Letters to the Editors

Decreased interleukin-35 levels are associated with higher risk of pregnancy morbidity in patients with antiphospholipid syndrome

Sirs,

Interleukin (IL)-35, a cytokine belonging to IL-12 family, is composed of p35 and Epstein-Barr virus-induced gene 3 (EBI3) (1). It could attenuate inflammation by expanding Treg and suppressing T cell activation, including Th17 (2-4). Persistent expression of subunits of IL-35 was observed in embryo maternal syncytiotrophoblasts and extravillous trophoblasts throughout human pregnancy (5, 6). Most recently, Ozkan *et al*. found that plasma IL-35 was decreased in women with history of idiopathic recurrent pregnancy loss (7). These results prompted the present study focusing on the potential pathogenic role of IL-35 in patients with antiphospholipid syndrome (APS), which is characterised mainly by recurrent pregnancy failures and/ or thrombosis.

We enrolled 126 APS patients fulfilling the revised Sydney Criteria (8) and 107 controls, including 38 patients with primary pregnancy morbidity and 69 healthy donors. Major clinical/ laboratory features of the APS patients are presented in Supplementary Table 1. Informed consents were obtained from all participants. Serum IL-35 was tested with enzyme linked immunosorbent assay (ELISA) and expressed as median (interquartile range, IQR). Values of <12.988 pg/ml were considered decreased according to the ROC curve.

IL-35 was detectable in 72.2% (91/126) of APS patients, 97.4% (37/38) of patients with primary pregnancy morbidity and all healthy controls (Chi-square (χ^2), all p<0.001). The sensitivity of the IL-35 cut-off value for recognising APS from controls was 47.6% and the specificity was 86.0%. The concentration of serum IL-35 was also significantly decreased in APS patients (14.73, 0–28.09 pg/ml) and patients with primary pregnancy morbidity (16.00, 10.56–25.00 pg/ml), compared to healthy controls (28.87, 20.16–47.47 pg/ml; Mann-Whitney, all p<0.001) (Fig. 1A).

IL-35 in the 41 APS patients who had pregnancy morbidity history (8.28, 0–17.63 pg/ml) was significantly lower than the other 85 APS patients (17.71, 7.39–33.81 pg/ml) (Mann-Whitney, p=0.008), and the healthy controls (p<0.001) (Fig. 1B). The similar results were observed in female APS patients (Fig. 1C). However, IL-35 levels were not significantly different among APS patients with different pregnancy morbidity events (Kruskal-Wallis H, p=0.232). Female APS patients with decreased IL-35 had increased risk of pregnancy morbidity (OR=2.565, 95% CI 1.138–5.785; Pearson, p=0.022), especially events of foetus death ≥ the 10th week (OR=6.545, 95% CI 6.03, 21.101; p<0.001). APS patients with decreased IL-35 had over 2 times higher risk of total pregnancy morbidity and nearly 7 times higher risk of events of fetus death at or beyond the 10th week, indicating that IL-35 might play a protective role in the immune tolerance during pregnancy in patients with APS.

In contrast, IL-35 was significantly higher in APS patients with thrombosis history (n=72, 18.97, 7.94–33.92 pg/ml) as compared with those without (n=54, 11.02, 0–18.45 pg/ml, Mann-Whitney, p=0.005), while both of which were lower than healthy controls (all p<0.001) (Fig. 1D). The OR of the risk of thrombosis in IL-35-decreased patients was 0.382 (95%CI 0.185, 0.789; Person, p=0.009). APS patients with thrombosis history had higher IL-35, suggesting that IL-35 might affect outcomes of pregnancy through mechanisms independent of thrombosis in APS.

In conclusion, serum IL-35 was decreased in patients with APS and was associated with pregnancy morbidity and thrombosis, raising the hypothesis of its complicated pathogenic roles in APS.

Table I. Clinical and laboratory characteristics of the 126 patients with APS in our study.

Characteristics	Patients with APS (n=126)
Age, years	42.0±15.9
Gender (M/F)	23/103
Clinical manifestations	
Disease duration, median (range), years	1.5 (0.3-34.0)
Pregnancy morbidity, n/N (%)[$]	41/103 (39.8)
Abortion <10th w, n/N (%)	16/41 (43.9)
Foetal death ≥10th w, n/N (%)	22/41 (53.7)
Premature ≤34th w, n/N (%)	1/41 (2.4)
Multi-types, n/N (%)	2/41 (4.9)
Thrombosis, n/N (%)	72/126 (57.1)
Both pregnancy morbidity and thrombosis, n/N (%)	9/126 (7.1)
Thrombocytopenia, n/N (%)	58/126 (46.0)
Frequency of autoantibodies, n/N (%)	
aCL (IgG/ IgM/ IgA)	88/126 (69.8)
aβ2GPI (IgG/ IgM/ IgA)	88/125 (71.2)
LA	49/121 (40.5)
ANA (IgG)	81/126 (64.3)
aPLs titer	
aCL (IgG/ IgM/ IgA), U/ml	19.8, 8.05-55.9
aβ2GPI (IgG/ IgM/ IgA), RU/ml	42.0, 16.5-123.0
Laboratory measurement parameters	
PLT, ×10^9/L	151.69±98.83
IgA, g/L	2.65, 1.87-3.61
IgG, g/L	14.31±5.47
IgM, g/L	1.44±0.93
C3, g/L	0.83±0.31
C4, g/L	0.17±0.13
CRP, mg/dL	3.78, 1.68-13.97
ESR, mm/h	24.00, 11.00-54.00
Treatment	
Prednisone, n/N (%)	58/126 (46.0)
Prednisone, mg/d	17.5, 7.5-41.25
Immunosuppressors, n/N (%)	32/126 (25.4)

[$]The frequency of pregnancy morbidity was calculated in the 103 female APS patients. Normal data were expressed as mean ± SD, while non-normal data were expressed as median (IQR).

Acknowledgements

This study was funded by the National Basic Research Programme of China (973 programme, no. 2012CB517702) and National Natural Science Foundation of China (no. 31170840 and 81471536).

We thank Chun Li, Hua-qun Zhu, Lei Zhu from Peking University People's Hospital, and Ali Al-Hussaini from Cardiff University for their contribution to this work. We also thank all the patients and healthy donors whom took part in this study.

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Competing interests: none declared.

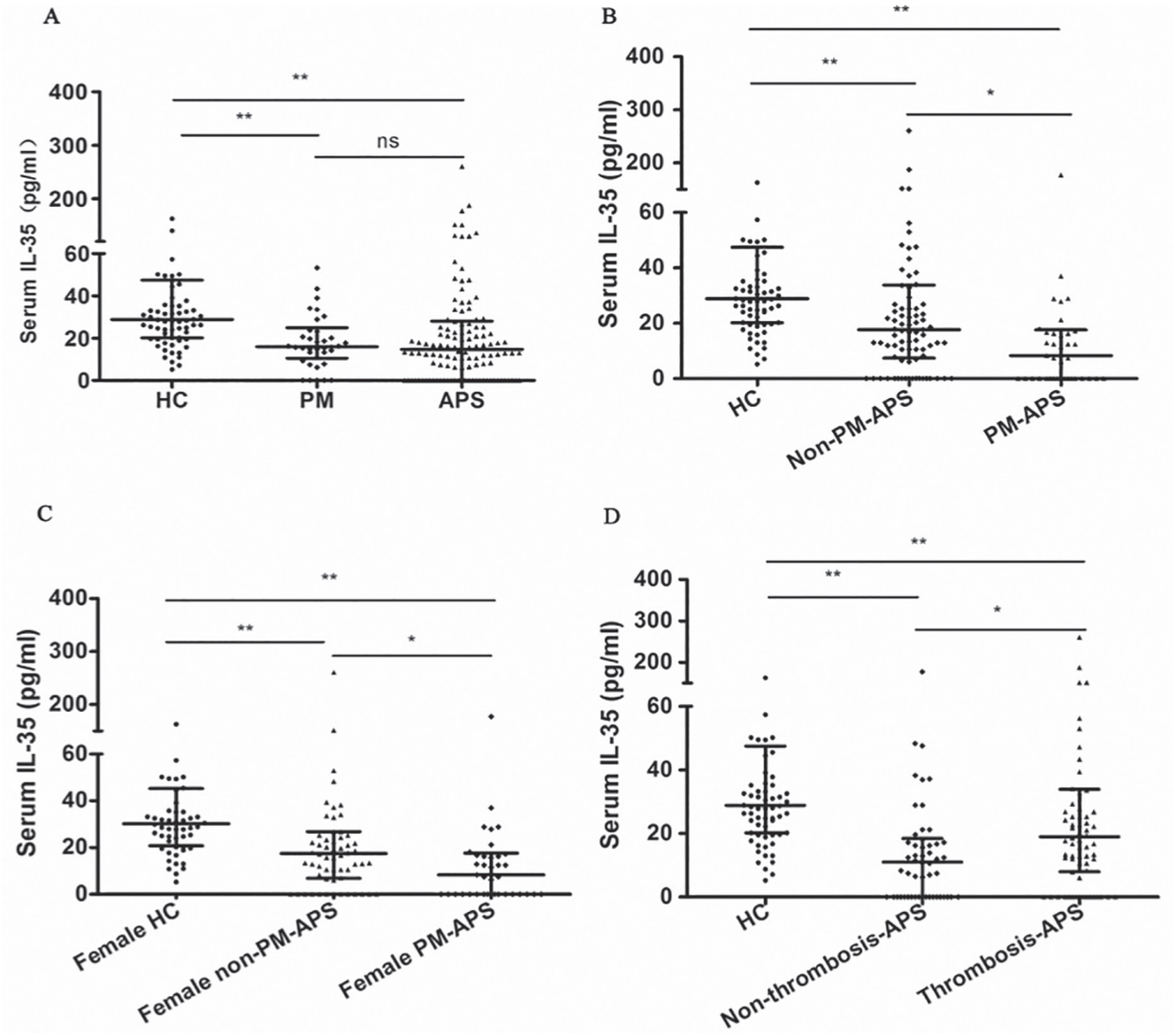

Fig. 1. Serum IL-35 concentration was decreased in patients with APS.
A. IL-35 in patients with APS (14.73, 0-28.09 pg/ml) and patients with primary pregnancy morbidity (16.00, 10.56-25.00 pg/ml) was significantly lower than healthy controls (28.87, 20.16-47.47 pg/ml) (all p <0.001).
B. Serum IL-35 in the 41 APS patients with history of pregnancy morbidity (8.28, 0-17.63 pg/ml) was significantly lower than the other 85 APS patients (17.71, 7.39-33.81 pg/ml; p=0.008) and healthy controls (p<0.001).
C. The 41 APS patients with history of pregnancy morbidity had significantly lower IL-35 level compared to the other 62 female patients (17.47, 6.84-26.80 pg/ml; p=0.021) and female healthy controls (n=56; 31.49, 24.85-41.78 pg/ml; p<0.001).
D. IL-35 concentration in APS patients with thrombosis history (n=72; 18.97, 7.94-33.92 pg/ml) was significantly higher than in patients without thrombosis (n=54; 11.02, 0-18.45 pg/ml; p=0.005), while both of which were lower than in healthy controls (all p<0.001).
The Mann-Whitney U-test was used. HC: healthy controls; APS: antiphospholipid syndrome; PM: primary pregnancy morbidity; Non-PM-APS: APS patients without history of pregnancy morbidity; PM-APS: APS patients with history of pregnancy morbidity; Non-thrombosis-APS: APS patients without thrombosis; Thrombosis-APS: APS patients with thrombosis; *p<0.05, **p<0.001, ns: no significance. Bar: median, IQR.

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