**Table 5** Multivariate model of predictors of infusion reaction by logistic regression

| | Estimates | OR | 95% CI | p Value |
|---|---|---|---|---|
| *FCGR3B* NA1/1: NA2/2+NA1/2 | 0.90 | 6.1 | 1.9 to 24.3 | <0.01 |
| Glucocorticoid use | −0.68 | 0.26 | 0.08 to 0.84 | 0.03 |

We found that 19% of the patients with RA developed AIA during the 52-week study period. The reported prevalence of AIA in patients with RA varies from 12% to 44% and appears to be inversely proportional to the serum levels of infliximab and therapeutic response.[28] The dose of MTX did not significantly affect the development of AIA in our study (data not shown). In this study, infusion reactions frequently occurred between 14 and 30 weeks after initiating infliximab. Since the infusion interval was increased to 8 weeks after induction at 0, 2 and 6 weeks, the serum trough levels of infliximab probably lowered at the infusions with 8-week intervals, supporting the above observation in this study. However, the precise mechanisms related to AIA and the development of infusion reactions are not fully understood and require further study.

Numerous studies have analysed the possible association between *FCGR2A* and *FCGR3A* polymorphisms and the efficacy of biological agents against TNFα in patients with RA.[29–31] However, the association between these polymorphisms and the adverse effects of anti-TNFα has not been fully explored. In this regard, the *FCGR3B* NA2 allele has been shown to be associated with urinary tract infections in patients with RA treated with MTX or etanercept.[32] FcγRIIIb is expressed exclusively on neutrophils, eosinophils[12] and basophils.[33] The isoform containing the *FCGR3B* NA1 allele produces larger phagocytic, oxidative burst and degranulation responses than the *FCGR3B* NA2 allele.[32] Thus, the *FCGR3B* NA1/NA1 genotype with high affinity to Ig present on the surface of neutrophils, eosinophils and basophils may account for the higher incidence of infusion reactions to infliximab. In addition, optimal ligand concentrations leading to formation of immune complexes may allow binding to FcγRIIIb and subsequent activation of cells.[34] Recently, variation in the copy number of *FCGR3B* has been shown to be associated with susceptibility to systemic autoimmunity.[35] Copy number variation of *FCGR3B* may play a role in infusion reactions and warrants further examination.

One may speculate that glucocorticoids may interfere with the binding, activation and effector function of immune cells, thereby reducing the severity and frequency of infusion reactions. Some of these effects may be shared with MTX. In this study, the concomitant use of oral glucocorticoids was significantly associated with a reduced risk of developing an infusion reaction, consistent with recent reports.[9 36] Therefore, not only the moderate- to high-dose glucocorticoids given as a premedication, but also the low-dose daily glucocorticoids may be a potent inhibitor of infusion reactions. FcγRIIIb is a phosphatidyl inositol-linked cell surface protein and thus lacks any self-kinase activity. Instead, FcγRIIa is coupled with FcγRIIIb to transduce signals. As one of the functional polymorphisms in FcγRIIa is the 131H allele which confers receptor affinity to IgG2 subclass, it is unlikely that this receptor plays a direct role in binding to the IgG1 monoclonal antibody infliximab. Nevertheless, the possibility that other polymorphisms of *FCGR2A* associated with receptor function contribute to infusion reactions should be examined.

There are a number of limitations that warrant mention. First, the number of patients examined in this study was insufficient to demonstrate an association between *FCGR3A* polymorphism and infusion reactions. Second, in addition to the small number of patients, other aspects of the study design also imposed limitations. For example, the study was designed to monitor infusion reactions during 52 weeks, meaning that infusion reactions appearing after 52 weeks could not be addressed. Third, this was an open-label study which may have affected the incidence and severity of infusion reactions and the response and retention rates. However, the incidence and the types of infusion reactions in this study were comparable to those of previous reports, as were the other results related to the efficacy and retention rate of infliximab.[37 38] Also, AIA is typically developed during the treatment period and this information would not be available before starting infliximab; it is apparent that the presence of AIA is limited in its use as a predictive marker. When the two risk factors excluding AIA are used as predictive variables, they were still associated with approximately 40% of infusion reactions observed in this study (data not shown).

In summary, we have shown that the *FCGR3B* NA1/NA1 genotype and the absence of glucocorticoid usage are predictive factors of infusion reactions in patients with RA. Premedication for infliximab may therefore not be necessary for all patients but only for those with the *FCGR3B* NA1/NA1 genotype without daily glucocorticoids.

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## IgG4-positive multi-organ lymphoproliferative syndrome manifesting as chronic symmetrical sclerosing dacryo-sialadenitis with subsequent secondary portal hypertension and remarkable IgG4-linked IL-4 elevation

Sir, Immunoglobulin G (IgG) 4-positive multi-organ lymphoproliferative syndrome (IgG4 + MOLPS), also known as IgG4-related systemic disease [1–3] and others, is an emerging clinical entity [2, 3] that exhibits a variety of clinical manifestations, including autoimmune pancreatitis, inflammatory pseudotumour and chronic symmetrical sclerosing dacryo-sialadenitis [CSSD; or Mikulicz disease (MD)] [2–8]. We report a patient initially diagnosed with SS, but subsequently recognized with CSSD-manifesting IgG4 + MOLPS after re-presenting with symptoms of secondary portal hypertension (SPH).

A 55-year-old female was admitted in September 2004 with a 2-month history of abdominal distention against a 18-month background of SS satisfying the 2002 Revised American–European Classification Criteria [9], with dryness of the eyes and mouth, bilateral lacrimal and salivary gland enlargement, lymphocyte infiltration on salivary gland biopsy, positive Schirmer's test and decreased salivary gland function on $^{99m}$Tc scintigraphy. Abdominal findings confirmed ascites, and lacrimal and salivary glands were enlarged bilaterally. Serum autoantibodies including anti-Ro or SSA and anti-La or SSB were negative and abdominal CT showed multiple abdominal masses in the hepatic hilar and mesenteric regions. Pathological findings are shown in Figure 1A–C.

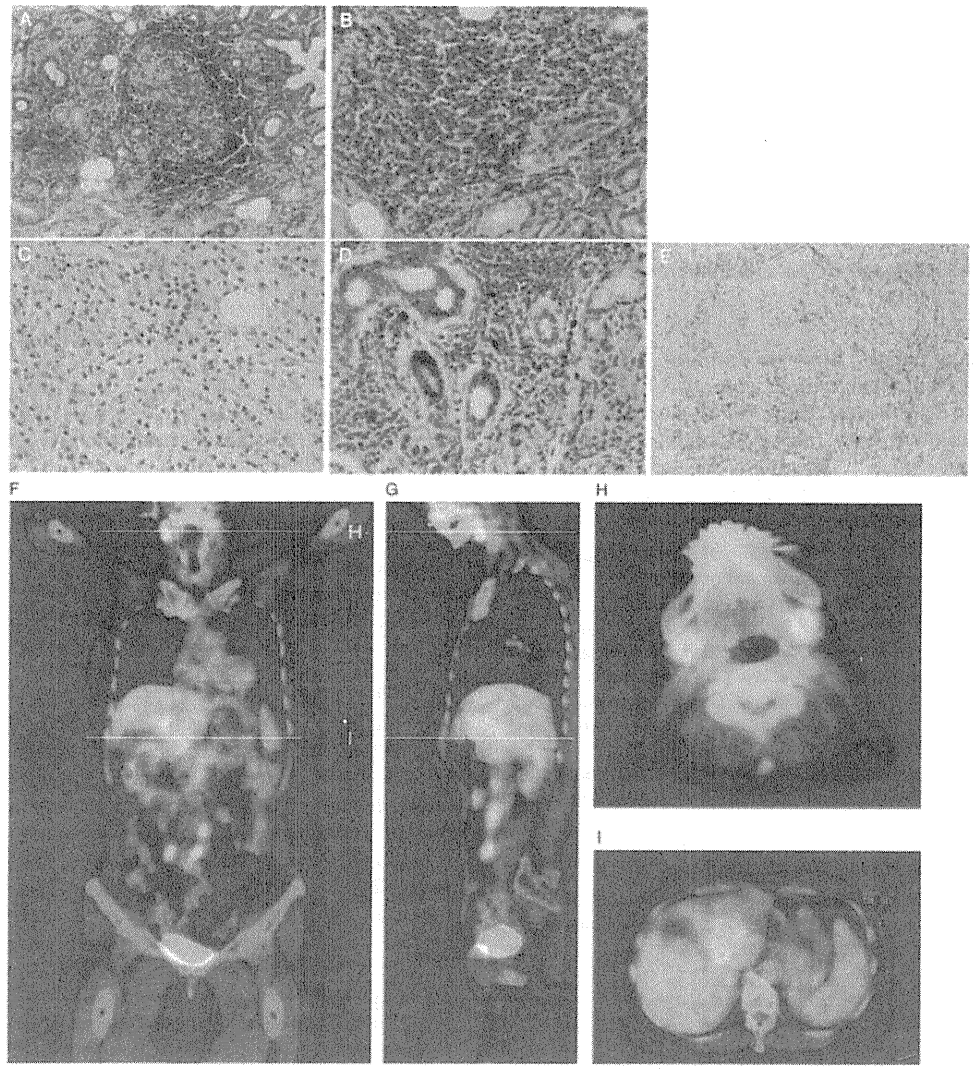
Discharging against medical advice, she subsequently re-presented in December 2005 and August 2006 for upper gastrointestinal variceal bleeding. Imaging results are shown in Figure 1F–I. Further literature search revealed a histopathological similarity with IgG4-related autoimmune pancreatitis, and a then-recent case series describing raised serum IgG4 levels and infiltration of lacrimal and salivary glands and lymphoid tissue by IgG4-expressing cells in 7 so-called MD patients but not 14 SS patients [1]. Subsequent investigation showed elevated serum IgG4 (1100 mg/dl; normal range 4.8–105 mg/dl). Immunohistochemistry of the biopsied lesions for IgG4 is shown in Figure 1D and E. We consequently diagnosed CSSD-manifesting IgG4 + MOLPS rather than SS [2].

Corticosteroid (CS) therapy was started with oral prednisolone [60 mg/day (= 1 mg/kg/day)]. The glandular swelling showed complete resolution at 2 weeks, whereas the abdominal distention, abdominal masses and varices showed partial but significant regression on CT and endoscopy at 3 months. Serum IgG4 decreased, but remained elevated at 4 weeks (530 mg/dl). Further, immune complexes involving all IgG subtypes, including IgG4, were also decreased at 4 weeks. Among pro-inflammatory cytokines measured at the start of treatment, two were significantly elevated, with IL-4 at 51.9 pg/ml (normal range 6.0) and IL-6 at 12.5 pg/ml (normal range 4.0). While IL-6 quickly decreased to normal (0.7 pg/ml), IL-4 also decreased but remained high (31.7 pg/ml) at 2 weeks. This decrease in IL-4 was largely consistent with the decreased but still elevated levels of IgG4 at 4 weeks. CS was tapered to 6 mg/day of oral methylprednisolone (mPSL) at 6 months. Subsequent serology showed a declining but still elevated IgG4 (386 mg/dl) and continued elevation of IL-4 (32.5 pg/ml). Presently, a mild, persistent elevation of IgG4 (282 mg/dl) remains. Lacrimal and salivary gland size and function are normal, and the patient is well on maintenance mPSL at 4 mg/day.

This case fulfilled the proposed diagnostic criteria for both MD [1] and IgG4 + MOLPS [2], strengthening the validity of the diagnosis, but also fulfilled the diagnostic criteria for SS [9]. This case therefore highlights the need for comprehensive diagnostic criteria that provide both high sensitivity for this emerging clinical syndrome and specificity from potentially clinically similar entities such as SS.

Establishing an early diagnosis of IgG4 + MOLPS lessens unnecessary investigations and offers the possibility of an effective treatment. Consistent with previous reports, IgG4 appeared to be a useful serological marker in both diagnosis and monitoring [2, 4, 5]. PET-CT was more informative [8] than conventional CT or $^{67}$Ga scintigraphy, providing valuable information on sites that were not suitable for biopsy, although this is still required to exclude malignancy. Further, CS had a significant effect on the lacrimal and salivary gland lesions, but only a partial effect on the mesenteric and hepatic hilar masses. Other studies have reported significant and often complete normalization of glandular size and function with CS, further emphasizing the importance of timely diagnosis [2, 4–6, 10]. Concerning the extra-glandular

FIG. 1 Investigational findings on initial and subsequent presentations leading to a diagnosis of CSSD-manifesting IgG4 + MOLPS. Initial pathological examination on haematoxylin and eosin staining of minor salivary gland [(**A**) Low magnification and (**B**) High magnification], hepatic hilar (**C**) and mesenteric lesion (not shown) biopsies shows significant plasma cell infiltration with fibrosis in all specimens, with lymphocytes predominating over plasma cells in the glandular lesions, whereas the reverse is seen in the extra-glandular lesions, accompanied by an increase in fibrosis. No evidence of malignancy was detected in any specimen, and the initial pathological diagnosis was inflammatory pseudotumour. Co-registered [$^{18}$F]fluorodeoxyglucose PET (FDG-PET) and CT images on first re-admission show the clear persistence of the bilateral lacrimal and salivary gland, hepatic hilar and multiple mesenteric masses in coronal (**F**) and sagittal (**G**) views of the whole body and axial views at two levels (**H** and **I**). Immunohistochemical examination shows IgG4-expressing plasma cells in salivary gland (**D**) and hepatic hilar (**E**) lesions.




lesions, the differing histopathological patterns of lymphocyte: plasma cell ratios and accompanying fibrosis and differing response levels to CS may suggest a specific window of therapeutic opportunity and CS sensitivity [3].

Importantly, by demonstrating analogous immunohistochemical findings in both glandular and extra-glandular lesions, our case provides a clear illustration of the systemic nature of IgG4 + MOLPS [2, 3, 6, 7]. Further, the role of IgG4 in IgG4 + MOLPS remains unclear [3, 5] and a finding of hyper-IgG4 may not necessarily indicate a central aetiological role for IgG4. Interestingly, the elevated IL-4 in the active phase decreased with CS, largely consistent with changes in IgG4 levels.

This case highlights the systemic nature of IgG4+ MOLPS and the importance of distinguishing it from clinically similar entities such as SS. Clinical recognition of IgG4+MOLPS is important, as CS may provide complete relief from symptoms and reduce morbidity, particularly if instigated early in the course of the disease. Comprehensive, validated diagnostic criteria and a greater understanding of the pathogenesis of IgG4+ MOLPS are required.

**Rheumatology key message**

- CSSD-manifesting IgG4+MOLPS can present with SPH and remarkable IgG4-linked IL-4 elevation.

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### Severe pneumonia by aciclovir-resistant varicella-zoster virus during etanercept therapy

Sir, Patients treated with antagonists of TNF-α (anti-TNF-α) are at increased risk of bacterial infections [1]. Compared with bacterial infection, little is known about the risk of viral infections in patients undergoing therapy with anti-TNF-α agents. Immunodeficiency is known to trigger the reactivation of varicella-zoster virus (VZV), one of the most common adverse events reported in clinical trials of anti-TNF-α agents [1]. Recent studies observed a mildly increased risk of herpes zoster reactivation in patients treated with anti-TNF-α agents [2–4]. Nevertheless, therapy with the anti-TNF-α agent etanercept was not associated with an enhanced risk of herpes zoster [2, 3].

During therapy with etanercept, disseminated or invasive disease by reactivation of the VZV, has seldom been reported [5], and no case of VZV pneumonia has been published. We report a case of severe pneumonia by aciclovir-resistant VZV, occurring in a patient with PsA during therapy with etanercept.

A 63-year-old male with PsA, treated with etanercept during 6 months, was admitted for a multi-dermatomal herpes zoster and fever (up to 38.5°C). Creatinine was

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ORIGINAL ARTICLE

# Single center prospective study of tacrolimus efficacy and safety in the treatment of various manifestations in systemic lupus erythematosus

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**Abstract** The aim of this study was to prospectively evaluate the efficacy and safety of tacrolimus (TAC) in various manifestations of systemic lupus erythematosus (SLE) patients in daily clinical practice. Each of the 21 TAC-treated patients with SLE in our care over 2 years was enrolled in this open-label trial. Patients were administered TAC at a dosage of 1–6 mg once daily, followed up for 24 weeks. Efficacy and safety were evaluated utilizing clinical and laboratory findings. As treatment targets, TAC was preferentially used with oral corticosteroid administration for mild active manifestations such as arthritis, skin eruptions, or asymptomatic nephritis. In efficacy, the mean value of the SLE disease activity index was significantly reduced to 4.1, 2.7, 1.8, and 1.2 ($N = 21$, 20, 16 and 13) at 0, 4, 12, and 24 weeks, respectively. In eight cases, treatment was discontinued within 24 weeks due to insufficient effects (6 cases) and side effects (2 cases). Non-serious side effects were observed in only five cases (23.8%) over 24 weeks. TAC can be considered both effective and safe for the treatment of various manifestations of SLE.

**Keywords** Tacrolimus · Systemic lupus erythematosus · T cell

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## Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by an aberrant production of autoantibodies and immune complexes [1–3]. While corticosteroids (CS) and immunosuppressants are widely used for treatment, thereby creating a certain effect toward a higher rate of induction and maintenance therapy, more than a few resistant cases have been experienced. A new treatment has been strongly sought in the clinical setting [4].

Tacrolimus (TAC) is a relatively new type of medication that induces an immunosuppressive effect, specifically inhibiting the calcineurin pathway in T cells and reducing accompanying inflammatory cytokine production [5–7]. TAC has been approved for worldwide use in organ transplantation [8–11] and for treating autoimmune diseases, including myasthenia gravis [12]. In 2004, the use of TAC for the treatment of rheumatoid arthritis (RA) had been approved in Canada and Japan. A great deal of clinical evidence was accumulated, including our reports on RA [13–16]. More recently, in 2006, TAC was approved for lupus nephritis with intolerance to be the previously normal treatment in Japan, first in the world. However, its efficacy and safety for various manifestations of SLE have not been clearly investigated. We have prospectively evaluated the efficacy and safety of TAC in SLE, focusing especially on various lupus manifestations beyond nephritis in the clinical setting; this report forms the basis for the appropriate practical use of TAC in various conditions of SLE.

## Patients and methods

This study is a single-center open-label prospective 24-week observational study which took place from April

2005 to March 2007 in a typical clinical practice. Twenty-one SLE patients, fulfilling the American College of Rheumatology's 1997 revised criteria for the classification of SLE [17] were enrolled. They all exhibited a resistance to or intolerance to other treatments, or potential difficulties with other treatments were foreseen because of anticipated complications. Patients were administered TAC at a dose of 1–3 mg (with the exception of 6 mg in one case) once a day in the evening for 24 weeks. The use of other drugs in combination was not restricted in any way.

Disease activity and clinical responses were evaluated utilizing the SLE disease activity index (SLEDAI) [18] at 0, 4, 12, and 24 weeks. The final efficacy evaluation of TAC was assessed by satisfying all of the following criteria: (1) a decrease of SLEDAI at 24 weeks from week 0 (baseline), (2) the continuation of TAC at 24 weeks, and (3) no increase of CS and no addition of immunosuppressive drugs. Safety was evaluated by examining clinical signs and symptoms in combination with laboratory findings, including the following: complete blood counts, general biochemistry and serum glucose, urinalysis, chest X-ray, and appropriate additional tests in cases of suspected adverse events. Each physician involved with this study decided independently on the continuation or discontinuation and the dosage adjustments of TAC.

Blood concentrations of TAC at 12 h after administration were measured at 4 weeks after starting the TAC regimen, with the exception of three cases; these levels were used for determining dose adjustments of TAC. After 4 weeks, they were measured if necessary.

For statistical analysis, we utilized a paired $t$ test for the comparison of two groups and a non-repeated ANOVA test for the comparison of multiple groups. A $P$ value of $<0.05$ was considered statistically significant ($^{*}P < 0.05$, $^{**}P < 0.01$).

## Results

### Patient background

Twenty-one SLE patients were enrolled in this study. Summarized demographics and baseline characteristics of all cases are shown in Table 1. The mean age was 34.8 years and 20 patients were females. Regarding subtypes of the disease, 24% of the patient had renal involvement. A few cases of syndromes overlapping with other collagen vascular diseases, including RA, were enrolled and all of them were used for lupus manifestations and evaluated. Regarding causes of administration, 16 cases of recurrences of disease, 4 cases of maintenance therapy-seeking for reduction of CS amounts, and only one case of first induction were included. Most cases had previously only used CS with the mean dose (± standard deviation) being prednisone at 13.4 ± 8.3 mg/day. Immunosuppressants, such as cyclosporine A (CyA), had also previously been used in eight cases.

**Table 1** Summarized demographics and baseline characteristics of 21 SLE cases

| | |
|---|---|
| Number of cases | 21 |
| Age (mean ± SD, range) | 34.8 ± 11.4 (16–75) |
| Sex (male/female) | 1/20 |
| Subtype of disease | Renal 5 |
| | Non-renal 16 |
| Cause of administration | First induction 1 |
| | Recurrence 16 (insufficient 14, side effects 2) |
| | Maintenance 4 (for reduction of CS amount 4) |
| Just previous treatment | CS 20 (PSL 13.4 ± 8.3 (2–35) mg/day) |
| | IS 8 (CyA 6, CPA 1, MZB 1) |
| | IVIG 1 |
| Disease activity index (SLEDAI) at start | 4.2 ± 2.0 (1–8) |
| Target manifestation | Arthritis 8, skin eruption 5, nephropathy 5 |
| | Immunological abnormality 4, cytopenia 1 |
| | Alopecia 1, myositis 1 |
| Combination therapy at start (mean ± SD) | CS 21 [PSL: 13.3 ± 7.9 (mg/day)] |
| | IS 1 (CPA 1) |
| Dose of TAC at start (mean ± SD, range) | 2.5 ± 1.0 (1–6) |

*CS* Corticosteroids, *CPA* cyclophosphamide, *CyA* cyclosporin A, *IS* immunosuppressant, *IVIG* intravenous immunoglobulin, *MZB* mizoribine, *SLEDAI* SLE disease activity index, *TAC* tacrolimus

Mean scores of SLEDAI at baseline were 4.2 ± 2.0. The disease activity of enrolled patients was mild to moderate. Target manifestations of TAC treatments were diverse, including arthritis in 8; skin eruptions in 5; nephropathy in 5; immunological abnormalities in 4; and cytopenia, alopecia, and myositis in one case. All cases used CS in combination with TAC from the onset and the mean dose of predonisone was 13.3 ± 7.9 mg/day, almost equal to that prior to the start of TAC treatments. Only one case was orally concomitant with cyclophosphamide (CPA). The mean dose of TAC from the start was 2.5 ± 1.0 mg/day.

### Efficacy

As a result of efficacy assessments, changes in SLEDAI scores in all cases are shown (Fig. 1a). Mean SLEDAI scores improved significantly at each of 4, 12, and 24 weeks from baseline in continuing cases. TAC continuation rates at

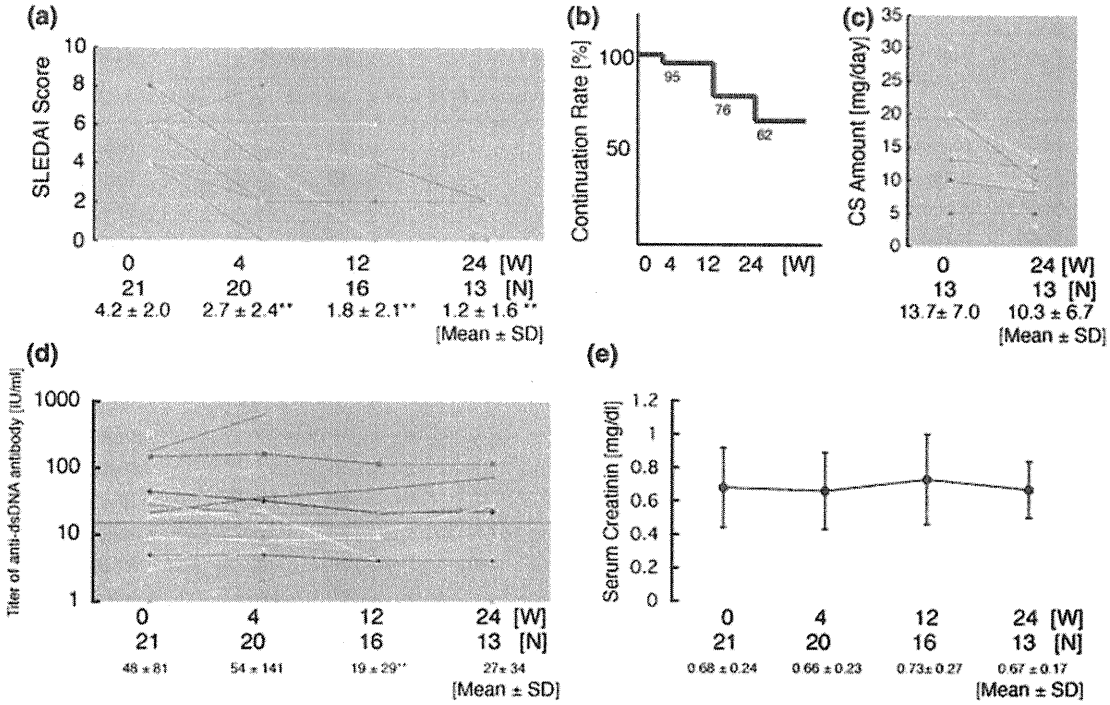


**Fig. 1** Efficacy and safety of tacrolimus in 21 SLE cases. **a** Time courses of SLEDAI scores after start of tacrolimus are shown. Mean values and standard deviations of SLEDAI and the number of assessed patients are also indicated. **b** Continuation rates (%) of tacrolimus at 0, 4, 12, and 24 weeks are shown. **c** Corticosteroid amounts at 0 and 24 weeks in all 13 cases that received 24 weeks of tacrolimus. Mean amounts and standard deviations of prednisolone amounts for the 13 cases are also indicated. **d** Time courses of serum anti-dsDNA antibody titers after start of tacrolimus are shown. Mean values and standard deviations of the titers and number of assessed patients are also indicated. Normal range of serum anti-dsDNA antibody titers is below 20 IU/ml in our laboratory. **e** Time courses of serum creatinine after start of tacrolimus are shown. Mean values and standard deviations of the values and the number of assessed patients are also indicated. Normal range of serum creatinine is from 0.4 to 1.1 mg/dl in our laboratory. $*P < 0.05$ and $**P < 0.01$, respectively

4, 12, and 24 weeks are shown (Fig. 1b). In the 13 cases that continued for 24 weeks, mean CS amounts were successfully reduced at 24 weeks compared with baseline (Fig. 1c). Only one of those 13 cases required an increase of CS at 24 weeks (Table 2). Although there was no significant difference in CS amounts between at start and at 24 weeks in those 13 cases, in the sub group of patients except one exacerbated case, there were significant differences ($P < 0.05$) by paired $t$ test.

The mean titer amounts of anti-dsDNA antibodies, as a representative indicator of immunological parameters reflecting disease activity, were also reduced at 12 and 24 weeks compared with baseline (Fig. 1d).

In all cases, we also assessed efficacy by the last observation carried forward (LOCF). Ten of 21 cases (47.6%) satisfied efficacy criteria for mild to moderate disease activity, including various manifestations of SLE patients at 24 weeks.

We searched predictors for the continuation or the effectiveness, but there were no significant differences in age, sex, major target organ, initial dose of TAC, SLEDAI (at start) and CS, between those two groups.

## Safety

Physician-determined definitive or undeniable adverse drug reactions relating to TAC were observed in five cases (23.8%) over 24 weeks (Table 3). Three of these cases were symptomatic events in the gastrointestinal tract, including epigastric discomfort, nausea, and diarrhea. Cervical lymphadenopathy was observed in one case. Finger tremors were observed in one case, although this had previously appeared with CyA treatment in the patients, and the physician continued TAC. In the four other cases, treatments were stopped at the onset of the events. None of the cases were considered to be serious and each was completely ameliorated by appropriate countermeasures [drug discontinuation (4) or careful observation (1)]. In four of five cases (80%), these events occurred within 2 months.

In three of the above five cases, the blood concentration levels of TAC were measured at the adverse events. In two cases, the levels were below 10 ng/ml, but in the one case that was administered 6 mg/day, the level was 38.0 ng/ml and highly elevated at the time of the adverse events. Nausea turned out to be caused by high concentrations of TAC.

**Table 2** List of detailed information on the TAC treatment for various manifestations

| Case # | Age/sex | Major target manifestations | Initial dose of TAC (mg/day) | SLEDAI | | CS amount | | Efficacy assessment |
|---|---|---|---|---|---|---|---|---|
| | | | | At start | At 24 weeks | At start | At 24 weeks | At 24 weeks |
| SLE1 | 28/F | Immunological abnormality | 2 | 4 | 2 | 5 | 5 | Effective |
| SLE2 | 30/F | Arthritis | 3 | 6 | – | 10 | – | Insufficient (discontinue) |
| SLE3 | 34/F | Arthritis | 3 | 4 | 0 | 10 | 9 | Effective |
| SLE4 | 30/F | Arthritis | 1.5 | 4 | 5 | 10 | 30 | Insufficient (continue) |
| SLE5 | 37/F | Arthritis, alopecia | 2 | 6 | 2 | 13 | 8 | Effective |
| SLE6 | 71/F | Arthritis, proteinuria | 1 | 8 | 0 | 13 | 12 | Effective |
| SLE7 | 34/F | Skin eruption | 3 | 4 | 0 | 10 | 8 | Effective |
| SLE8 | 21/F | Skin eruption | 3 | 2 | 2 | 5 | 5 | Insufficient (continue) |
| SLE9 | 44/F | Cytopenia | 2 | 1 | 3 | 20 | 10 | Insufficient (continue) |
| SLE10 | 42/F | Skin eruption | 2 | 2 | 2 | 10 | 7 | Insufficient (continue) |
| SLE11 | 36/F | Immunological abnormality | 2 | 2 | – | 10 | – | Adv. events (discontinue) |
| SLE12 | 48/M | Proteinuria | 2 | 6 | – | 10 | – | Insufficient (discontinue) |
| SLE13 | 28/F | Immunological abnormality | 3 | 6 | 0 | 30 | 8 | Effective |
| SLE14 | 29/F | Skin eruption | 2 | 2 | 0 | 20 | 3 | Effective |
| SLE15 | 38/F | Proteinuria | 2 | 4 | – | 10 | – | Insufficient (continue) |
| SLE16 | 16/F | Proteinuria, cytopenia | 6 | 6 | – | 8 | – | Adv. events (discontinue) |
| SLE17 | 31/F | Immunol. abnormality, arthritis | 2 | 4 | – | 5 | – | Insufficient (discontinue) |
| SLE18 | 41/F | Arthritis | 3 | 8 | 0 | 20 | 13 | Effective |
| SLE19 | 23/F | Proteinuria | 2 | 4 | 0 | 12.5 | 12.5 | Effective |
| SLE20 | 30/F | Arthritis | 3 | 4 | – | 5 | – | Insufficient (discontinue) |
| SLE21 | 41/F | Skin eruption | 3 | 2 | – | 16 | – | Insufficient (discontinue) |

*Adv. events* Adverse events, *CS* corticosteroid, *ILD* interstitial lung disease, *SLEDAI* SLE disease activity index

**Table 3** Definitive or undeniable adverse events relating to tacrolimus in 21 SLE cases

| Case # | Age/sex | Adverse events | | | | Dose of TAC at event (mg/day) | Blood conc. of TAC at event (ng/ml) |
|---|---|---|---|---|---|---|---|
| | | Onset (M) | Type | Measures | Outcomes | | |
| SLE4 | 30/F | 2 | Epigastric discomforts | Stop | Improved and restart | 1.5 | 1.4 |
| SLE8 | 21/F | 1 | Diarrhea | Continue | Improved | 3 | ND |
| SLE11 | 36/F | 4 | Finger tremor | Stop | Discontinue (previously appeared in CyA treatment) | 2 | ND |
| SLE16 | 16/F | 2 | Nausea | Stop | Improved and restart in lower dose | 6 | 38.0 |
| SLE21 | 41/F | 1 | Cervical lymphadenopathy | Stop | Improved and restart | 3 | 6.1 |

*Blood conc.* Blood concentration, *ND* no data

After TAC was disrupted, this symptom improved and TAC was restarted at a lower dose.

Mean serum creatinine levels at 0, 4, 12, and 24 weeks were $0.68 \pm 0.24$, $0.66 \pm 0.23$, $0.73 \pm 0.27$, and $0.67 \pm 0.17$, respectively. There were no statistically significant differences (i.e. non-repeated measures of ANOVA) among the four groups (Fig. 1e).

## Blood concentration

Blood concentration levels of TAC were measured in 18 cases, with the exception of 3 cases (SLE8, 11, and 20), and correlations of initial doses with blood concentration levels of TAC are shown in Fig. 2a. The mean concentration of 18 cases was $4.3 \pm 2.4$ ng/ml. The mean concentration of

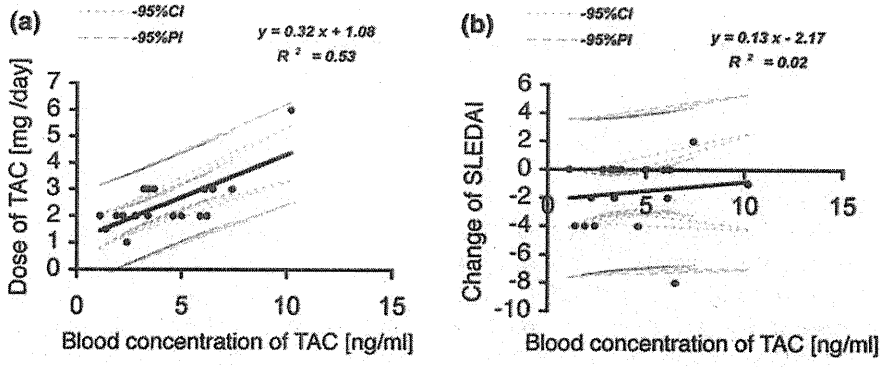


**Fig. 2** Correlation between initial dose of TAC and blood concentration of TAC or change of SLEDAI. **a** Correlation between initial dose of TAC and blood concentration of TAC is shown ($N = 18$). **b** Correlation between initial dose of TAC and change of SLEDAI is shown ($N = 18$). *Closed circles* show each patient. The linear regression equations (including 95% confidence interval and 95% prediction interval) are also indicated as *solid* and *dashed lines*, respectively

TAC in four dose groups (<2, 2, 3, and 6 mg/day) was $1.9 \pm 0.7$ ($N = 2$), $3.7 \pm 1.8$ ($N = 9$), $5.1 \pm 1.8$ ($N = 6$), and $10.6 \pm 0.0$ ng/ml ($N = 1$), respectively. Blood concentration levels were basically dose-dependent, but these levels had a wide range in each individual patient within the same dose groups. There were positive correlations ($R^2 = 0.52$) and statistically significant differences ($P = 0.0006$).

On the other hand, no significant correlation was observed between the blood concentration levels of TAC, the dose of TAC (Fig. 2b), and SLEDAI improvement at 4 weeks from base line. Only in the 6 mg/day administration case was a high concentration level of TAC (10.2 ng/ml) observed, without any signs or symptoms. In this case, the medication was continued for 2 months, nausea appeared, and then TAC was discontinued as described in the earlier subsection on safety.

## Discussion

In this report, we have demonstrated that TAC is both effective and safe in the treatment of various manifestations of SLE patients in a clinical setting. In our case series, for nearly half of the patients, 10 of 21 cases (47.6%), TAC was effective for mild to moderate disease activity, including various manifestations of SLE at 24 weeks without an increase of CS. Moreover, regarding safety, non-serious side effects were observed in only five cases (23.8%) over 24 weeks. The results of our prospective study support the finding that TAC is a new, effective agent for treating SLE.

While various immune response abnormalities were identified in SLE patients, especially aberrant and persistent T cell abnormalities, involving signal transduction defects, have been recognized as important factors in disease formation in the most recent decade [3]. Thus, a T cell blockade is considered to be quite reasonable as a potential therapeutic target for SLE.

Upon reviewing past reports of TAC treatment for SLE, a case series on three refractory SLE was first described by Duddridge and Powell [19]. Two of three cases were well-controlled and previous persistent vasculitis had been resolved; other features of active disease were controlled. The third patient's vasculitis had not improved significantly after 2 months of treatment, and TAC was discontinued because of nephrotoxicity. Second, a case series on the successful usage of TAC in eight SLE cases, including six patients with refractory nephritis and two with antiphospholipid syndrome, had been reported [20]. After that, several successful reports on TAC's efficacy for nephritis [21–27], nephropathy after kidney transplantation for SLE [28, 29], pediatric lupus nephritis [30], lupus cystitis [31], and neuromyelitis optica [32], were accumulated. Topical use of TAC, which had been approved for atopic dermatitis, had also begun to be tried for cutaneous lesions in lupus [33–36]. A few clinical studies on TAC for collagen vascular diseases other than RA have also been reported [37–40].

In designing our study, all patients at a specific period were involved, to avoid selection bias, which is different from many of the aforementioned case series. Patients were assigned according to the decisions of the involved physicians, as opposed to assignation by randomization. Our study focused on the real-world ordinary practice of TAC treatments for SLE; thus a full randomization study was not well suited to our purposes, nor would it have been suitable for our investigation into the optimization of efficacy and safety for all patients. Each physician carefully determined the dosage of TAC upon considering multiple factors for every individual patient, such as disease activity, complications, age, and renal function.

Looking for the patient background in our study, age and sex were typical for lupus patients, and baseline disease activity was mild to moderate; however, many cases were refractory for CS or recurrent with therapeutic difficulty. In daily clinical practice, we often encounter such cases of being refractory to multiple immunosuppressive agents with an intermediate dose of CS. Although we unwillingly increased CS for such cases in the past, in more than a few cases complications were forced due to CS. For the blind alley cornered patients with a mild flare of disease, such as arthritis, skin eruption, or immunological abnormalities, TAC was effective. Drop-out rate may not be low simply; however, the rate itself strongly depends on multiple factors, such as patient background, study protocol, existance of alternative choice and objective symptoms.

Although we think therapeutic effects should be basically dose dependent, the reason why dose dependency was not statically clear might be considered to be number of cases, heterogeneous group, and narrow declined distribution of TAC dose. We were able to confirm a drug response for these manifestations at 4 weeks, the same as in our report on RA [16]. Our results also indicate that it possesses steroid-sparing effects and makes a good combination partner in a subgroup of patients that responded well to the therapy. However, for severe manifestations, including cytopenia, current doses of TAC were not enough to improve their activities. Higher doses of TAC might be effective for induction, maintenance, or prevention of relatively severe disease manifestations in some cases. Longer observation and a double-blind, randomized controlled trial might be needed for dealing with this problem.

Regarding safety, TAC was generally considered to be safe except in the high-dose case, where we used it while paying special attention to renal toxicity, gastrointestinal dysfunction, glucose intolerance, and infection. Live vaccine, CyA, bosentan, and potassium-sparing diuretics are prohibited to use concomitant with TAC. Furthermore, we should be also careful to use TAC with following drugs such as macrolides, antifungal azoles, some of calcium channel blockers because of possibility of increase in blood concentration [16, 22, 23, 41]. A lower incidence of adverse events than in past clinical reports may be due to an appropriate and flexible dose regimen. From a safety perspective, it is important to consider dosages of TAC for each individual.

On the measurement of blood concentrations of TAC in SLE patients, current recognition and proposed new practice guidelines were garnered from our previous and current studies are as follows: (1) basically, efficacy is dose dependent, but individual differences exist and we should be checking that the concentration is in an appropriate range, at least once, soon after initial administration; (2) especially in the case of (a) liver and/or renal dysfunction, (b) an increase of dose in elderly patients, (c) more than 3 mg/day administration, and/or (d) a combination with drugs interacting with TAC, the monitoring of blood concentration levels is efficacious for preventing serious adverse events caused by an increased concentration; (3) it is difficult to predict the initial clinical response, drug concentration, and independent adverse events.

As a suggestion for further studies, it is necessary to establish evidence for the following: (1) appropriate applicants for the treatment of TAC; (2) appropriate dose, methods, periods, and blood concentration levels to maximize effect and minimize side effects for each manifestation; (3) sensitive indicators of monitoring and assessment methods for the treatment; and (4) long-term efficacy and safety, and contribution to prognosis.

In conclusion, TAC can be considered both effective and safe for treating various manifestations in SLE patients. For mild disease activation of SLE, it could become one of the new pragmatic options. However, for severe active conditions, its efficacy is considered to be limited at current dose settings and usage.

**Conflict of interest statement** None.

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SPECIAL ARTICLE

# 'Non-criteria' aPL tests: report of a task force and preconference workshop at the 13th International Congress on Antiphospholipid Antibodies, Galveston, TX, USA, April 2010

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**Abstract:** Current classification criteria for definite APS recommend the use of one or more of three positive standardized laboratory assays, including anticardiolipin antibodies (aCL), lupus anticoagulant (LA), and antibodies directed to $\beta_2$glycoprotein I (anti-$\beta_2$GPI) to detect antiphospholipid antibodies (aPL) in the presence of at least one of the two major clinical manifestations (i.e., thrombosis or pregnancy morbidity) of the syndrome. Several other autoantibodies shown to be directed to phospholipids and/or their complexes with phospholipids and/or to proteins of the coagulation cascade, as well as a mechanistic test for resistance to annexin A5 anticoagulant activity, have been proposed to be relevant to APS. A task force of worldwide scientists in the field discussed and analyzed critical questions related to 'non-criteria' aPL tests in an evidence-based manner during the 13th International Congress on Antiphospholipid Antibodies (APLA 2010, 13–16 April 2010, Galveston, Texas, USA). This report summarizes the findings, conclusions, and recommendations of this task force. *Lupus* (2011) **20,** 191–205.

**Key words:** autoantibodies; prothrombin; phosphatidylethanolamine; IgA

## Introduction

Current classification criteria for definite antiphospholipid syndrome (APS) recommend the use of one or more of three positive standardized laboratory assays to detect antiphospholipid antibodies (aPL) in the presence of at least one of the two major clinical manifestations (i.e., thrombosis or pregnancy morbidity) of the syndrome.[1] Anticardiolipin antibodies (aCL), anti-$\beta_2$glycoprotein I ($\beta_2$GPI) antibodies, and lupus anticoagulant (LA) are the laboratory tests included in the revised criteria for the classification of APS.

A number of issues regarding the definition of 'aPL positive' are under discussion. For example, there are in daily practice many in vitro 'false positives' for aPL, due to the lack of specificity of the tests, particularly the aCL ELISA. APL antibodies are found in patients with a variety of diseases, such as infectious, malignant, or autoimmune diseases (clinical false positive), but in those cases they are

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not associated with clinical manifestations of APS. Furthermore, increasing evidence demonstrates that aPL antibodies are heterogeneous in function and specificity, and individual tests may recognize various subtypes of antibodies, some of which may be 'pathogenic'. In addition, there are patients strongly suspected of having APS by their clinical phenotype, but persistently negative for any currently tested aPL (laboratory and/or clinical false negative). These findings have nurtured the concept of 'seronegative APS' (SNAPS), a much contended setting that is based on a clinical picture highly suggestive of the syndrome in the absence of conventional aPL antibodies, leading investigators to maintain their efforts to identify 'true aPL' in an attempt to better recognize APS patients.

Several autoantibodies shown to be directed to negatively charged phospholipids other than cardiolipin, to other proteins of the coagulation cascade (i.e., prothrombin and/or phosphatidylserine–prothrombin complexes), to some domains of $\beta_2$GPI, or to interfere with the anticoagulant activity of annexin A5 (A5), have been proposed to be relevant to APS.[2] In some cases, these assays appear to detect specific subsets of pathogenic antibodies, or a particular mechanism in APS. However, the clinical utility of these newly developed assays and their diagnostic value remains elusive. The issue of the value of IgA aPL antibodies and whether this test should be part of the routine diagnostic algorithm has also been a subject of debate. A worldwide task force of scientists in the field – divided into subgroups – discussed and analyzed critical questions related to 'non-criteria' aPL tests in an evidence-based manner during the 13th International Congress on Antiphospholipid Antibodies (APLA 2010, 13–16 April 2010, Galveston, TX, USA). This report summarizes the findings, conclusions, and recommendations of this task force.

## Antibodies to phosphatidylethanolamine

*(Presented by Drs Sanmarco, Lambert, and Matsubayashi)*

### *Introduction and questions addressed by the task force*

Antibodies directed toward phosphatidylethanolamine (anti-PE) deserve particular attention, since they have been described in some instances as the sole aPL in patients that have manifestations of APS. Thus, the goal of this session was to highlight the clinical interest of anti-PE investigation through a brief review of the literature of their clinical associations and clinical experience. Another point opened to the debate was the methodological problems of the anti-PE assays.

Regarding obstetrical complications, anti-PE have been reported to be significantly more frequent in women with unexplained early fetal loss (UFL) than in either those with explained early fetal loss or healthy mothers. Two different studies have shown that anti-PE are a higher independent risk factor for early UFL than either aCL or anti-$\beta_2$GPI antibodies.[3,4] Interestingly, anti-PE have also been described as the only aPL found in the majority of cases (73%). Likewise, anti-PE have been reported as significantly the most frequent aPL in infertile women (67.5% of aPL-positive sera), where they were found to be the sole aPL in 85% of cases.

Recently, a murine model has reinforced the interest in anti-PE investigation in obstetric complications. Indeed, as reported by Dr Matsubayashi in this session, passive immunization of anti-PE or anti-LDC27 (antigen site in the third domain of kininogen) in pregnant mice causes increased fetal resorption, which correlated with significant increases in apoptosis in the placenta (study in progress). He claimed that this study supports the pathogenic role of anti-PE in pregnancy complications and also suggests the importance of LDC27, the target antigen site for kininogen-dependent anti-PE.

The relationship between anti-PE and thrombosis, the other clinical feature of APS, has also been reported in several studies. In particular, in a multicenter study set up within the framework of the European Forum on aPL, the prevalence of anti-PE was 15% in patients with unexplained venous thromboses and mainly found as the sole aPL.[5] In this retrospective study, IgG-anti-PE were found to be an independent risk factor for venous thrombosis, with an odds ratio of 6:1. Interestingly, Dr Lambert reported that in a selected population of 243 outpatients consulting for idiopathic arterial and/or venous thrombosis, negative for conventional aPL antibodies, 58 were positive for anti-PE (IgM mainly and IgG rarely). Other thrombophilic disorders were not frequently found. During a median follow-up of 34 months, thrombotic recurrence was found in 25% of patients.

Importantly, the task force recognized that no consensual standardized method exists for the measurement of anti-PE and that the heterogeneity of these antibodies increases the difficulties in attempting such a goal. This problem significantly limits

the clinical utility of this assay. The impact of the various ELISA components on the interlaboratory variability of results was analyzed, the conclusion being that the buffer supplement represents the critical factor in anti-PE measurement. To that regard, the results from a recent study showing that buffer supplements with a high lipid content decrease anti-PE reactivity in a dose-dependent manner were presented at this meeting.[6]

*Recommendations of the task force*

Based on published evidence and the additional studies presented during this session, the detection of anti-PE antibodies may be useful in 'seronegative' APS, in spite of the absence of a consensual method for their detection. The task force recognized that further steps must be made in order to ascertain the place of these antibodies in the diagnostic algorithm of APS, including standardization and proper validation of an anti-PE ELISA test and a prospective study on a broad population with well-documented clinical and biological features of APS (Table 1a).

## Antibodies to domains of β2 glycoprotein I

*(Presented by Dr Bas de Laat)*

*Introduction and questions addressed by the task force*

APL antibodies form a heterogeneous population of antibodies recognizing different antigens.[7] $\beta_2$GPI is recognized as the most important antigen in APS, but anti-β2GPI antibodies are also regarded as a heterogeneous population of antibodies with reactivity towards different epitopes on $\beta_2$GPI.[8] During the last decade evidence has accumulated for a central role for domain I of $\beta_2$GPI as a primary epitope for aPL antibodies. Iverson et al. were the first to show that a specific population of aPL antibodies showed reactivity towards domain I, with glycine40-arginine43 as the major epitope.[9,10] Recently Ioannou et al. reported that the epitope possibly comprises a larger region on domains I and II.[11]

Two studies have been conducted to investigate the clinical significance of the detection of anti-domain I antibodies. The first of these showed that the presence of anti-domain I antibodies was associated more with (predominantly venous) thrombosis compared with anti- $\beta_2$GPI antibodies with reactivity towards other domains.[12] This observation was recently confirmed in a double-blinded multicenter study including 442 patients, all positive for anti- $\beta_2$GPI antibodies.[13] Anti-domain I antibodies were shown to be present in the plasma of 243/442 patients (55%). From these patients with anti-domain I antibodies in their plasma, 83% had a history of thrombosis resulting in an odds ratio of 3.5:1 (2.3–5.4, 95% confidence interval, CI) for thrombosis. Interestingly, it was also found that anti-domain I antibodies were associated with pregnancy morbidity. Furthermore, recently in vivo data have been generated with respect to domain I. Ioannou et al. conducted a study in which mice were injected with IgG purified from patients diagnosed with APS.[14] After standardized vessel injury, mice injected with antiphospholipid-related IgG displayed increased thrombus size that could be inhibited by domain I of $\beta_2$GPI.

This task force subgroup was charged with investigating whether there is sufficient scientific evidence to recommend the incorporation of the assay to measure anti-domain I antibodies for implementation in the official guidelines for diagnosis of patients with APS.

*Recommendations of the task force*

The general opinion of the task force was that detection of anti-domain I antibodies is of major importance. This was predominantly based on a double-blinded multicenter study in which it was shown that anti-domain I antibodies were associated more with thrombosis and pregnancy morbidity compared with antibodies with reactivity towards other domains of $\beta_2$GPI.[13] One of the problems that can also be applied to (some of) the other assays that are already included in the official guidelines is lack of prospective data (a) and causality (b):

(a) Several prospective studies have been performed with regard to the clinical significance of the presence of aPL antibodies regardless of specificity, but there is no consensus as to whether the presence of aPL antibodies is a risk factor for thrombosis (either first or second event).[15–21]
(b) The causality of anti-domain I has been demonstrated only by the use of animal models, and additional clinical studies are needed.[14]

Therefore, this task force recommended that the anti-domain I assay may be used in a research-based setting and that more prospective and in vivo data are needed before the anti-domain I

**Table 1** Questions and recommendations of the non-criteria aPL task force

*1a. Anti-PE antibodies and antibodies to negatively charged phospholipids other than cardiolipin*

| Test | Questions addressed by task force | Recommendations |
|---|---|---|
| Anti-PE antibodies | Is the anti-PE ELISA standardized? What are the challenges with the assay? | Standardization of anti-PE ELISA needed |
| | Are anti-PE antibodies clinically relevant? | Well-designed clinical studies needed to confirm the diagnostic value of anti-PE antibodies |
| Antibodies to negatively charged phospholipids other than cardiolipin | | |
| *a) Perspectives and experiences from a large reference laboratory in the USA* | Are antibodies to negatively charged phospholipids other than cardiolipin important in the diagnosis of APS? | Important to establish whether these antibodies recognize additional APS patients, currently missed with traditional assays<br>Address existing technical problems and inconsistencies with the tests<br>Anti-PS may be best candidate with respect to relevance and association with recurrent pregnancy loss |
| Antibodies to negatively charged phospholipids other than cardiolipin | | |
| *b) In the obstetric population* | Do non-criteria aPL exist and are they found in women with RPL? | Based on clinical studies = yes |
| | Are there sufficient clinical data to warrant a change in the 2006 Classification criteria | Not at the moment; more conclusive clinical studies are needed |
| | Do women with RPL who have early pregnancy losses and no thrombosis constitute a unique subgroup of APS with different diagnostic criteria of APS? | Obstetric populations should be stratified (with or without prior thrombosis and third-trimester losses from first trimester) |

*1b. Anti-domain I antibodies, IgA aCL and anti-$\beta_2$GPI antibodies, anti-prothrombin, and anti-prothrombin-phosphatidylserine antibodies*

| Test | Questions addressed by task force | Recommendations |
|---|---|---|
| Anti-domain I antibodies | Does the anti-domain I antibodies test recognize 'pathogenic' anti-$\beta_2$GPI antibodies? | Clinical data available encouraging |
| | Is there convincing evidence to include this test in the diagnostic algorithm of APS? | In vivo data with anti-domain I antibodies needed.<br>Standardized consensus protocol for this assay needed<br>Additional clinical studies needed |
| IgA aCL and IgA anti-$\beta_2$GPI antibodies | Are IgA aPL (particularly IgA anti-$\beta_2$GPI) clinically significant in patients with clinical manifestations of APS? | IgA anti-$\beta_2$GPI antibodies should be tested in the presence of clinical signs and symptoms of SLE and/or APS, particularly when other aPL tests are negative<br>Evaluation and comparison of multiple, commercially available IgA aPL assays in A larger and well-characterized population of patients needed to confirm the diagnostic value of isolated anti-$\beta_2$GPI positivity<br>Studies needed to determine the role of IgA anti-$\beta_2$GPI antibodies in the pathogenesis of APS<br>IgA anti-$\beta_2$GPI antibodies that bind to domains IV/V of $\beta_2$GPI might represent an important subgroup of clinically relevant aPL antibodies |
| Anti-prothrombin and anti-prothrombin-PS antibodies; antibodies to negatively charged phospholipids other than cardiolipin | What is the role of anti-prothrombin and anti-prothrombin/phosphatidylserine antibodies in APS? Are antibodies to negatively charged phospholipids other than cardiolipin important in the diagnosis of APS? | aPT-A test in conjunction with other tests may be a good risk marker for thrombosis<br>aPT-A and particularly the anti-PS/PT are good specific tests to confirm APS<br>aPT-A and anti-PS/PT not ready to be included in the diagnostic criteria (standardization of the tests needed)<br>Collaborative studies needed to confirm clinical associations with these tests |
| Annexin A5 (A5R) resistance test; anti-prothrombin and anti-prothrombin-PS antibodies | What is the role of the Annexin A5 resistance test in the diagnosis of APS? What is the role of anti-prothrombin and anti-prothrombin/phosphatidylserine antibodies in APS? | Data on the utility of AnxA5 resistance assay as a mechanistic diagnostic marker for APS are highly promising<br>Developing mechanistic clinical assays that measure APS disease mechanisms is an important and appropriate avenue to pursue<br>Additional data are needed before recommending A5R as a standard component of aPL testing panels |

assay can be added to the official diagnostic guidelines. This assay needs to be made available to other centers for testing before any recommendation can be made (Table 1b).

## Antibodies to negatively charged phospholipids other than cardiolipin: perspectives and experiences from a large reference laboratory in the USA (*Presented by Dr Tebo*)

### *Introduction and questions addressed by the task force*

Antibodies directed against negatively charged phospholipids such as phosphatidic acid (PA), phosphatidylinositol (PI), and phosphatidylserine (PS) have been reported in patients with APS. However, the use of these antibodies in addition to the currently recommended laboratory markers for the diagnosis of APS remains controversial. Some investigators have suggested that testing for these aPL antibodies may help to identify women with recurrent pregnancy loss (RPL) with clinical features of APS who may benefit from treatment, a topic discussed in detail in the next section.[22–26] In other such studies, as well as in the context of thrombosis associated with systemic lupus erythematosus, no improvement in the diagnosis performance was observed when these were measured simultaneously with aCL and LA,[23–25,27] Therefore, these assays were not included in the 2006 revised criteria for the classification of APS.[1] In a review of the literature since the laboratory criteria for APS were revised, very few studies have been carried out to examine the relevance for these antibody markers. As such, most of the discussion and recommendations in this article will focus on the few recent investigations on this topic, with reference to some earlier key findings.

Early investigations by Gharavi and colleagues showed that aCL antibodies broadly cross-react to both antiphosphatidylserine (anti-PS) and antiphosphatidylinositol (anti-PI) antibodies.[28] Of the three major negatively charged aPL antibodies (anti-PA, anti-PI, and anti-PS), anti-PS has been most extensively investigated in thrombosis- and pregnancy-related morbidity APS.[22–27,29,30] These antibodies, particularly anti-PS, have been shown to be more specific for APS when compared with aCL, since aCL is often found to be positive in infectious diseases and other disorders.[31,32] However, the conditions necessary to achieve optimal clinical and analytical performance in these assays are yet to be determined.[1,29] Using aPS assays from two different manufacturers, Tebo et al. could not document a consistent diagnostic utility for this marker for both the IgG and IgM isotypes.[29] In addition, the combined use of these 'non-criteria' aPL antibodies differed significantly between manufacturers, especially for IgM specificities, and their overall combined diagnostic performance was not significantly higher than that of aCL and anti-$\beta_2$GPI assays.[29,30] Of clinical importance, no difference in the magnitude and prevalence of these antibodies was documented between healthy controls and women with recurrent pregnancy loss.[30]

### *Recommendations of the task force*

In the evaluation of additional diagnostic markers for APS:

(a) It is important to determine critically whether, indeed, these antibodies contribute to the identification of additional patients who would otherwise be missed by the current assays or, alternatively, they would be better predictors of disease due to improved analytical and clinical performance. Anti-PA, anti-PI, and anti-PS antibodies in their current format pose significant diagnostic and analytical challenges. First, when they occur, they do so in high association with aCL antibodies and in isolation, and their clinical relevance is questionable and has not been fully investigated.
(b) In the case of anti-PS antibodies, the conditions required to detect this antibody remain controversial. Even for assays using the same reagents, the results are discordant as there are no formal calibrators or agreed methods of detection. Thus, in addition to not being cost-effective, to choose assays with the best medical benefit rather than a collection of tests with overlapping properties and equivalent or questionable clinical value may be the best practice.
(c) Based on the current evidence, it would appear that testing for anti-PA, anti-PI, and anti-PS antibodies in the initial diagnostic work-up for APS is not clinically useful, as these antibodies may have overlapping properties with the markers considered diagnostic for this disease.
(d) It would appear that the anti-PS marker may be the best candidate for further investigation of its relevance and significance, especially in the area of recurrent pregnancy loss, provided an accepted and standardized method is in place. In this case, more prospective studies using an agreed-upon protocol for patient recruitment,

follow-up, and testing for the presence of these antibodies are critical (Table 1a).

## Antiphospholipid antibodies other than anticardiolipin antibodies in obstetric APS

*(Presented by Dr. Kutteh)*

*Introduction*

Several investigators worldwide have advocated the use of a panel of aPL antibodies (aPL) to screen for APS.[33,34] This panel of tests includes not only cardiolipin (CL, diphosphatidyl glycerol) but also phosphatidyl inositol, phosphatidyl glycerol, phosphatidyl serine, and other negatively charged phospholipids. These phospholipids are found in various proportions on virtually every cell in the body, on the inner and outer surface membranes. Controversy has arisen as to the significance of these antibodies and whether treatment should be based solely on positive results of aCL or on positive results of any other aPL.

This ongoing debate of the clinical significance of aCL and other aPL has prompted some clinicians to screen recurrent pregnancy loss (RPL) patients and indentify those that might be missed if only aCL were considered significant. For example, Branch et al. analyzed the 95th and the 99th percentiles of the positive and negative cut-off for a panel of phospholipids among 147 women with RPL, APS, and fertile controls.[23] By using the 99th percentile, they found that 26/147 (17.7%) of women with RPL had positive antibodies to CL and 13/147 (8.8%) with RPL demonstrated binding against phospholipids other than CL or lupus anticoagulant (LA). The cut-off value in phospholipid units was determined by using the 99th percentile of the normal population, approximately threefold the median value. Based on comparison with controls, they concluded that this difference was not clinically significant.

In a much larger, earlier study, Yetman and Kutteh determined the prevalence of aPL among 866 women with RPL 5. In this population, 150 of 866 (17.3%) women with RPL were positive for IgG and/or IgM aCL while only 12 of 288 (4%) of control women without a history of poor obstetrical outcome were positive for the same antibodies ($p < 0.001$). The same study identified 87 of 866 women with RPL who were negative for aCL but positive for one of the other aPL, considering patients with more than one positive aPL only once.[35] Although this study was retrospective, it suggests that a significant number of women with RPL would not have been identified if they had been tested solely for aCL. The same group recently reported on another group of 872 women with RPL.[36] Positive aCL were detected in 132 of 872 women with RPL (15.1%), LA was detected in 31 of 872 (3.6%), and aPS was identified in 49 of 872 (5.6%) of women with RPL who were negative for aCL and LA.[36] Anti-PS antibodies were found in the absence of aCL and LA in women with RPL and two consecutive losses (18/391 or 4.6%), women with three consecutive losses (16/288 or 5.6%), and women with four or more consecutive losses (15/193 or 7.8%). In control women without a history of poor obstetric outcome, positive aCL were detected in 4.9%, positive LA in 1.0%, and positive aPS in 2.8%. Differences in aCL and anti-PS when comparing women with RPL to controls were significant using the two-tailed Fisher exact test.

The lack of standardization among different laboratories has made it difficult for physicians to identify patients with APS and those at risk for a miscarriage.[37–39] This has been used as a reason for not using other aPL as APS criteria, but in fact a great deal of variation exists between laboratories even when assaying aCL. For example, IgG aCL, considered by almost all clinicians and laboratory professionals as the 'gold standard', is still not standardized to the level of uniform agreement in all labs and all assays. In 2009, the College of American Pathologists survey results for sample ACL-06 showed that only 78% of labs could even agree that the sample was positive, while 5.5% of the labs determined the sample was negative, and the remaining 16.5% of the labs indicated that the result was indeterminate! Thus, an international group of investigators has established both clinical and laboratory criteria for the diagnosis of APS.[1] Yet, problems still exist when pregnancy loss patients are referred to fertility clinics that may have had testing performed at different laboratories using different control values and cut-off values to determine positive results. Also, standard testing may exclude a population of aPL patients who have had significant obstetric problems but test positive for other aPL and negative for the most commonly assayed aCL and LA.

Basic science supports the significance of aPL other than aCL. Anti-PS antibodies have been shown to inhibit trophoblast development and invasion using an in vitro model system.[40] Anti-PS retard syncytiotrophoblast formation and

decrease the synthesis of hCG. Both low-molecular weight and unfractionated heparin have been shown to reduce the in vitro binding of anti-PS as well as aCL.[41] Furthermore, some clinical data have been published suggesting that some women with a diagnosis of RPL and aPL positivity may benefit from treatments that have assisted women with RPL and aCL to deliver healthy offspring.[26]

*Questions and answers from the task force*

1. *Do non-criteria aPL exist and are they found in women with RPL?*

The task force generally felt that enough studies had been performed on large populations of patients to demonstrate that these 'non-criteria' aPL do indeed exist.[23,33–35]

2. *Are there sufficient clinical data to warrant a change to the 2006 criteria for the diagnosis of APS?*

The task force acknowledged that several studies have suggested that 'non-criteria' aPL may have clinical significance, but that the current level of evidence did not warrant any changes to the current criteria. Obviously, the task force would like to see more prospective, randomized trials, but acknowledged that a number of obstacles exist to make these types of studies difficult. These challenges include both clinical and laboratory inclusion criteria and the need to use an experienced laboratory in a multicenter study.

3. *Do women with recurrent pregnancy loss who have predominantly early pregnancy losses (prior to ten gestational weeks) and no history of thrombosis constitute a unique population that warrants different diagnostic criteria to APS?*

Considerable discussion on this topic was generated. It was felt that obstetric populations should be stratified to distinguish women based on their history of prior thrombotic events from those without this history. It was also felt that women with predominantly later-trimester losses (beyond 13 gestational weeks) should be distinguished from those women who had losses that were predominantly in the first trimester.[42] This population of women with early pregnancy losses may be affected differently by the non-criteria aPL through mechanisms other than thrombosis.[40] The task force felt that this should receive strong consideration at the next consensus conference.

*Recommendations of the task force*

The 'non-criteria' aPL task force agreed that studies from several different investigators clearly demonstrate that there are women with RPL who are negative when tested for aCL and LA but who are positive for other 'non-criteria' aPL. In fact, some of the task force members reiterated previous suggestions that women with RPL without a history of thrombosis should be placed in a separate classification when considering the diagnosis of APS, and that a treatment algorithm be constructed to address this group. However, the task force is uncertain and unwilling at this time to make any changes in the current criteria for the diagnosis of APS. It was agreed that some clinical studies show promise and need to be repeated by other groups, as those available do not have enough power to be considered significant. The task force felt that the significance of a panel of aPL antibodies to diagnose APS is an ongoing debate, with many complex questions that can only be addressed with larger study groups using an experienced central laboratory and multiple sites (Table 1a)

## IgA anticardiolipin (aCL) and IgA anti-$\beta_2$GPI antibodies

*(Presented by Dr Murthy on behalf of Dr Pierangeli's group and by Dr Petri)*

*Introduction and questions addressed by the task force*

The current laboratory criteria for APS include the presence of positive lupus anticoagulant (LA) and/or IgG or IgM isotypes of aCL and/or anti-β2GPI antibodies, but omit the IgA isotypes for both tests.[1]

*a) IgA aCL antibodies*

Studies have shown data on the prevalence and significance of IgA aCL antibodies. In unselected patients with systemic lupus erythematosus (SLE), the prevalence of increased titers of IgA aCL has been reported to vary from 1% to 44%.[43–51] The lowest reported frequency was that found by Selva-O'Callaghan et al., who detected IgA aCL in only 2 of their 200 (1%) patients with SLE.[52] Alarcon-Segovia et al., in an earlier study that included 500 patients with SLE, found increased titers of IgA aCL in 16.6% of their patients.[53] In another study, Spadaro et al. found that IgA aCL was positive in 13 (20%) of their 65 SLE patients.[54]

In contrast, Weidmann et al. found IgA aCL to be positive in 44% of 92 SLE patients and also found IgA to be the most frequent aCL isotype.[45] The reported frequency for raised IgA aCL was higher (52.5%) in an earlier study by Wilson et al., where patients were preselected for being IgG or IgM aCL positive and/or having APS-associated clinical complications.[46] A prevalence of 83.3% was reported by Lopez et al. in a group of patients with SLE and thrombocytopenia.[47] As noted, the ethnic group composition of patients can influence the isotypic distribution of aCL. Molina et al. studied African-American, Afro-Caribbean, and Hispanic patients with SLE and found elevated levels of IgA aCL in 16%, 21%, and 14%, respectively.[48] The most important finding was that IgA aCL was the only aCL isotype present in 82% of aCL-positive Afro-Caribbean patients. In contrast, IgA aCL was found to be positive only in 4.4% of Chinese patients with SLE.[49] In another study, Cucurull et al. found that, although IgA aCL antibodies were present in 51% to 55% of patients with APS, most were also IgG or IgM positive, suggesting that measurement of IgA aCL would add little to IgG and IgM determination.[50]

There is some experimental evidence that IgA aCL antibodies are pathogenic. In a mouse model designed to study thrombus formation, injected IgA immunoglobulins with aCL activity from patients with APS were shown to cause thrombosis. The mean thrombus size using two different IgA immunoglobulin preparations was found to be significantly larger compared with control IgA.[55]

Numerous studies have also investigated possible associations between raised levels of aCL and clinical manifestations of APS attributed to these autoantibodies. Several of these studies reported a significant association for IgA aCL with one or more of the main clinical manifestations of APS. Cucurull et al., studying both aCL and anti-β2GPI antibodies in African-American patients with SLE, found an association between thrombotic events and raised levels of both these autoantibodies.[50] However, the number of their patients with thrombotic events was very small: only 5% of their 100 patients had documented evidence of thrombosis.[50] An association between raised IgA aCL levels and thrombocytopenia in patients with SLE or other collagen vascular diseases has also been reported.[56] Finally, an association between IgA aCL and recurrent fetal loss and with unexplained spontaneous abortions has been reported in women with SLE.[57] In a study that tested over 700 samples from an APS registry (APSCORE), only five samples were positive for IgA aCL alone and four of those were from patients who had presented with at least one of the two major manifestations of APS, according to the Sapporo revised criteria (unpublished observations). Furthermore, although the number of APS patients with IgA aCL positive results only – in the absence of IgG and/or IgM aCL-positive results – is low, its presence seems to be associated with clinical manifestations for the APS.[57] At this preconference workshop, Dr Michelle Petri showed data from her own laboratory, indicating that isolated IgA aCL positivity is rare but is associated with venous and arterial thrombosis.

*b) IgA anti-$\beta_2$GPI antibodies*

Previous studies have raised the possibility that IgA anti-$\beta_2$GPI might be associated with clinical manifestations of APS; those observations showed that SLE patients with APS are more prone to be positive for the IgA isotypes.[58–61] Furthermore, it seems that IgA anti-$\beta_2$GPI antibodies are independent risk factors of acute myocardial infarction and atherosclerotic disease in populations without APS (OR 3.4, CI 1.3–9.1),[62] and the same positive association was found for acute cerebral ischemia.[63–66] A concise report by Yamada et al. also showed anti-$\beta_2$GPI positivity in the absence of IgG anti-$\beta_2$GPI in a subgroup of women with unexplained recurrent pregnancy loss (particularly in the first trimester).[67] Similar findings were reported by Lee et al., indicating that IgA anti-$\beta_2$GPI positivity is more common in women who experience unexplained recurrent spontaneous abortion and unexplained fetal death and whose initial test results for other isotypes and LA were negative.[68] Further characterization of IgA anti-$\beta_2$GPI positivity in the absence of IgG anti-$\beta_2$GPI positivity associated with vascular morbidity showed that these antibodies may recognize domain IV of $\beta_2$GPI as their epitope.[69,70] In patients with SLE, the IgA anti-$\beta_2$GPI that recognizes domains IV and V seems to be positively correlated with thrombosis.[69–71]

Recently, Kumar et al. (from Dr Pierangeli's group) reported five isolated cases of individuals who were *exclusively* positive for IgA anti-$\beta_2$GPI and had concomitant clinical manifestations of APS.[72] Subsequently, Sweiss et al. reported that the presence of isolated IgA anti-$\beta_2$GPI positivity is associated with an increase in thromboembolic events, especially among patients with SLE. In that study – which included only a small group of SLE patients – IgA anti-$\beta_2$GPI was associated with an increased prevalence of morbidities involving