POTENTIAL OF SOLUBLE IMMUNE CHECKPOINTS AS PROGNOSTIC AND PREDICTIVE BIOMARKERS IN ENDOMETRIAL CANCER

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Abstract

Soluble immune checkpoints (sICs) have recently been studied as diagnostic, prognostic and predictive biomarkers in a range of cancers. Aim of presented study was to discover potential diagnostic, prognostic and predictive biomarkers among a set of sICs in endometrial cancer (EC). We included 50 patients diagnosed with EC prior to surgery and 26 women undergoing surgery for benign gynaecologic conditions as controls. We measured plasma concentrations of 16 sICs using Luminex XMAP Multiplex immunosorbent assay, 8 inhibitory (sPD-1, sPD-L1, sPD-L2, sCTLA-4, sLAG3, sTIM3, sBTLA, sHVEM), 6 stimulatory (sICOS, sGITR, sGITR-L, sCD40, sCD27, sCD2) and 2 with mixed role (sCD80, sCD86). Study cohort included 66% of EC patients who had non-aggressive histological type, while 33% of tumors exhibited MMR d eficiency and 14% had aberrant p53 expression. Majority of tumors were confined to the uterus, with 16% of patients having stage cancer of stage IIIA or higher. sICs levels did not significantly differ between control patients and EC patients. Also, sICs levels did not significantly differ by aggressiveness of histological type. Levels of sTIM3 tended to be higher in more advanced stage cancers, similarly, its levels were higher in higher risk group patients. Levels of sPD-1, sPD-L1, sLAG-3, sICOS, sGITR, sGITRL, sCD86 were higher in MMR deficient tumors. Contrary to published findings in a small cohort of EC patients and in other malignancies, we did not detect differing sICs levels in EC patients against patients without cancer. This may be due to majority of the patients in presented study having localised disease. Further studies are needed before ruling out sICs as diagnostic biomarkers in endometrial cancerEC. Biological foundation of higher levels of some sICs in MMR deficient tumors may be their immunoreactivity, stimulating immune response. This finding may be clinically significant, as blood-based prognostic and predictive biomarkers could preclude the need for repeat biopsies and overcome tissue biomarker limitations.

Introduction

Soluble immune checkpoints (sICs) are proteins measurable in peripheral blood, closely related to transmembrane forms, the latter have established role in immune regulation and are targets of oncological immunotherapies.[1,2] These molecules have been studied as diagnostic, prognostic and predictive biomarkers in a range of cancers. Simplistically, sICs can be divided into inhibitory and stimulatory. Elevated levels of the former have been associated with cancer diagnosis, less favourable prognosis and worse response to therapy, mainly immunotherapy. Studies of stimulatory immune checkpoints are few and yield inconsistent findings.[2–5] Recently, levels of 3 sICs – sPD-1, sPD-L1 and sPD-L2 were examined in two small cohorts of endometrial cancer (EC) patients, observing higher sPD-L1 and sPD-L2 levels in EC patients compared to controls and finding higher sPD-L1 levels in patients with higher stage and grade tumors.[6,7] Aim of presented study was to discover potential diagnostic, prognostic and predictive biomarkers among a larger set of sICs in endometrial cancer (EC).

Methods

In this case-control study we included 50 patients diagnosed with endometrial cancer before undergoing surgical treatment and 26 women without significant comorbidities before undergoing surgery for benign gynaecologic conditions (mainly pelvic organ prolapse, leiomyomas and urinary incontinence). Patients with active malignant disease at other site, with recurrent endometrial cancer or receiving immunosuppressive therapy were excluded. We obtained and processed peripheral venous blood into plasma according to standardized protocol. We measured plasma concentrations of 16 sICs using Luminex XMAP Multiplex immunosorbent assay, 8 inhibitory (sPD-1, sPD-L1, sPD-L2, sCTLA-4, sLAG3, sTIM3, sBTLA, sHVEM), 6 stimulatory (sICOS, sGITR, sGITR-L, sCD40, sCD27, sCD2) and 2 with mixed role (sCD80, sCD86) – schematically represented in Figure 1.

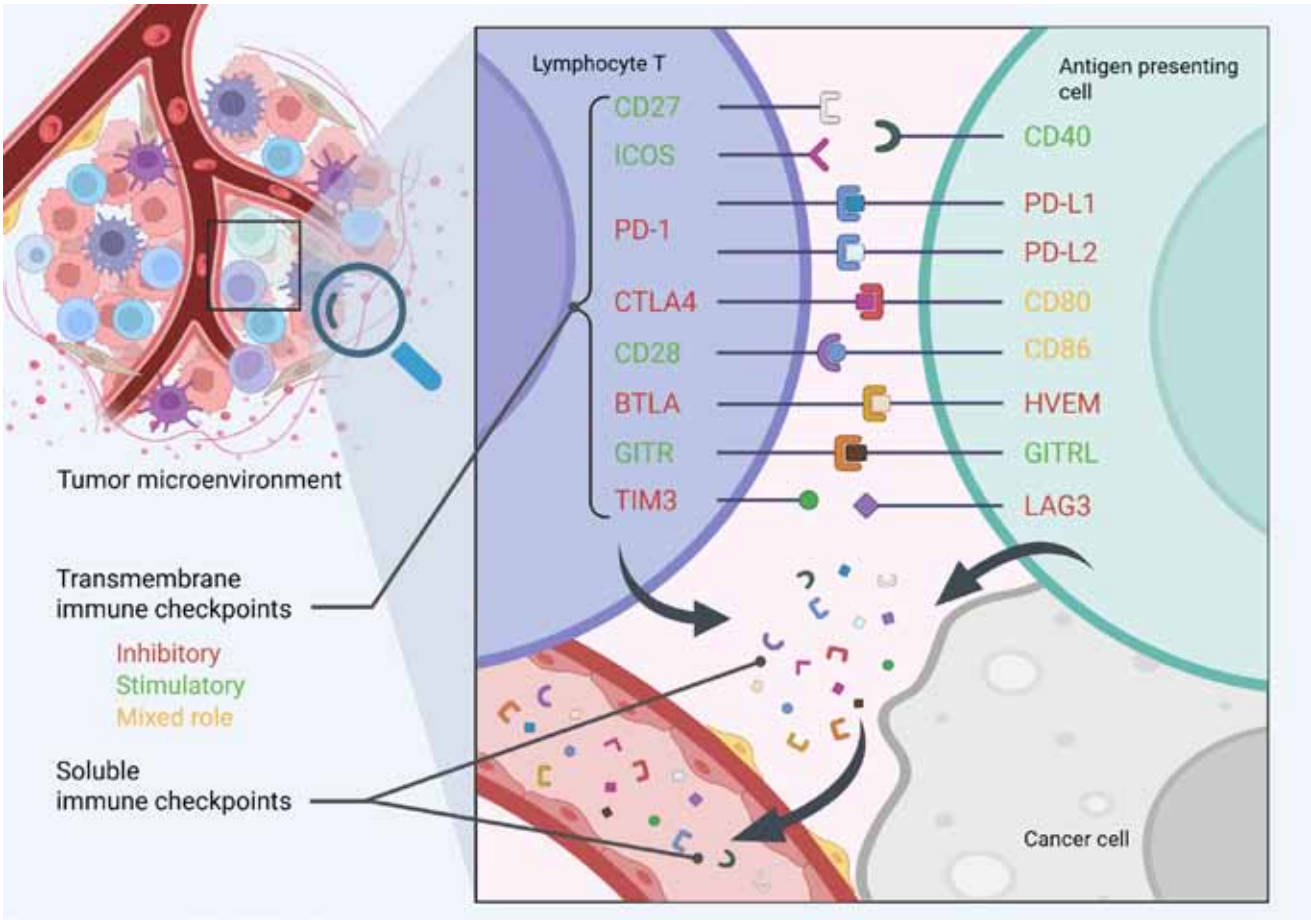

Figure 1: Schematic representation of studied soluble immune checkpoints (sICs).

Results

Groups of endometrial cancer patients and control subjects were well balanced in respect to age, BMI, waist-to-hip ratio, amount of physical activity, alcohol consumption, smoking status and presence of obesity (Table 1). Study cohort included 66% of EC patients who had non-aggressive histological type, while 33% of tumors exhibited MMR deficiency and 14% had aberrant p53 expression. Majority of tumors were confined to the uterus, with 16% of patients having stage cancer of stage IIIA or higher (Table 2). sIC levels did not significantly differ between control patients and EC patients. Also, sICs levels did not significantly differ by aggressiveness of histological type. Levels of sTIM3 tended to be higher in more advanced stage cancers, similarly, its levels were higher in higher risk group patients (Figure 3). Levels of sPD-1, sPD-L1, sLAG-3, sICOS, sGITR, sGITRL, sCD86 were higher in mismatch repair (MMR) deficient tumors (Figure 2).

Table 1: Characteristics of study patients. Obesity defined as BMI > 30 kg/m^2 as per WHO.

	All patients (N=76)	Control patients (N=26)	EC patients (N=50)
Age	64.0 (55.0, 70.0)	63.5 (55.2, 68.0)	64.0 (55.2, 70.0)
BMI (kg/m^2)	29.1 (25.4, 33.1)	29.8 (26.8, 33.0)	28.9 (25.0, 33.0)
Waist to hip ratio	0.860 (0.810, 0.920)	0.865 (0.795, 0.915)	0.860 (0.825, 0.915)
Menopausal status			
Postmenopausal	62 (81.6%)	19 (73.1%)	43 (86.0%)
Premenopausal	14 (18.4%)	7 (26.9%)	7 (14.0%)
Age at menopause	52.0 (50.0, 53.0)	52.0 (50.0, 53.0)	52.0 (50.0, 53.0)
History of infertility			
No	69 (94.5%)	25 (96.2%)	44 (93.6%)
Yes	4 (5.5%)	1 (3.8%)	3 (6.4%)
Parity (N)			
0	3 (4.1%)	1 (3.8%)	2 (4.3%)
1	15 (20.5%)	2 (7.7%)	13 (27.7%)
2	41 (56.2%)	16 (61.5%)	25 (53.2%)
>2	14 (19.2%)	7 (26.9%)	7 (14.9%)
Physical activity (average hrs/week)	27.8 (20.0, 35.2)	27.0 (19.3, 33.1)	27.8 (22.1, 35.8)
Alcohol consumption			
No	58 (80.6%)	21 (80.8%)	37 (80.4%)
Yes	14 (19.4%)	5 (19.2%)	9 (19.6%)
Smoking status			
Non-smoker	62 (86.1%)	22 (84.6%)	40 (87.0%)
Smoker	10 (13.9%)	4 (15.4%)	6 (13.0%)
Obesity			
No	40 (52.6%)	13 (50.0%)	27 (54.0%)
Yes	36 (47.4%)	13 (50.0%)	32 (46.0%)

Table 2: Tumour characteristics in EC patients.

EC patients (N = 50)		
Histological type	Endometrioid	41 (82.0%)
	Serous	6 (12.0%)
	Mixed	2 (4.0%)
	Other	1 (2.0%)
Endometrioid cancer grade	1	20 (47.6%)
	2	13 (31.0%)
	3	9 (21.4%)
Histological type (FIGO 2023)[a]	non-aggressive	33 (66.0%)
	aggressive	17 (34.0%)
MMR status	dMMR	16 (32.0%)
	pMMR	34 (68.0%)
p53 status	Abberant	7 (14.0%)
	Wild type	43 (86.0%)
Stage (FIGO 2023)	IA1	11 (22.0%)
	IA2	7 (14.0%)
	IA3	0 (0.0%)
	IB	2 (4.0%)
	IC	6 (12.0%)
	IIA	3 (6.0%)
	IIB	8 (16.0%)
	IIC	3 (6.0%)
	IIC(IICmp53ab)	2 (4.0%)
	IIIA	0 (0.0%)
	IIIB	0 (0.0%)
	IIIC1	3 (6.0%)
	IIIC2	2 (4.0%)
	IVA	0 (0.0%)
	IVB	3 (6.0%)
	IVC	0 (0.0%)
Risk group (ESGO 2020)[b]	LR	18 (36.0%)
	IR	8 (16.0%)
	HIR	14 (28.0%)
	HR	7 (14.0%)
	AM	3 (6.0%)

Legend: a - endometrioid grade 1 or 2 cancers are regarded as non-aggressive and others as aggressive. b - Risk groups according to ESGO 2020 guidelines, LR - low risk, IR - intermediate risk, HIR - high intermediate risk, HR - high risk, AM - advanced/metastatic disease (stage III or higher with residual disease after surgery or stage IV)

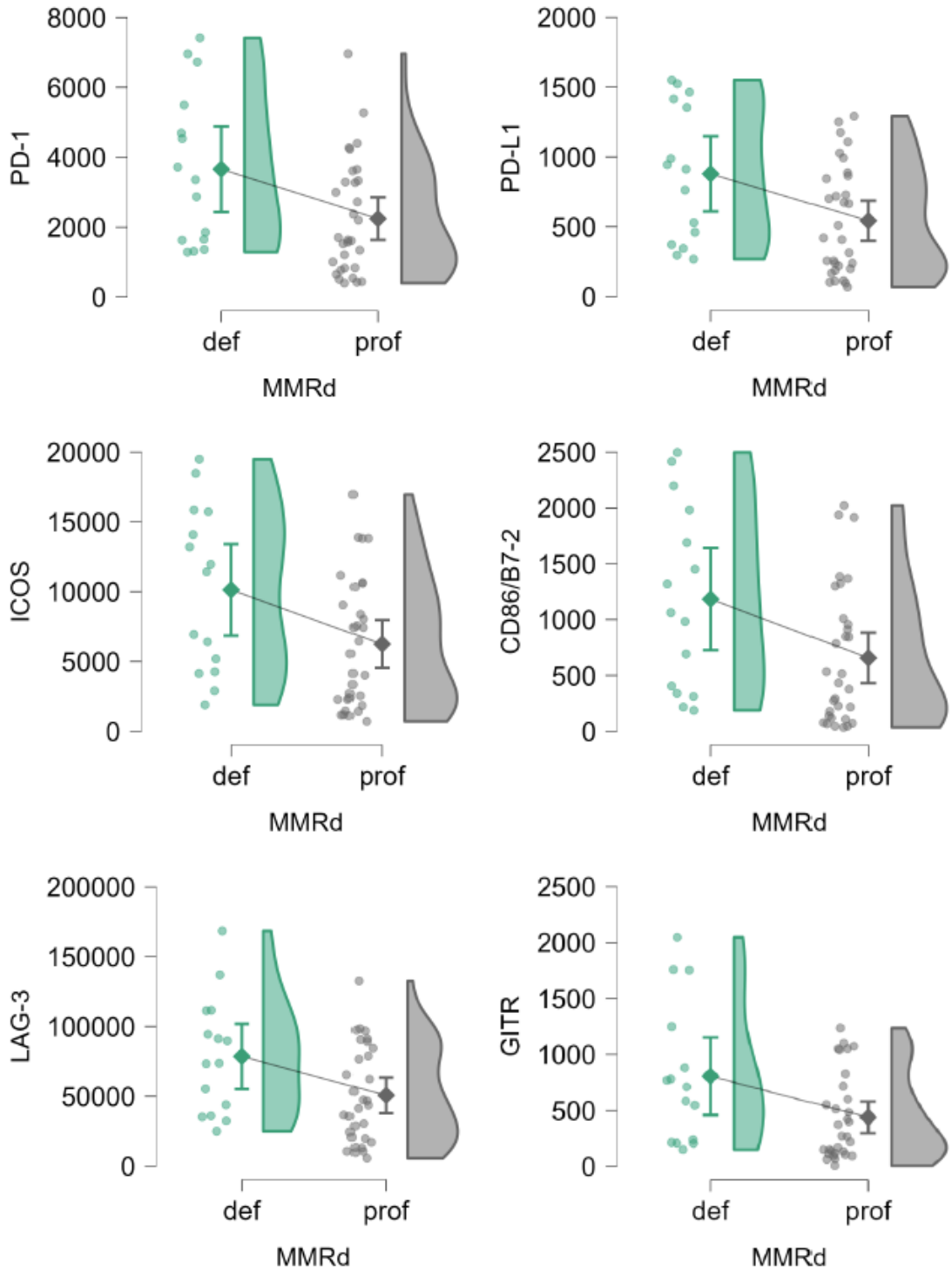

Figure 2: Raincloud plots of significant differences ($p<0.05$) in sIC levels by tumor MMR status. Levels are expressed in pmol/L. Plotted from left to right are jittered data points, mean and 95% CI, kernel density plot.

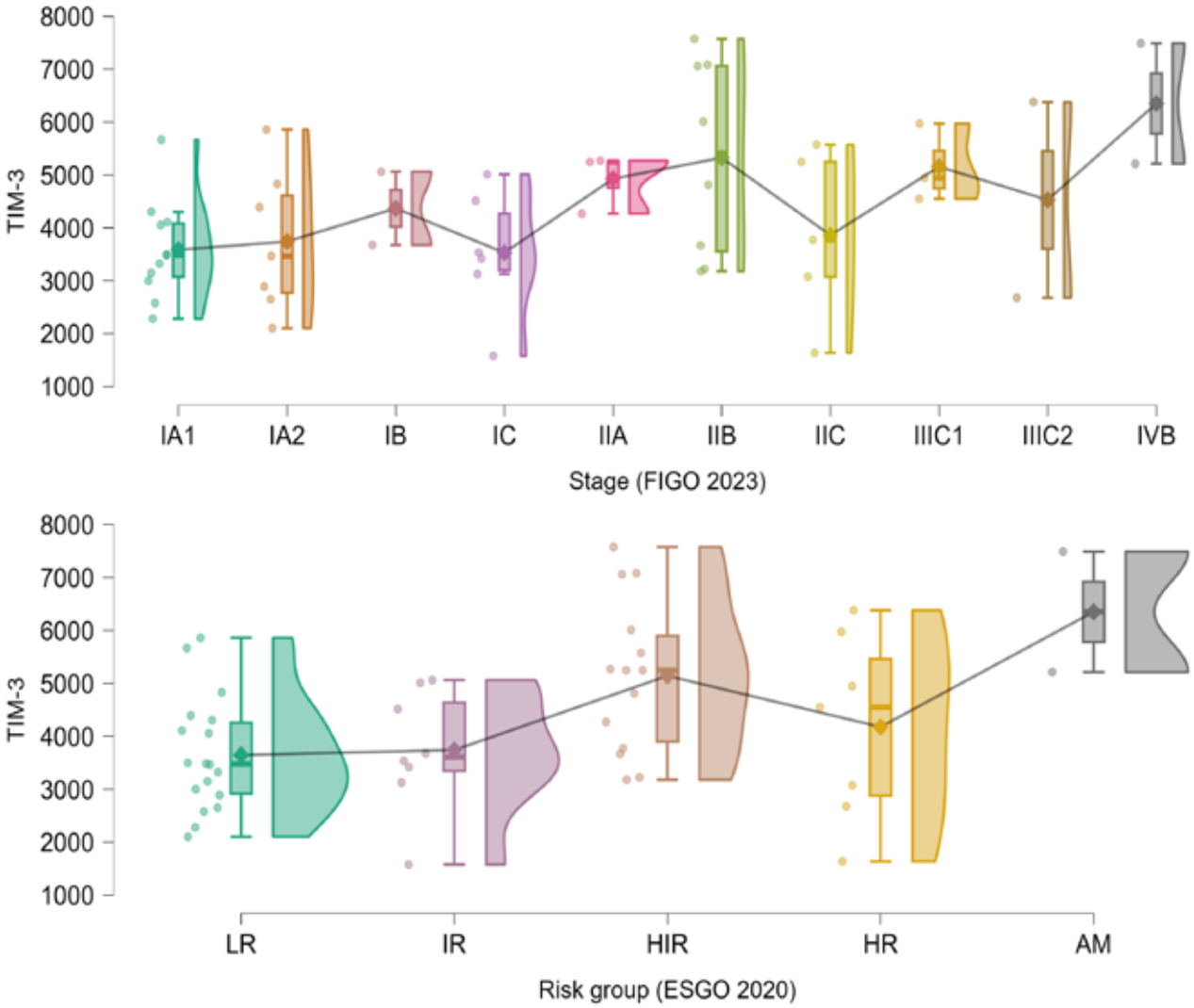

Figure 3: Raincloud plot of differing TIM3 levels by FIGO 2023 stage (upper) and ESGO 2020 guidelines risk group (lower). Levels are expressed in pmol/L. Plotted from left to right are jittered data points, box and whiskers plot with added mean, kernel density plot. LR – low risk, IR – intermediate risk, HIR – high intermediate risk, HR – high risk, AM – advanced/metastatic disease (stage III or higher with residual disease after surgery or stage IV)

Conclusion

Contrary to previously published findings in a small cohort of EC patients and findings in other malignancies, we did not detect differing sICs levels in EC patients against patients without cancer. This may be due to majority of the patients in presented study having localised disease. Further studies are needed before ruling out sICs as diagnostic biomarkers in endometrial cancer. Biological foundation of higher levels of some sICs in MMR deficient tumours may be their immunoreactivity, stimulating immune response. This finding may be clinically significant, as blood-based prognostic and predictive biomarkers could preclude the need for repeat biopsies and overcome tissue biomarker limitations.

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