## Serum soluble Fas and Fas ligand (FasL) in primary Sjögren's syndrome

Sirs,

The Fas/Fas ligand (FasL) apoptotic pathway play a potential role in the pathogenesis of primary Sjögren's syndrome (pSS) (1), an idiopathic systemic autoimmune disease (2, 3). A very limited number of studies examined the potential role of the soluble form of Fas (sFas) and FasL (sFasL) in pSS, and were rarely focused on organ involvement. Serum sFas concentrations are reported higher in pSS compared to healthy subjects in some studies (4-6), and in patients with extra-glandular disease compared to those without (6). One study has investigated the relationship between serum sFas and pSS disease activity, reporting a lack of association (7). Serum sFasL is also reported higher in SS compared to healthy subjects in one study (4), while not or decreased in others (8, 9). Serum sFasL has also been reported related to overall disease activity, as well as glandular and liver involvement in one study (9). No published study investigated the ratio between serum sFas and sFasL as a potential pSS biomarker. Here, we aimed to examine the clinical relevance of serum sFas, sFasL and their ratio in pSS, particularly at the phenotypic level.

Adult patients fulfilling the 2002 revised American-European Consensus Criteria for Sjögren's syndrome classification were prospectively enrolled at The Queen Elizabeth Hospital (TQEH) between April 2013 and August 2014, as previously described (10). The European League Against Rheumatism (EULAR) SS disease activity index (ESSDAI) was used to assess disease activity, as previously described (10). pSS patients were classified as having low (ESSDAI<5), moderate (5≤ESSDAI≤13) or high pSS disease activity (ESSDAI≥14) (10). Healthy subjects were enrolled as a healthy control (HC) group. All participants gave their written, informed consent. This study was approved by the Human Research Ethics Committees from TQEH, Monash University and Monash Health. After whole blood collection during routine clinical examination, serum was separated and stored at -80°C. A Luminex screening assay (Cat# LXSAH, R&D Systems) was used to quantify serum sFas and sFasL concentrations, following the manufacturer's protocol. Statistical analyses were performed using Stata version 14 (StataCorp, College Station, Texas, USA). Difference in continuous variable between two groups were examined by the Wilcoxon rank-sum test. Fisher exact test was used to assess difference in proportions. Serum sFas, sFasL and their ratio were $\log_{10}$ transformed to perform linear regression analysis. A $p$-value of <0.05 was considered statistically significant.

Forty-eight pSS patients and 17 HC were included in this study (Supplementary Table 1). As the HC cohort was not age-matched to the pSS cohort, age was including in multivariable model to account for this difference. Serum sFas and sFasL concentrations were detectable in all samples. Serum sFas and sFasL concentrations were significantly higher in pSS compared to HC, using either non-parametric test or univariable linear regression (Fig. 1A-D). The associations between increased serum sFas and sFasL with pSS were, however, attenuated after adjusting for age (data not shown). No significant difference in sFasL/sFas ratio was observed between pSS and HC (Fig. 1E-F). Serum sFas and sFasL concentrations were not correlated in pSS (r=0.19; $p$=0.2).

We examined differences in serum sFas, sFasL and their ratio according to clinical parameters in pSS. Serum sFasL and sFasL/sFas ratio were both significantly lower in pSS patients with active lymphadenopathy disease (Table I). We also observed a significant decrease in serum sFas in pSS patients with active biological domain (Table I). No significant difference in these serum biomarkers was observed according to overall or other organ-specific disease activity, or anti-Ro/La antibodies positivity (Table I). This is the first study evaluating the clini-

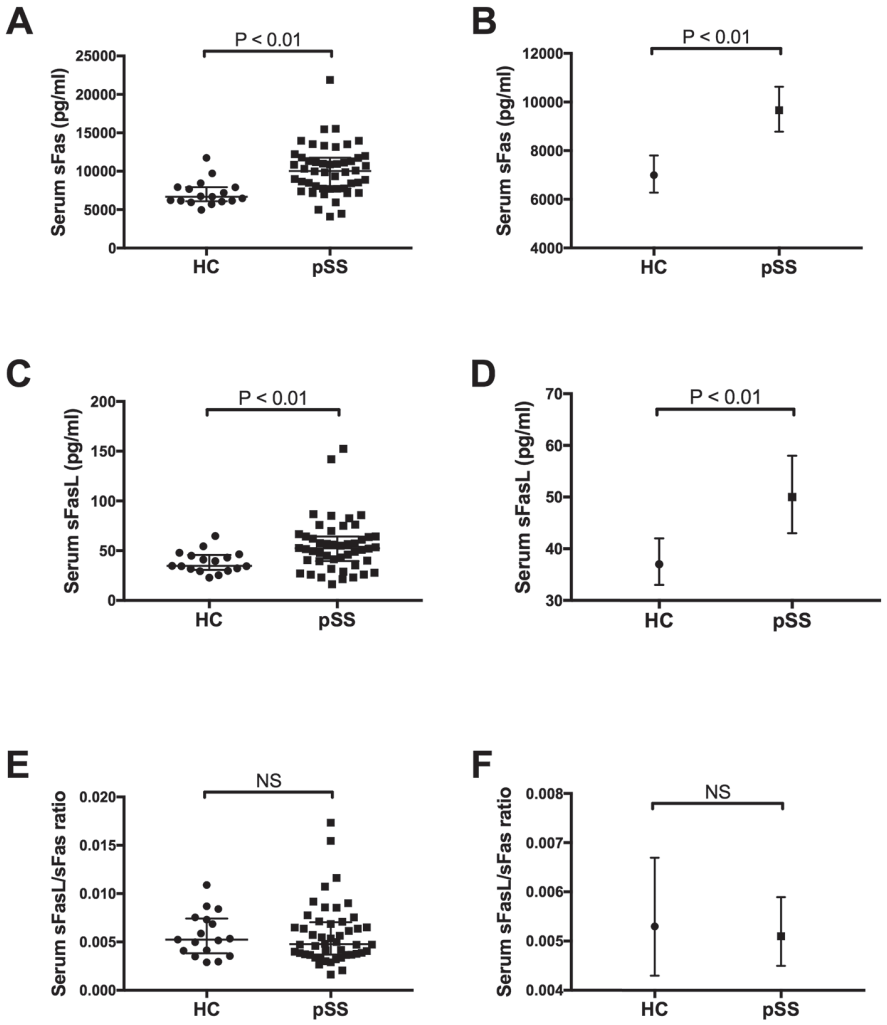


**Fig. 1.** Serum sFas, sFasL, and sFasL/sFas ratio in pSS.
**A**: Serum sFas concentrations in HC (median [interquartile range] [IQR]: 6676 [6153, 7913] pg/ml; n=17) compared to pSS (median [IQR]: 10035 [7753, 11756] pg/ml; n=48) cohorts.
**B**: Geometric mean (GM) of serum sFas concentrations in HC (GM (95%CI): 6998 (6277, 7802) pg/ml; n=17) compared to pSS (GM (95%CI): 9661 (8783, 10628) pg/ml; n=48) cohorts.
**C**: Serum sFasL concentrations in HC (median [IQR]: 35 [32, 45] pg/ml; n=17) compared to pSS (median [IQR]: 53 [40, 64] pg/ml; n=48) cohorts.
**D**: GM of serum sFasL concentrations in HC (GM (95%CI): 37 (33, 42) pg/ml; n=17) compared to pSS (GM (95%CI): 50 (43, 58) pg/ml; n=48) cohorts.
**E**: Serum sFasL/sFas ratio in HC (median [IQR]: 0.0052 [0.0041, 0.0073]; n=17) compared to pSS (median [IQR]: 0.0048 [0.0037, 0.007]; n=48) cohorts.
**F**: GM of serum sFasL/sFas ratio in HC (GM (95%CI): 0.0053 (0.0043, 0.0067) pg/ml; n=17) compared to pSS (GM (95%CI): 0.0051 (0.0045, 0.0059) pg/ml; n=48) cohorts.
Panels A, C and E: horizontal bars indicate the median [IQR], and difference in medians was examined using Wilcoxon rank-sum test. Panels B, D and F: horizontal bars indicate the GM (95%CI) derived using univariable linear regression analysis.

**Table I.** Serum concentrations of sFas, sFasL, and sFasL/sFas ratio according to demographics and clinical parameters in pSS.

| | n | pSS patients (N=48) Serum sFas (pg/ml) Median [IQR] | *p*-value | Serum sFasL (pg/ml) Median [IQR] | *p*-value | sFasL/sFas ratio Median [IQR] | *p*-value |
|---|---|---|---|---|---|---|---|
| Demographics | | | | | | | |
| Age (years) | | | 0.05 | | 0.37 | | 0.69 |
| <60 | 17 | 7758 [7132, 10948] | | 51 [29, 62] | | 0.0064 [0.0034, 0.0078] | |
| ≥60 | 19 | 10980 [8660, 12225] | | 53 [40, 76] | | 0.0047 [0.0037, 0.0065] | |
| Gender | | | 0.77 | | 0.8 | | 0.95 |
| Female | 31 | 9985 [7184, 11772] | | 52 [32, 70] | | 0.0048 [0.0037, 0.0071] | |
| Male | 5 | 10889 [7758, 11316] | | 54 [41, 55] | | 0.0047 [0.0037, 0.0071] | |
| Clinical Features | | | | | | | |
| Overall disease activity§ | | | 0.86 | | 0.3 | | 0.33 |
| Low (ESSDAI<5) | 15 | 10085 [7184, 11741] | | 57 [40, 76] | | 0.0056 [0.0041, 0.0071] | |
| Moderate to high (ESSDAI≥5) | 21 | 9985 [7726, 11772] | | 49 [29, 67] | | 0.004 [0.0034, 0.0071] | |
| Organ-specific disease activity§ | | | | | | | |
| Lymphadenopathy | | | 0.55 | | 0.05 | | 0.05 |
| Inactive | 30 | 10862 [7381, 11772] | | 54 [43, 70] | | 0.0056 [0.0039, 0.0071] | |
| Active | 6 | 8827 [7725, 9347] | | 27 [26, 32] | | 0.0032 [0.0027, 0.004] | |
| Glandular | | | 0.81 | | 0.32 | | 0.13 |
| Inactive | 24 | 9716 [7438, 11756] | | 54 [45, 69] | | 0.0056 [0.0039, 0.0074] | |
| Active | 12 | 10437 [7553, 12234] | | 36 [27, 68] | | 0.0039 [0.0028, 0.0064] | |
| Articular | | | 0.5 | | 0.73 | | 0.5 |
| Inactive | 22 | 9170 [7381, 11316] | | 53 [32, 76] | | 0.0051 [0.0037, 0.0086] | |
| Active | 14 | 10460 [7725, 12225] | | 52 [43, 58] | | 0.0044 [0.0037, 0.0064] | |
| Cutaneous | | | 0.52 | | 0.64 | | 0.83 |
| Inactive | 30 | 9716 [7381, 11741] | | 51 [32, 67] | | 0.0048 [0.0037, 0.0071] | |
| Active | 6 | 10650 [8881, 11772] | | 53 [51, 75] | | 0.0056 [0.0039, 0.0071] | |
| Pulmonary | | | 0.38 | | 0.97 | | 0.7 |
| Inactive | 26 | 10862 [7693, 11772] | | 53 [39, 67] | | 0.0051 [0.0037, 0.0069] | |
| Active | 10 | 9170 [6981, 11181] | | 48 [29, 76] | | 0.0043 [0.0037, 0.0092] | |
| Haematological | | | 0.38 | | 0.22 | | 0.06 |
| Inactive | 28 | 10460 [7709, 11998] | | 52 [28, 60] | | 0.0047 [0.0035, 0.0064] | |
| Active | 8 | 9114 [7256, 10918] | | 64 [44, 73] | | 0.0086 [0.005, 0.0091] | |
| Biological | | | 0.02 | | 0.7 | | 0.21 |
| Inactive | 22 | 10934 [8495, 13328] | | 52 [40, 58] | | 0.0047 [0.0037, 0.0065] | |
| Active | 14 | 8359 [6981, 10948] | | 57 [28, 75] | | 0.0064 [0.0037, 0.009] | |
| Laboratory markers | | | | | | | |
| Anti-La Ab | | | 0.89 | | 0.28 | | 0.44 |
| Negative | 6 | 9539 [7693, 11121] | | 59 [56, 76] | | 0.0063 [0.0046, 0.0086] | |
| Positive | 28 | 9666 [7282, 11756] | | 52 [34, 68] | | 0.0048 [0.0037, 0.0071] | |
| Anti-Ro52 Ab | | | 0.92 | | 0.96 | | 0.9 |
| Negative | 4 | 10057 [8073, 11673] | | 53 [39, 66] | | 0.0057 [0.0038, 0.007] | |
| Positive | 31 | 9985 [7381, 11772] | | 53 [32, 70] | | 0.0048 [0.0037, 0.0078] | |
| Anti-Ro60 Ab | | | 0.8 | | 0.41 | | 0.76 |
| Negative | 4 | 10057 [8343, 11673] | | 66 [42, 79] | | 0.0057 [0.0038, 0.0088] | |
| Positive | 31 | 9985 [7184, 11772] | | 52 [32, 67] | | 0.0048 [0.0037, 0.0071] | |

Data are presented as median [interquartile range] [IQR]. §Calculated in the 36 pSS patients in whom ESSDAI was assessed.
Ab: antibody; ESSDAI: European League Against Rheumatism Sjögren's syndrome Disease Activity Index; FasL: Fas ligand; IQR: interquartile range; pSS: primary Sjögren's syndrome; sFas: soluble Fas; sFasL: soluble FasL.

cal relevance of both sFas and sFasL at the phenotypic level in pSS. In line with some published studies (4-6), significantly higher concentrations of serum sFas and sFasL were found in pSS patients compared to HC, albeit not confirmed after adjusting for age. sFasL/sFas ratio was not significantly different between pSS and HC. We report, for the first time, a significant decreased in serum sFasL in pSS patients with active lymphadenopathy manifestations. Although no causal link can be drawn from the present study, since sFasL act as decoy blocking Fas-mediated apoptosis (11, 12), it could be speculated that a negative relationship between serum sFasL and lymphadenopathy in pSS may reflect a modulation of the Fas/FasL system, where decrease in serum sFasL levels might lead to increase membrane-bound Fas-mediated apoptosis in membrane-bound Fas-bearing autoreactive immune cells, particularly when they accumulate in the secondary lymphoid organs. While bearing in mind this small phenotypic subset, this preliminary finding is of special importance in pSS, which is associated with a marked increase in risk of lymphoma (13). Further research would be of interest to evaluate the kinetics of serum sFasL levels in relation to lymphadenopathy manifestations, and its potential as biomarker for monitoring pSS patients with such phenotype at risk of lymphoma; analysis of matching salivary gland tissues in such study would be of value.

**Acknowledgments**
The authors wish to thank the patients and healthy individuals for their participation. We also thank the clinical staff of the the Department of Rheumatology at TQEH and staff of the Immunology Department at Monash University, particularly those involved in biological samples and/or data collection, including Ms Emma Dunstan and Ms Susan Lester. The authors wish to thank the French Society of Rheumatology for its support.

F.B. VINCENT[1], *MD, PhD*
M. BUBICICH[2], *BSc (Hons)*
S. DOWNIE-DOYLE[2], *PhD*
F. MACKAY[3,4], *PhD*
E.F. MORAND[1], *MBBS, FRACP, PhD*
M. RISCHMUELLER[2,5], *MBBS, FRACP*

*[1]Centre for Inflammatory Diseases, Monash University School of Clinical Sciences at Monash Health, Melbourne; [2]Rheumatology Department, The Queen Elizabeth Hospital, Woodville, South Australia; [3]Department of Immunology and Pathology, Monash*

*University, Central Clinical School, Alfred Medical Research and Education Precinct (AMREP), Melbourne; [4]Department of Microbiology and Immunology, School of Biomedical Sciences, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne; [5]Discipline of Medicine, University of Adelaide, Australia.*

*Please address correspondence to:*
*Fabien Vincent, MD, PhD,*
*Centre for Inflammatory Diseases,*
*School of Clinical Sciences at Monash Health,*
*Faculty of Medicine, Nursing & Health Sciences,*
*Monash Medical Centre, 246 Clayton Rd,*
*Clayton VIC 3168, Australia.*
*E-mail: fabien.vincent@monash.edu*

*Funding: E.F. Morand is recipient of fellowships from the NHMRC of Australia, and was supported by the Kim Jolly Lupus Research Trust.*

*Competing interests: E.F. Morand has been consultants to GSK and Eli Lilly. The other co-authors have declared no competing interests.*



## References

1. SINGH N, COHEN PL: The T cell in Sjögren's syndrome: force majeure, not spectateur. *J Autoimmun* 2012; 39: 229-33.
2. NOCTURNE G, MARIETTE X: Advances in understanding the pathogenesis of primary Sjögren's syndrome. *Nature Rev Rheumatol* 2013; 9: 544-56.
3. FERRO F, MARCUCCI E, ORLANDI M *et al.*: One year in review 2017: primary Sjögren's syndrome. *Clin Exp Rheumatol* 2017; 35: 179-91.
4. NOZAWA K, KAYAGAKI N, TOKANO Y *et al.*: Soluble Fas (APO-1, CD95) and soluble Fas ligand in rheumatic diseases. *Arthritis Rheum* 1997; 40: 1126-9.
5. TREVINO-TALAVERA BA, PALAFOX-SANCHEZ CA, MUNOZ-VALLE JF *et al.*: FAS -670A>G promoter polymorphism is associated with soluble Fas levels in primary Sjögren's syndrome. *Genetics Mol Res* 2014; 13: 4831-8.
6. FUJIHARA T, TAKEUCHI T, TSUBOTA K *et al.*: Serum soluble Fas/APO-1 is increased in patients with primary Sjögren's syndrome. *Clin Rheumatol* 1998; 17: 496-9.
7. SAHIN M, AYDINTUG O, TUNC SE *et al.*: Serum soluble Fas levels in patients with autoimmune rheumatic diseases. *Clin Biochem* 2007; 40: 6-10.
8. COPPO P, SIBILIA J, MALOISEL F *et al.*: Primary Sjögren's syndrome associated agranulocytosis: a benign disorder? *Ann Rheum Dis* 2003; 62: 476-8.
9. LUO J, WANG Y, YU B, QIAN H, HE Y, SHI G: A Potential of sFasL in Preventing Gland Injury in Sjögren's Syndrome. *Biomed Res Int* 2017; 2017: 5981432.
10. VINCENT FB, LANG T, KANDANE-RATHNAYAKE R *et al.*: Serum and urinary macrophage migration inhibitory factor (MIF) in primary Sjögren's syndrome. *Joint Bone Spine* 2019; 86: 393-5.
11. SUDA T, HASHIMOTO H, TANAKA M *et al.*: Membrane Fas ligand kills human peripheral blood T lymphocytes, and soluble Fas ligand blocks the killing. *J Exp Med* 1997; 186: 2045-50.
12. HOHLBAUM AM, MOE S, MARSHAK-ROTHSTEIN A: Opposing effects of transmembrane and soluble Fas ligand expression on inflammation and tumor cell survival. *J Exp Med* 2000; 191: 1209-20.
13. NOCTURNE G, MARIETTE X: Sjögren Syndrome-associated lymphomas: an update on pathogenesis and management. *Br J Haematol* 2015; 168: 317-27.