	6.2 $\pm$ 4.4***#	9.7 $\pm$ 7.4
Current QMG	9.3 $\pm$ 5.9	6.6 $\pm$ 4.9*	7.9 $\pm$ 5.2
Current MG-QOL 15-J	19.0 $\pm$ 15.4	13.6 $\pm$ 12.4*#	16.8 $\pm$ 12.8
Current dose of PSL (mg/day)	6.1 $\pm$ 6.0	5.0 $\pm$ 6.0#	6.2 $\pm$ 5.7
Total dose of PSL during the last 1 year (mg)	2166.6 $\pm$ 1971.5	1738.6 $\pm$ 1836.3#	2626.5 $\pm$ 2409.0
Maximum dose of PSL (mg/day)	27.4 $\pm$ 18.8	21.5 $\pm$ 17.4***#	35.6 $\pm$ 16.8
Duration of PSL $\geq 20$ mg/day (years)	1.5 $\pm$ 3.6	0.3 $\pm$ 0.5***#	1.6 $\pm$ 3.8
PP and/or IVIg (%)	52.3	44.6##	62.7
Achieved 5 mg-MM or better status (%)	25.8	40.6*#	30.1

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.0001$  (Mann–Whitney  $U$ -test or  $\chi^2$ -test) compared with early-onset myasthenia gravis (MG) patients; # $P < 0.05$ , ## $P < 0.01$ , ### $P < 0.0001$  (Mann–Whitney  $U$ -test or  $\chi^2$ -test) compared with thymoma-associated MG patients. CNI, calcineurin inhibitors; IVIg, intravenous immunoglobulin; MG, myasthenia gravis; MG-QOL15-J, Japanese version of the 15-item myasthenia gravis-specific quality of life scale; MM, minimal manifestations; PP, plasmapheresis; PSL, prednisolone; QMG, Myasthenia Gravis Foundation of America quantitative myasthenia gravis score; QOL, quality of life.

**Table 4** Among patients not treated with calcineurin inhibitors, comparison of early-onset, late-onset and thymoma-associated myasthenia gravis groups regarding treatment course and current status

	Type of patients		
	Early-onset MG (n = 90)	Late-onset MG (n = 76)	Thymoma-associated MG (n = 37)
Time since onset (years)	15.8 ± 12.6	7.2 ± 5.9 <sup>**#</sup>	11.3 ± 9.5
Current QMG	5.5 ± 4.1	5.7 ± 4.7	6.5 ± 4.7
Current MG-QOL 15-J	10.1 ± 4.0	11.8 ± 11.9	11.4 ± 8.9
Current dose of PSL (mg/day)	4.9 ± 6.1 <sup>#</sup>	3.5 ± 4.2 <sup>#</sup>	7.7 ± 8.7
Total dose of PSL during the last 1 year (mg)	1649.7 ± 1834.7	1247.9 ± 1206.5 <sup>#</sup>	2044.0 ± 1876.4
Maximum dose of PSL (mg/day)	32.3 ± 17.3	24.7 ± 19.4 <sup>*#</sup>	35.1 ± 20.2
Duration of PSL ≥20 mg/day (years)	0.9 ± 1.1	0.4 ± 1.2 <sup>**##</sup>	1.2 ± 2.7
PP and/or IVIg (%)	22.2	10.5 <sup>***#</sup>	40.5
Achieved 5 mg-MM or better status (%)	45.6 (41/90)	51.3 (39/76)	37.8 (14/37)

\* $P < 0.05$ , \*\* $P < 0.0001$  (Mann–Whitney  $U$ -test or  $\chi^2$ -test) compared with early-onset myasthenia gravis (MG) patients; <sup>#</sup> $P < 0.05$ , <sup>##</sup> $P < 0.01$ , <sup>###</sup> $P < 0.0001$  (Mann–Whitney  $U$ -test or  $\chi^2$ -test) compared with thymoma-associated MG patients. CNI, calcineurin inhibitors; IVIg, intravenous immunoglobulin; MG, myasthenia gravis; MG-QOL15-J, Japanese version of the 15-item myasthenia gravis-specific quality of life scale; MM, minimal manifestations; PP, plasmapheresis; PSL, prednisolone; QMG, Myasthenia Gravis Foundation of America quantitative myasthenia gravis score; QOL, quality of life.

early stages of disease and/or thymoma-associated disease.<sup>24</sup> However, in the present participants, CNI were given to patients with severe symptoms, but not necessarily to those more likely to respond, probably because the use was limited to poor-responder patients to oral steroids under the national health insurance up to 2009. Even currently, patients with CNI, compared with those without CNI, showed higher severity and worse HRQOL despite taking more than equivalent levels of PSL during the course of treatments. Naturally, this patient group would have had a much more unfavorable status if they had not taken CNI. However, it is also true that CNI therapy, in the way presently administered, has failed to exert a strong impact with regard to ensuring favorable disease status and HRQOL for MG patients. When a patient is recognized to not have a good response to CNI, combination therapy with high-dose intravenous methylprednisolone, plasmapheresis and/or intravenous immunoglobulin should be started earlier. Even if the oral PSL dose is increased, longer treatment with a higher oral PSL dose not ensure a better outcome for intractable MG patients.<sup>21</sup>

Higher age of MG onset is one of the susceptibility factors for CNI therapy.<sup>24</sup> Consistently, in the group treated with CNI, late-onset MG patients, compared with the other MG subtypes, achieved less severe disease and better HRQOL despite lower levels of PSL dosage. Such better outcome in late-onset MG was not observed in the group without CNI, but possibly could have been achieved if CNI had been

given. In other words, these late-onset MG patients could have achieved better outcome than the present results if with CNI. Furthermore, MG patients in early stages of the disease are known to be susceptible to CNI, but the drugs were not often used for such patients.<sup>24</sup> Achieving a treatment target (MM or better-5 mg) was also not frequent enough in the patient group treated without CNI (44.8%), even with their lower baseline severity. Considering these present and previously reported findings, it is better to give CNI more aggressively to patients having susceptibility factors to the drugs, such as higher age at onset and the early stages of disease, regardless of baseline severity.<sup>24</sup>

Thymoma-associated MG patients are known to have a larger reduction in severity in response to CNI, and were more frequently included in the patient group treated with CNI.<sup>24</sup> However, thymoma-associated MG is the most severe subtype at worst condition, and requires the most aggressive immune treatment of the three MG subtypes, thus the higher frequency of this subtype in the CNI group in and of itself would not necessarily lead to a more favorable overall outcome.<sup>3</sup> The doses of CNI used for MG treatment are often lower and thus safer than those used for preventing organ rejection in clinical organ transplantation, which could be one of the reasons why CNI therapy failed to exert a strong impact on the MG population.

In conclusion, CNI were given to MG patients with severe symptoms with no attempt to select those more likely to respond, and thus failed to exert

a strong impact on MG therapy. As the frequency of CNI use was not high, CNI should be given more aggressively to patients with higher age at onset and in the early stages of disease, regardless of baseline severity.

### Acknowledgements

We thank Dr S. Ohji and Dr K. Nomura (Saitama Medical Center), Dr E. Tsuda (Sapporo Medical University Hospital), and Dr S. Nakane (Nagasaki Kawatana Medical Center) for the collection of patient data. This work was supported by the Japan MG registry group and funded by Astellas Pharmaceutical under the sponsored research contact. Astellas Pharmaceutical had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

### Conflict of interest

None declared.

### References

- Patrick J, Lindstrom J. Autoimmune response to acetylcholine receptor. *Science*. 1973; **180**: 871–2.
- Meriggioli MN, Sanders DB. Autoimmune myasthenia gravis: emerging clinical and biological heterogeneity. *Lancet Neurol*. 2009; **8**: 475–90.
- Murai H, Masuda M, Utsugisawa K, et al. Clinical features and treatment status of adult myasthenia gravis in Japan. *Clin Exp Neuroimmunol*. 2014; **5**: 84–91.
- Utsugisawa K, Suzuki S, Nagane Y, et al. Health-related quality of life and treatment targets in myasthenia gravis. *Muscle Nerve*. 2014; **50**: 493–500.
- Higuchi O, Hamuro J, Motomura M, Yamanashi Y. Autoantibodies to low-density lipoprotein receptor-related protein 4 in myasthenia gravis. *Ann Neurol*. 2011; **69**: 418–22.
- Fujii Y, Lindstrom J. Regulation of antibody production by helper T cell clones in experimental autoimmune myasthenia gravis. *J Immunol*. 1988; **141**: 3361–9.
- Mokhtarian F, Pino M, Ofosu-Appiah W, Grob D. Phenotypic and functional characterization of T cells from patients with myasthenia gravis. *J Clin Invest*. 1990; **86**: 2099–108.
- Kino T, Hatanaka H, Miyata S, et al. FK506, a novel immunosuppressant isolated from a streptomycetes. II. Immunosuppressive effect of FK506 in vitro. *J Antibiot (Tokyo)*. 1987; **40**: 1256–65.
- Schreiber SL, Crabtree GR. The mechanism of action of cyclosporin A and FK506. *Immunol Today*. 1992; **13**: 136–42.
- Dunn CJ, Wagstaff AJ, Perry CM, Plosker GL, Goa KL. Cyclosporin; An updated review of the pharmacokinetic properties, clinical efficacy and tolerability of a micro-emulsion-based formulation (Neoral®) in organ transplantation. *Drug*. 2001; **61**: 1957–2016.
- Utsugisawa K, Nagane Y, Suzuki S, Suzuki N. Monitoring treatment with cyclosporine microemulsion in myasthenia gravis. *Eur J Neurol*. 2008; **15**: 598–604.
- Tindall RSA, Rollins JA, Phillips JT, Greenlee RG, Wells L, Belendiuk G. Preliminary results of a double-blind, randomized, placebo-controlled trial of cyclosporine in myasthenia gravis. *N Engl J Med*. 1987; **316**: 719–24.
- Ciafaloni E, Nikhar NK, Massey JM, Sanders DB. Retrospective analysis of the use of cyclosporine in myasthenia gravis. *Neurology*. 2000; **55**: 448–50.
- Evoli A, Di Schino C, Marsili F, Punzi C. Successful treatment of myasthenia gravis with tacrolimus. *Muscle Nerve*. 2002; **25**: 111–4.
- Konishi T, Yoshiyama Y, Takamori M, Saida T. Long-term treatment of generalised myasthenia gravis with FK506 (tacrolimus). *J Neurol Neurosurg Psychiatry*. 2005; **76**: 448–50.
- Nagane Y, Utsugisawa K, Obara D, Kondoh R, Terayama Y. Efficacy of low-dose FK506 in the treatment of myasthenia gravis-A randomized pilot study. *Eur Neurol*. 2005; **53**: 146–50.
- Yoshikawa H, Kiuchi T, Saida T, Takamori M. Randomised, double-blind, placebo-controlled study of tacrolimus in myasthenia gravis. *J Neurol Neurosurg Psychiatry*. 2011; **82**: 970–7.
- Gilhus NE. Autoimmune myasthenia gravis. *Expert Rev Neurother*. 2009; **9**: 351–8.
- Masuda M, Utsugisawa K, Suzuki S, et al. The MG-QOL15 Japanese version: validation and associations with clinical factors. *Muscle Nerve*. 2012; **46**: 166–73.
- Suzuki Y, Utsugisawa K, Suzuki S, et al. Factors associated with depressive state in patients with myasthenia gravis: a multicenter cross-sectional study. *BMJ Open*. 2011; **1**: e000313.
- Imai T, Suzuki S, Tsuda E, et al. Oral corticosteroid therapy and present disease status in myasthenia gravis. *Muscle Nerve*. 2015; doi: 10.1002/mus.24438.
- Grob D, Brunner N, Namba T, Pagala M. Lifetime course of myasthenia gravis. *Muscle Nerve*. 2008; **37**: 141–9.
- Jaretzki A 3rd, Barohn RJ, Ernstoff RM, et al. for the Task Force of the Medical Scientific Advisory Board of Myasthenia Gravis Foundation of America. Myasthenia gravis: recommendations for clinical research standards. *Neurology*. 2000; **55**: 16–23.
- Nagane Y, Suzuki S, Suzuki N, Utsugisawa K. Factors associated with response to calcineurin inhibitors in myasthenia gravis. *Muscle Nerve*. 2010; **41**: 212–8.