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Calcineurin inhibitors in a cohort of patients with antisynthetase-associated interstitial lung disease

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ABSTRACT

Objectives. *The aim of this paper is to assess the effect of calcineurin inhibitors (tacrolimus or cyclosporine) for treating patients with interstitial lung disease (ILD) associated with antisynthetase autoantibodies.*

Methods. *Sixty patients with antisynthetase autoantibodies were identified in our myositis cohort of 179 patients. The medical records of 15 patients with antisynthetase autoantibody-associated ILD treated with tacrolimus/cyclosporine (11 for refractory disease and 4 as first-line therapy) between 1980 and 2011 were retrospectively reviewed. Serial pulmonary function tests were used to assess the clinical response. Qualitative data are presented as a number and percentage, and quantitative data as the median and interquartile range (IQR).*

Results. *Patients were classified as having probable or definite idiopathic inflammatory myopathy (8 dermatomyositis and 4 polymyositis), and pure interstitial lung disease (3 cases). The 15 patients had received tacrolimus/cyclosporine for an average of 19 (IQR 14–30) months. Median age at onset of ILD was 42.3 (IQR 32.4–56.8) years and median duration of lung disease before administration of calcineurin inhibitors was 11 (IQR: 5–49) months. Median duration of follow-up was 24 (IQR 12–32) months. Thirteen patients had anti-histidyl-transfer RNA synthetase autoantibody (anti-Jo-1) and two had anti-alanyl-transfer RNA synthetase autoantibody (anti-PL-12). A more than 10% increase in FVC or stabilisation was observed in 13 (87%; 95%CI 56–98) patients who received calcineurin inhibitors (9 [81%] refractory cases and 4 [100%] as first-line therapy).*

Conclusion. *Calcineurin inhibitors seem to be a good therapeutic option for managing ILD associated with antisynthetase autoantibodies, not only in refractory cases, but also as first-line treatment.*

Introduction

Idiopathic inflammatory myopathies (IIM), including polymyositis (PM) and dermatomyositis (DM), are systemic autoimmune diseases characterised by skeletal muscle inflammation (1). It is estimated that 35% to 40% of patients diagnosed with PM/DM will develop interstitial lung disease (ILD) during the course of their illness (2). Moreover, up to 56% of patients with myositis are positive to various autoantibodies, the most common ones being antisynthetase antibodies, which are directed against aminoacyl-transfer-RNA synthetases, a group of cytoplasmic enzymes that catalyse binding of an amino acid to its cognate tRNA (3, 4).

Patients with antisynthetase antibodies develop a clinical condition known as antisynthetase syndrome, which is characterised by ILD, myositis, arthritis, fever, Raynaud's phenomenon and mechanic's hands (4, 5). The reported frequency of ILD in these patients varies from 42% to 93%, depending on patient selection and the sensitivity of the tests applied to detect ILD (4-6). Interstitial lung disease is a major cause of morbidity and mortality in IIM patients, and is often resistant to immunosuppressive drugs (2). Several reports have suggested that cyclosporine or tacrolimus may be useful in refractory ILD in patients with antisynthetase syndrome (7-10), but overall, few patients have been studied in this regard.

The purpose of this study was to investigate the effect of calcineurin inhibitors (cyclosporine and tacrolimus) in a cohort of patients with antisynthetase-associated ILD.

Patients and methods

Patients

This is a cohort study with retrospectively collected data. Sixty patients positive to antisynthetase antibodies were identified from a cohort of 179 adult patients with IIM, seen at Vall d'Hebron General Hospital in Barcelona (Spain) between 1983 and 2011. From this group, only patients with ILD who had been treated with calcineurin inhibitors continually for at least 3 months were included in the study.

All patients were followed up in our outpatient clinic a minimum of 4 times per year. Pulmonary function testing was usually performed at diagnosis and at each outpatient clinical appointment, using the MasterLab Pro lung function

Table I. Characteristics of patients treated with calcineurin inhibitors.

Patient	Autoantibody	Diagnosis	Age at ILD onset, years	ILD/CNI	ILD diagnosis	Immuno-suppressive therapy
1§/F	Jo-1	DM	32	51	HRCT + PFT	MTX/AZA
2/M	Jo-1	DM	41	18	HRCT	AZA/CYF
3/F	Jo-1	PM	43	9	HRCT+PFT	AZA
4/F	Jo-1	PM	32	2	HRCT	AZA/MTX
5/F	Jo-1	DM	63	1	HRCT+PFT	None
6§/F	PL-12	ILD#	65	11	HRCT+PFT	None
7/F	Jo-1	DM	57	11	HRCT	MTX/MYF*
8/M	PL-12	ILD#	52	7	HRCT	None
9/F	Jo-1	PM	30	120	HRCT+PFT	AZA
10/M	Jo-1	ILD#	46	4	HRCT+PFT	CYF
11/F	Jo-1	DM	64	49	HRCT+PFT	MTX
12§/F	Jo-1	DM	32	114	HRCT+PFT	AZA/MYF
13§/M	Jo-1	DM	38	2	HRCT	None
14§/F	Jo-1	PM	34	5	HRCT	MTX
15/F	Jo-1	DM	42	12	HRCT+PFT	MTX

AZA: azathioprine; CNI: calcineurin inhibitors; CYF: monthly pulses of cyclophosphamide; DM: dermatomyositis; F: female; ILD: interstitial lung disease; ILD/CNI: lag time between ILD onset and start of calcinerin inhibitors; M: male; MTX: methotrexate; MYF: mycophenolate mofetil; PM: polymyositis; #: without myositis; §: patients treated with cyclosporine. All patients initially received a course of corticoids (1 mg/kg/day). *This patient received concomitantly tacrolimus and mycophenolate mofetil. HRCT: high resolution CT scan. PFT: pulmonary function test (forced vital capacity).

Table II. FVC (%) values before tacrolimus/cyclosporine treatment (baseline) and at last follow-up after starting treatment.

Patient	Baseline	CNI	Improvement (%)	Months*
1§	63	75	19	14
2	37	40	9	43
3	54	51	-6	27
4	69	55	-19	25
5	44	66	48	12
6§	57	75	32	3
7	69	93	34	27
8	76	81	5	30
9	50	49	-1	17
10	66	86	29	44
11	62	50	-19	17
12§	64	65	2	13
13§	72	94	29	19
14§	73	80	9	48
15	62	59	-4	17

CNI: calcineurin inhibitors; FVC: forced vital capacity.
§patients treated with cyclosporine; *number of months of treatment.

measurement system (Jaeger GmbH, Wuerzburg, Germany), according to the European Respiratory Society guidelines. Theoretical values for the Mediterranean population were used as reference.

Data on the clinical and serologic features, pulmonary function test results, and therapies were obtained by retrospective review of the patients' medical records and laboratory databases. The diagnoses of DM and PM were based on the criteria of Bohan and Peter, and only patients with definite or probable disease were included (11, 12). The diagnosis of ILD had been established in all patients by high resolution computed tomography (HRCT) features of ground glass opacities, honeycombing, fibrosis, and/or interstitial thickening, and additionally in some cases, a restrictive pattern on pulmonary function testing (forced vital capacity [FVC] <80%, forced expiratory volume in 1 second [FEV1] <70%, diffusing capacity of the lung for carbon monoxide [DLCO] <75%). The onset of ILD was defined by the presence of pathological findings on HRCT or pulmonary function tests and/or clinical symptoms (dyspnea, non-productive cough). All patients gave oral consent to the analysis and publication of their data. The main characteristics of the cohort are shown in Table I.

Determination of autoantibodies and HLA typing

Myositis-specific and myosistis-associated autoantibodies were identified by line immunoassay (Myositis Profile Euroline, Euroimmun, Lübeck, Germany) (13) or RNA and protein immunoprecipitation assay with radiolabelled HeLa cells. Antisynthetase antibodies were confirmed by at least two of the following techniques: ELISA line immunoassay (Myositis Profile Euroline, Euroimmun, Lübeck, Germany) or RNA and protein immunoprecipitation assay.

Calcineurin inhibitor treatment

Oral tacrolimus was given twice daily at a total dose of 0.075 mg/kg of body weight. Oral cyclosporine was given twice daily at a dose of 2–5 mg/kg of body weight. Dosage was adjusted depending on the patient's clinical response or side effects produced. Refractory cases were defined as those failing to respond to corticosteroids and at least one immunosuppressive agent. Treatment with tacrolimus was administered concomitantly with other immunosuppressants in only one case.

Outcome of ILD after treatment

Serial pulmonary function tests were performed to assess the response to treatment. Results were recorded as total values (litres) and as a percentage of the predicted normal value. The outcomes were categorised as improvement, stabilisation or deterioration. Improvement was defined as a ≥10% increase in FVC, according to the American Thoracic Society criteria regarding idiopathic pulmonary fibrosis (14). Deterioration was established when FVC decreased by ≥10%. The remaining results were considered stabilisation.

Statistical analysis

Sample size was not calculated: the patients included in the study were a con-

venience sample. Qualitative data are presented as number and percentage, and quantitative data as the median and IQR. Improvement plus stabilisation, as defined above, was the composite outcome analysed; the main percentages were calculated with the 95% confidence interval (95%CI). The Fisher exact test and Mann-Whitney U-test were used to compare clinical and laboratory chracteristics between patients with ILD improvement and the remaining patients. We followed the STROBE statement to improve the quality of reporting in observational studies (15).

Results

From 179 patients with IIM, 15 (11 females) fulfilled the inclusion criteria. Median age at onset of ILD was 42.3 years (IQR: 32.4–56.8), and the median duration of treatment with calcineurin inhibitors was 19 months (IQR: 14–30). Thirteen patients tested positive to anti-Jo-1 and two to anti-PL-12. Patients were ultimately classified as having PM (4 cases), DM (8 cases), or antisynthetase-related pure ILD, without myositis (3 cases).

Eleven of the 15 patients analysed received calcineurin inhibitors, because of failure to respond to corticosteroids and at least 1 immunosuppressive agent. Six patients had been treated previously with azathioprine, 6 with methotrexate, 2 with cyclophosphamide pulses, and 2 with mycophenolate mofetil (Table I). One patient (case 7) developed Epstein Barr-associated Hodgkin lymphoma after 24 months of tacrolimus and mycophenolate therapy, and died. Tacrolimus was switched to cyclosporine due to gastrointestinal intolerance in one patient. No other side effects (*e.g.* hypertension, nephrotoxicity) were seen in the others. Because of the good tolerance and efficacy observed in these cases with the use of calcineurin inhibitors, these drugs were given as the first therapeutic option in the remaining 4 patients.

In the overall cohort, the median duration of ILD follow-up was 24 months (IQR: 12–32), whereas the median time between ILD onset and start of treatment with calcineurin inhibitors was 11 months (IQR: 5–49). Before beginning treatment, patients had the following median (IQR) pulmonary function values: FVC 63% (IQR 53.9–69.6) and 2.02 L (IQR 1.67–2.79), FEV1 69% (IQR 59.5–83.1) and 1.83 L (IQR 1.48–2.63), and DLCO 47% (IQR 34.4–53.5). After tacrolimus or cyclosporine therapy, improvement (≥10% increase in FCV) was achieved in 6 patients, pulmonary function values stabilised in 7 others, and deterioration (≥10% drop in FCV) was observed in 2 patients. Thus, lung function improved or stabilised in 13 patients treated with calcineurin inhibitors (87%; 95%CI 56–98). When patients were analysed separately according to whether they had receive these drugs as first-line therapy (4 patients) or because of refractory disease (11 patients), 100% of the former and 81% of the latter patients improved or stabilised. Comparison of clinical characteristics (fever, Raynaud phenomenon, arthritis) and laboratory data (creatine kinase levels) between patients with ILD improvement and the remainder of the series disclosed statistically significant differences only with regard to Raynaud phenomenon, which occurred more frequently in patients who did not improve ($p<0.05$).

Discussion

Our study shows that calcineurin inhibitors are useful in the treatment of antisynthetase-associated ILD, not only in refractory cases, but also as first-line therapy. Few reports have focused on use of the calcineurin inhibitors, cyclosporine and tacrolimus, in antisynthetase-associated ILD (7-10). Thus, we conducted this observational study in our large myositis cohort to assess the effect of these drugs in antisynthetase syndrome-associated ILD. We mention that all the patients reported here were affected by the classical antisynthetase syndrome-related ILD, and not the rare acute respiratory distress syndrome occasionally related with antisynthetase syndrome, which usually carries a much worse prognosis (16). We found out that pulmonary function improved or stabilised after therapy in 87% of our patients. All 4 patients who received calcineurin inhibitors as first-line therapy along with corticosteroids showed improved or stabilised lung function. In refractory cases, the results were also positive: only 2 of the 11 cases did not have a favourable outcome.

The immune mechanism that underlies antisynthetase-associated ILD is uncertain, although some authors have reported a predominance of $CD8^+$ activated T cells in bronchoalveolar lavage or lung tissue. Sauty *et al.* (17) described 4 patients with antisynthetase antibodies and nonspecific interstitial pneumonitis diagnosed by transbronchial biopsies, with bronchoalveolar lavage consistent on lymphocytosis and a very low CD4/CD8 ratio. $CD8^+$ expression has also been demonstrated by other research groups (18, 19).

Although the presence of autoantibodies in antisynthetase syndrome implies participation of humoral immunity, the role and pathogenicity of these autoantibodies remains unclear. Some data have indicated that there exists a T-cell response directed against histidyl-tRNA synthetase that might drive autoantibody formation and tissue damage (20). The findings, based on activated $CD8^+$ T cells described above, indicate that T-cell targeted therapies such as calcineurin inhibitors might be of benefit in these patients (21).

There are several limitations to this study, perhaps the most important being its retrospective nature. In addition, the small sample size due to the rarity of the syndrome likely precludes generalisation of the results. It may also be argued that some patients had been treated with corticosteroids and other immunosuppressive drugs, and it would be difficult to discern the effect of these therapies on the final result. Nonetheless, in all these cases therapy had been changed to calcineurin inhibitors because of a poor response or clinical worsening. Hence, it can be assumed that the final effect resulted from calcineurin inhibitor treatment.

In conclusion, despite the limitations of the study, our data indicate that calcineurin inhibitors can be considered an effective, relatively safe therapy for antisynthetase-associated ILD, not only in refractory cases, but also as first-line treatment along with glucocorticoids.

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