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ORIGINAL RESEARCH

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PD-L1 in a South African cohort of endometrial carcinomas: directions for the future

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Abstract

The Programmed Death-1(PD-1)/Programmed Death Ligand 1 (PD-L1) pathway is the focus of numerous clinical trials on various tumours using immunotherapy. Endometrial tumours with *Polymerase E* gene mutations and microsatellite unstable neoplasms have demonstrated strong immune responses against mutationally associated neoantigens, with favourable results using immune checkpoint inhibitors. We assessed PD-L1 on endometrioid endometrial carcinomas (EECs) in South Africa's state hospital sector, which, to the best of our knowledge, has not been investigated. We performed PD-L1 immunohistochemistry on 145 EECs and compared PD-L1 status to our data on cases that had previously undergone mismatch repair (MMR) immunohistochemistry, microsatellite instability assessment by polymerase chain reaction (PCR) and methylation analysis. PD-L1 was expressed in 13.1% (19/145) of EECs, of which, 7 (36.8%) showed MMR deficiency. There was MMR deficiency in 26.9% (34/126) of PD-L1 negative cases (p = 0.37). There were 47.4% (9/19) microsatellite unstable PD-L1 positive cases whereas 52.6% (10/19) of PD-L1 positive cases were microsatellite stable by PCR (p = 0.23). From the PD-L1 positive cases that underwent methylation testing, 80% (8/10) were methylated and 20% (2/10) were unmethylated (p = 0.54). Compared to a Jordanian study and a Chinese study, on populations also not extensively investigated, our study demonstrated lower PD-L1 expression (p = 0.0170; p-value $<$ 0.001 respectively). Our study showed that approximately two-thirds of cases were MMR proficient, which is more than twice the accepted published number of PD-L1 positive cases with MMR staining. This suggests that in our population PD-L1 staining should be considered in all EECs to identify cases that may derive benefit from immune checkpoint inhibitors. It is envisioned that this study may provide the impetus for future possible immune therapies for endometrial cancer patients in the state sector of South Africa. Our study provides data from a developing country which adds to current global data.

Keywords

PD-L1; Endometrial carcinoma; South Africa

1. Introduction

Globally, uterine corpus malignancies are the sixth most frequently occurring neoplasms, and endometrial carcinomas are the most commonly diagnosed malignancies of the female genital tract in developed countries [1, 2]. According to the most recent South African data (2019), uterine malignancies were responsible for 3.6% of all malignancies [3]. There has been an incremental increase in the incidence of this tumour in South Africa (SA), which mirrors global trends [4–8]. The worldwide increase may be due to exogenous and endogenous estrogen such as in the following situations: prolonged lifespans and thus increased time that females are exposed to circulating estrogen, hormonal estrogenic therapy without progesterone, increasing incidence of obesity and greater use of Tamoxifen for breast carcinoma treatment [7–9].

The Cancer Genome Atlas (TCGA) network classified endometrial carcinomas into 4 molecularly based groups, comprising the following: ultramutated (including mutations of the *Polymerase Epsilon* or *POLE* gene, Microsatellite instability (MSI) tumours (or hypermutated MSI tumours), copy number high tumours (in which *TP53* mutations are identified) and copy number low tumours (which lack *TP53* mutations, MSI and *POLE*) [10–13]. Since 2020, this molecular classification has been incorporated into guidelines by the National Comprehensive Cancer Network (NCCN) for endometrial carcinoma diagnosis and management [14]. This is an example of personalised medicine with targeted therapy based on the molecular signature of an individual's tumour and its associated inflammatory infiltrate [14].

It is well known that malignancies can escape host immunity by different means such as preventing the host from launching

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appropriate defence mechanisms against tumour cells. The Programmed Death-1 (PD-1)/Programmed Death Ligand 1 (PD-L1) pathway has been the focus of numerous clinical trials on various tumours using immunotherapy [15]. Physiologically, PD-1 can facilitate immune tolerance by tempering destructive immune responses [10, 16]. PD-1 is an immune checkpoint receptor that forms part of the cluster of differentiation (CD) 28 epitope on lymphocytes [17]. PD-L1 is also expressed on histiocytes, dendritic cells, some epithelial cells, and malignant cells. PD-L1 expression by neoplastic cells facilitates their evasion by host anti-tumoural activity [16, 18]. When PD-1 and its ligand PD-L1 or PD-L2 bind, by decreasing T-cell activity at peripheral sites, and thereby decreasing T-cell activity, there is an upsurge in immune resistance in the tumour microenvironment, which then decreases damage to host tissues [14, 17]. PD-L1 promotes self-tolerance by altering T-cell activity, preventing apoptosis, in addition to increasing apoptosis of antigen-specific T-cells [16]. Furthermore, intracellular signalling pathways may be affected by upregulated PD-L1 [16]. Neoplastic cells can increase PD-L1 expression, which then binds to PD-1 on T-cells resulting in stimulation of inhibitory pathways with subsequent prevention of cellular destruction, and thus tumour cell survival [14]. As such, increased PD-L1 may result in progression of malignant disease with an unfavourable prognosis [19].

Immune checkpoint inhibitor mechanisms of action thus enhance tumour identification by activating the host's immune system, resulting in a decrease and prevention of immune escape [14, 20]. Anti-PD-L1 immunotherapeutic agents have demonstrated favourable responses with tumour shrinkage and increased patient overall survival in several studies on metastatic or recurrent solid tumours [20].

In endometrial carcinomas, tumours with a multitude of *Polymerase E* gene mutations, (one of the 4 molecular categories) and microsatellite unstable neoplasms have shown a strong immune response directed against the large numbers of mutationally associated neoantigens [20, 21]. Such tumours have also demonstrated favourable results with use of immune checkpoint inhibitors, with resultant reduction in tumour burden [21]. The identification of MSI is now a biomarker for a positive outcome to immune checkpoint inhibitors [20].

Use of checkpoint inhibitors in endometrial carcinomas is currently not widespread and at present, is not available in the state/public health sector of South Africa. To the best of our knowledge, PD-L1 assessment on endometrial carcinomas has not been investigated to date in the state sector of South Africa and we aimed to compare these results to data previously collected on a cohort of endometrioid endometrial carcinomas that had undergone mismatch repair immunohistochemistry, MSI assessment by polymerase chain reaction (PCR) and methylation analysis [22].

2. Methods

Following ethical clearance (M2102100) immunohistochemistry (IHC) for PD-L1 (Dako, Agilent Technologies, Inc., Santa Clara, USA; Clone 22C3, 1:50) was performed on 4 μm deparaffinised whole tissue sections of 145 cases of endometrioid endometrial carcinoma, diagnosed at the Department of Anatomical Pathology, University of the Witwatersrand/National Health Laboratory Service, Johannesburg, South Africa. These 145 cases comprised endometrial curettage samples in some cases, and full thickness sections of tumour from the excision specimens for some patient samples. In a developing country such as South Africa, patients are often unfortunately lost to follow-up and as such not all patients who undergo an initial endometrial biopsy will have a hysterectomy. As such, in cases where only an endometrial curettage was available without the patient's corresponding hysterectomy specimen, only the curettage specimen was used for further testing. PD-L1 immunohistochemistry was performed according to departmental standard operating procedures and the manufacturer instructions, on the AS 48 Link. Briefly, after incubation with the primary monoclonal antibody to PD-L1, specimens were incubated with a linker antibody specific to the host species of the primary antibody. These were subsequently incubated with a ready-to-use visualization reagent, which comprised a secondary antibody molecule together with horseradish peroxidase (HRP), in addition to a dextran polymer backbone. A chromogen was then added which allowed for visualisation of the product at the antigen site. Thereafter, the tissue sections were counterstained and coverslipped [23].

Immunohistochemistry for the four mismatch repair markers (MLH1, PMS2, MSH2 and MSH6), PCR for MSI (using the mononuclear markers NR-21, NR-24, NR-27, BAT-25 and BAT26) were conducted on all 145 cases. Methylation analysis using MassARRAY EpiTYPER was performed on all MLH1/PMS2 deficient cases and 25 cases that showed discordance between MMR testing by IHC and by MSI by PCR. Thus, a total of 62 cases underwent methylation assessment with a single case harbouring insufficient DNA for analysis and was subsequently excluded for comparative purposes. These tests were undertaken as part of a prior research project by the principal investigator and have previously been described [22]. Tumours with methylation levels $\geq$10% were interpreted as hypermethylated [24]. The data from these previous investigations were used to compare against PD-L1 results in the current study.

2.1 PD-L1 assessment

Stained tissue sections of endometrial carcinoma were evaluated for PD-L1 staining using the 20$\times$ objective, on a BX43 Olympus microscope. Tumour cells were interpreted as positive if $\geq$1% of neoplastic cells demonstrated cytoplasmic and/or membranous staining, whilst inflammatory cells were read as positive if there was any PD-L1 staining in $\geq$1% of such cells (Fig. 1). The combined positive score (CPS) was determined by calculating the numerator (tumour cells, macrophages and lymphocytes)/denominator (total number of tumour cells) $\times$ 100. A value $\geq$1 was deemed positive as previously described [25].

2.2 Data analysis

Frequencies and percentage frequencies were used to describe categorical variables while normally distributed continuous

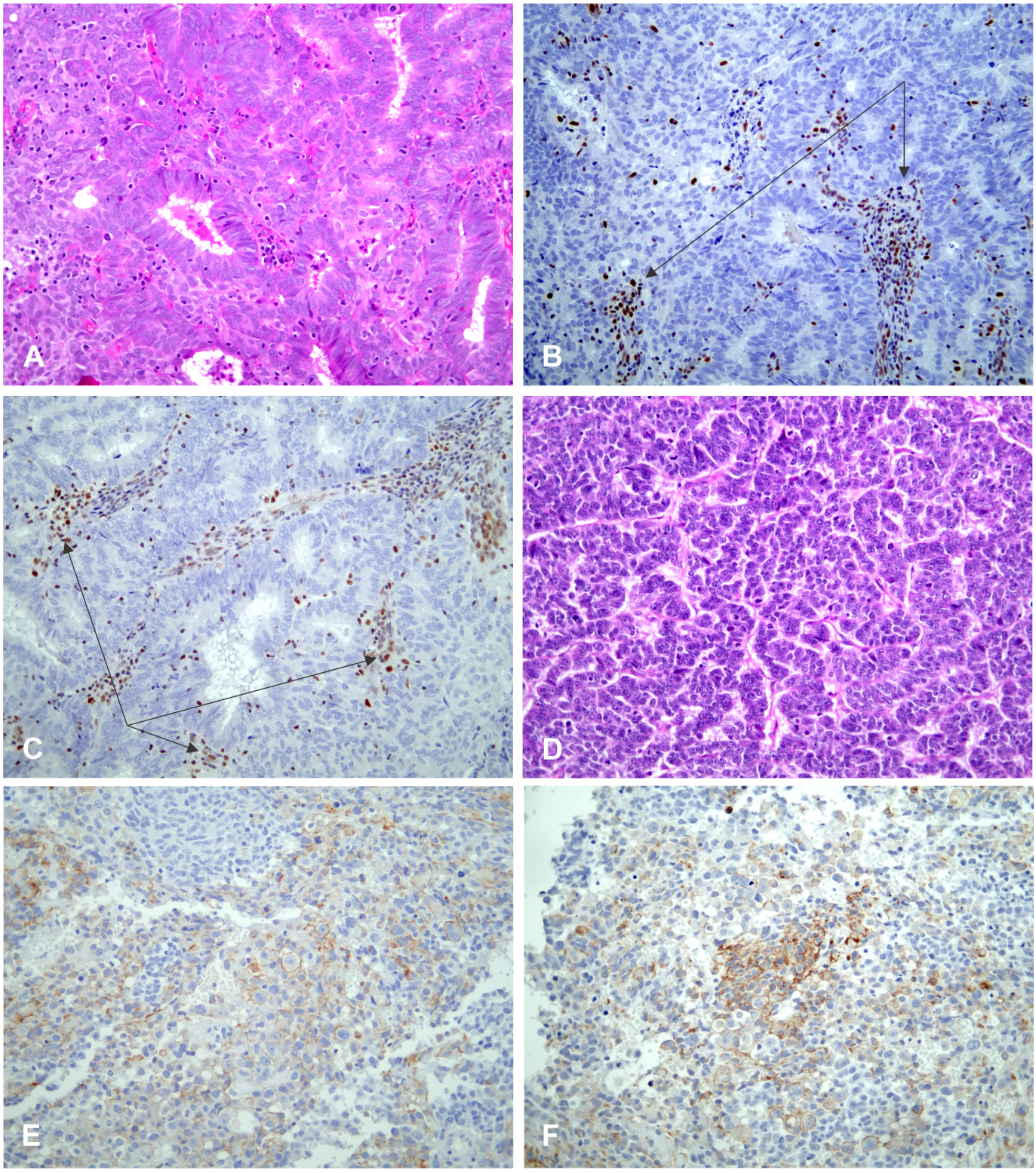


FIGURE 1. Microscopic images of endometrioid endometrial carcinomas and immunohistochemical stains. (A) Haematoxylin and Eosin-stained section of a grade 1 endometrioid endometrial carcinoma. (B) An MLH immunohistochemical stain showing loss of staining of tumour nuclei but retained staining of lymphocytes (arrows). (C) A PMS2 immunohistochemical stain demonstrates loss of staining of tumour nuclei but retained staining of stromal cells and lymphocytes (arrows). (D) Haematoxylin and Eosin-stained section of a grade 3 endometrioid endometrial carcinoma. (E and F) PD-L1 positive staining of tumour cells. All images at 200× magnification.

variables were described using means and standard deviations whereas for nonnormally distributed continuous medians and interquartile ranges were used. Patient cases were stratified according to PD-L1 CPS (<1 *vs.* ≥1) and clinicopathological characteristics were described while bivariate associations were assessed. The Fishers' Exact test was used to assess association between categorical variables and independent *t*-test was used for continuous variables. Kappa statistics were computed to estimate level of concordance between MMR IHC and PD-L1 results. These statistics were further computed and stratified by different age groups and by tumour grade. The agreement percentages with associated confidence intervals were also estimated. The logistic regression was used to assess the predictive ability of MMR IHC results for PD-L1 expression. Statistical analyses were undertaken utilising STATA version 18 (StataCorp LP, College Station, TX, USA). For all hypothesis testing, a p-value < 0.05 was considered statistically significant.

3. Results

Table 1 shows the prevalence of PD-L1 cases 19/145 (13.1%) from our cohort of endometrioid endometrial carcinomas (Fig. 1). There were 126 cases that were PD-L1 negative. Of the 19 PD-L1 positive cases, 4 (21.1%) had a CPS of 1, while the remainder (78.9%) had a CPS of ≥5. Table 2 shows that the average age of patients whose tumours showed PD-L1 positivity was 67.2 years, while the average age of patients who had PD-L1 negative tumours, was 64.9 years. The majority (36.8%) of PD-L1 positive tumours demonstrated International Federation of Gynecology and Obstetrics (FIGO) grade 2 histopathological features, while equal numbers of PD-L1 positive cases (31.6%) were diagnosed as FIGO grade 1 and 3 carcinomas. In contrast, within the PD-L1 negative group of tumours, most (44.4%) tumours were FIGO grade 2 tumours, followed by FIGO grade 1 and FIGO grade 3 tumours accounting for 30.2% and 25.4% of cases respectively, which is not statistically significant ($p = 0.79$). When the tumours were classified in a binary manner, in which grades 1 and 2 tumours were considered low grade tumours while FIGO grade 3 were considered high-grade tumours, 13/19 (68.4%) of tumours were PD-L1 positive, while 6/19 (31.6%) were PD-L1 negative, which is not statistically significant.

Regarding immunohistochemical mismatch repair (MMR) testing, 7 (36.8%) PD-L1 positive cases showed immunohistochemical mismatch repair deficiency, and of these 7 cases, 3 (42.9%) showed loss of staining for both MLH1 and PMS2, whilst 4 (57.1%) cases showed isolated MLH1 loss of staining, and none were deficient for MSH2 or MSH6. Twelve cases were mismatch repair proficient for all 4 markers. In the PD-L1 negative group of tumours, however, there was MMR deficiency in 27% of cases while 73% demonstrated MMR-proficiency, which is not statistically significant ($p = 0.37$).

Table 2 shows that of the PD-L1 positive cases, 47.4% were microsatellite unstable by PCR, whereas 29.4% of PD-L1 negative cases demonstrated MSI. In contrast, 52.6% of PD-L1 positive cases were microsatellite stable and 66.7% of PD-L1 negative cases were unstable, which was not statistically significant ($p = 0.23$). From the PD-L1 positive cases, 44.4% were MSI-High (as the tumours showed differences in allele sizes in at least two out of five markers when compared to the patient's non-tumour tissue). Of these 4, 3 cases showed differences in allele size for 2 markers, whilst a single case had allele size differences in three markers. The remaining 55.6% PD-L1 positive cases that were microsatellite unstable, were interpreted as being MSI-Low as these tumours had only 1 out of the 5 markers showing differences in allele sizes when compared to non-tumour patient tissue. The rest of the PD-L1 positive cases were microsatellite stable. From the PD-L1 negative tumours, 66.7% were stable, 29.4% were unstable and 4% had no PCR reaction (despite having been

TABLE 1. Clinicopathological patient characteristics.

Characteristic	Number (%)
Patients with PD-L1 positive tumours	19/145 (13.1%)
Age (yr) mean (SD)	65 (9.21)
<60	51 (35%)
≥60	94 (65%)
Tumour FIGO grade	
Grade 1	44 (30%)
Grade 2	63 (44%)
Grade 3	38 (26%)
Binary Tumour FIGO Grade	
Low grade	107 (73.8%)
High grade	38 (26.2%)
Immunohistochemistry	
Mismatch repair deficient	41 (28%)
Mismatch repair proficient	104 (72%)
Polymerase Chain Reaction	
Microsatellite stable	94 (65%)
Microsatellite unstable	46 (32%)
No reaction	5 (3%)
**Microsatellite Unstable	
MSI-Low	35 (76%)
MSI-High	11 (24%)
Microsatellite	
Stable (MSS and MSI-Low)	129 (89%)
High	11 (8%)
No reaction	5 (3%)
Methylation^{+}	
Methylated	44 (72%)
Not methylated	17 (28%)

$^{+}$*numbers are only for MLH1 deficient patients and cases of discordant MMR IHC and MSI PCR results and* ***depicts microsatellite unstable patients.*

PD-L1: Programmed Death Ligand 1; SD: Standard Deviation; FIGO: International Federation of Gynecology and Obstetrics; MSI: Microsatellite instability; MSS: Microsatellite stable.

TABLE 2. Clinicopathological characteristics of PD-L1 positive endometrial carcinomas.

Factor	Level	PD-L1 negative	PD-L1 positive	*p*-value
N		126	19	
Age, mean (SD)		64.9 (9.2)	67.2 (9.2)	0.32
Age category				
	<60 yr	47 (37.3%)	4 (21.1%)	0.17
	≥60 yr	79 (62.7%)	15 (78.9%)	
Tumour FIGO Grade				
	1	38 (30.2%)	6 (31.6%)	
	2	56 (44.4%)	7 (36.8%)	0.79
	3	32 (25.4%)	6 (31.6%)	
Binary Tumour FIGO Grade				
	Low grade	94 (74.6%)	13 (68.4%)	0.58
	High grade	32 (25.4%)	6 (31.6%)	
MMR IHC				
	Deficient	34 (27.0%)	7 (36.8%)	
	Proficient	92 (73.0%)	12 (63.2%)	0.37
	No reaction	5 (4.0%)	0 (0.0%)	
Microsatellite (MS)				
	MS Stable	84 (66.7%)	10 (52.6%)	0.23
	MS Unstable (MSI-Low and MSI-High)	37 (29.4%)	9 (47.4%)	
MS Unstable type**				
	MSI-Low	29 (78%)	6 (67%)	0.46
	MSI-High	8 (22%)	3 (33%)	
Microsatellite				
	Stable (MSS and MSI-Low)	113 (93.4%)	16 (84.2%)	0.17
	High	8 (6.6%)	3 (15.8%)	
Methylation+				
	Not methylated	15 (29%)	2 (20%)	0.54
	Methylated	36 (71%)	8 (80%)	

*+numbers are only for MLH1 deficient patients and cases of discordant MMR IHC and MSI PCR results and **depicts microsatellite unstable patients.*
The p-values were calculated from independent t-test for continuous age or Fishers' exact test for categorical variables.
PD-L1: Programmed Death Ligand 1; SD: Standard Deviation; FIGO: International Federation of Gynecology and Obstetrics; MMR: mismatch repair; MSI: Microsatellite instability; MSS: Microsatellite stable; IHC: Immunohistochemistry.

repeated), which is not statistically significant ($p = 0.46$). In additional assessments, we included the MSI-Low tumours in the category of microsatellite stable tumours (Table 2). In these assessments, the majority (84.2%) of tumours that were PD-L1 positive, were microsatellite stable, while 15.8% of tumours were MSI-High. Most (93.4%) of the microsatellite stable tumours were PD-L1 negative. The results were not statistically significant ($p = 0.172$).

Out of the 61 cases that underwent methylation testing, 29% of PD-L1 negative cases were unmethylated and 71% were methylated, while out of the 10 remaining cases that underwent methylation testing and were PD-L1 positive, 8 cases were methylated and 2 were unmethylated, which was not statistically significant ($p = 0.54$). Furthermore, all 7 PD-L1 positive cases that were mismatch repair deficient, were also hypermethylated. Similarly, 7 PD-L1 positive cases that showed mismatch repair deficiency by PCR were methylated (Fig. 2). Table 3 shows that there was high agreement between results from IHC and PD-L1 as well as between PD-L1 and overall MMR deficiency and/or microsatellite instability. However, the concordance was low and not significant. This was also similar for agreement between methylation and PD-L1 assessments which showed a low and insignificant concordance (kappa = 0.0398, p-value = 0.520). The agreement was also low (37.7%; 95% CI: 25.2–50.2%). Table 4 and Fig. 3 show that the predictive ability of MMR IHC independently for PD-L1 expression was just above random with an area under the curve (AUC) of 0.549 while improving to AUC =

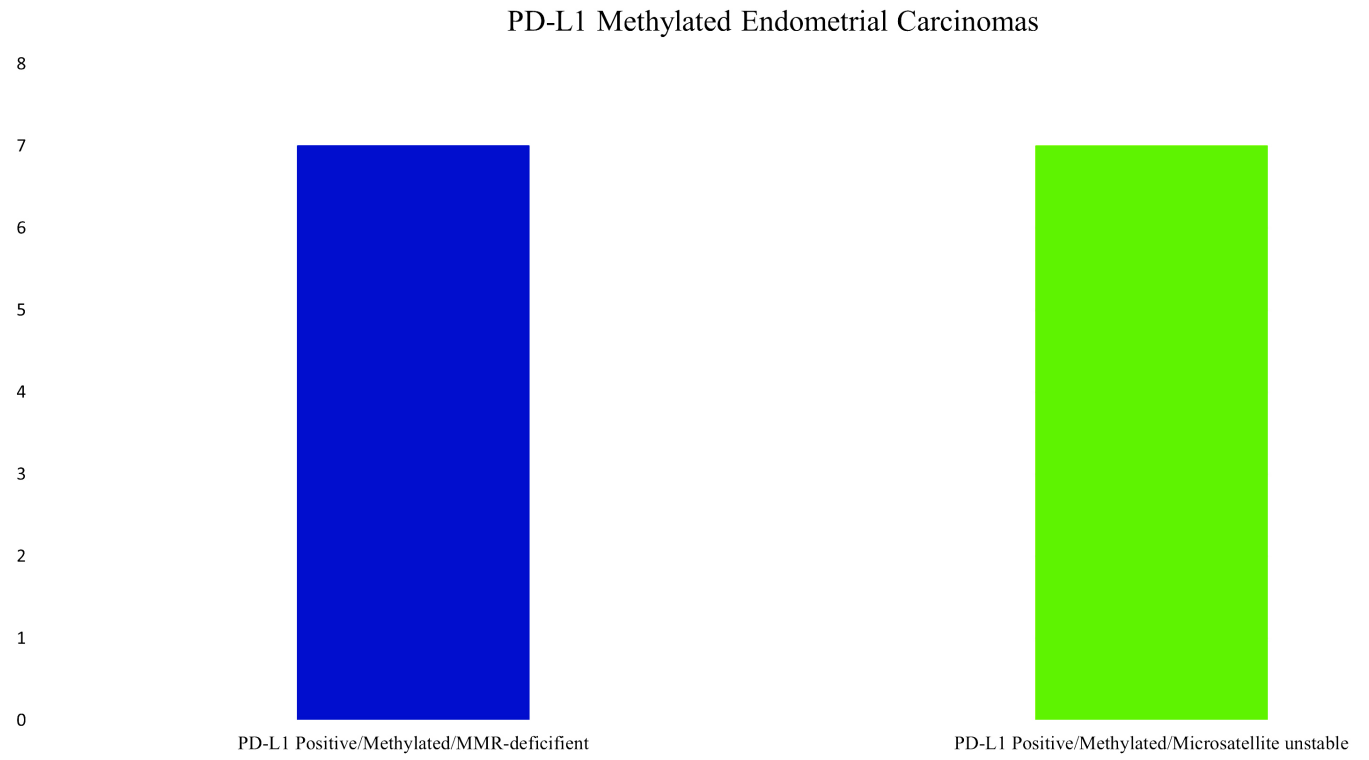


FIGURE 2. A graph demonstrating PD-L1 methylated mismatch repair deficient and microsatellite unstable endometrial carcinomas. PD-L1: Programmed Death Ligand 1; MMR: mismatch repair.

0.620 after adjusting for patient age and tumour grade. The association was, however, not statistically significant.

We compared our current results to results from a previous study by Amarin *et al.* [26] which was undertaken on a Jordanian population, (21.2% *vs.* 13.1%; p-value = 0.0170) and also compared our results to a study by Zong *et al.* [27] (45.1% *vs.* 13.1%; p-value < 0.001). Similar to South Africa, both Jordan and China are developing countries and to date have also not had endometrial carcinomas and PD-L1 expression extensively investigated. The difference was significantly different with PD-L1 expression in our cohort being much lower than the Jordanian and Chinese studies.

4. Discussion

The age of personalised medicine is upon us, yet for many patients in low to middle income countries such as South Africa, the possibility of patients in the public hospital setting accessing targeted therapies for malignancies is invariably not an option. Several studies assessing PD-L1 in endometrial carcinomas in developed countries have been conducted but there have been inconsistent findings [15]. It has been shown that approximately a third of endometrial carcinoma patients may have mismatch repair deficient or MSI tumours. In addition, the identification of PD-L1 positivity in endometrial carcinomas is associated with mismatch repair deficiency suggesting tumour-immune escape pathways which may be amenable to immune checkpoint inhibitors [28, 29]. Cho *et al.* [30] have demonstrated that there is a strong correlation between tumour mutational burden and PD-L1 positivity in endometrial carcinomas. It has been suggested that high PD-L1 positivity portends a favourable outcome [31]. Our study evaluated PD-L1 staining in a cohort of EECs in South Africa that had already been investigated for MMR deficiencies, MSI and *MLH1* methylation. We identified PD-L1 positivity in 13.1% of cases, which is similar to studies undertaken in developed countries by Mo *et al.* [17] and Li *et al.* [32] The present study did not show correlation between PD-L1 status and age, similar to that noted by Engerud *et al.* [20] Most of the PD-L1 cases in our cohort demonstrated FIGO grade 2 histological features, which is comparable to a study undertaken by Amarin *et al.* [26] Similarly, when using a binary classification of tumour grading in which FIGO grades 1 and 2 are classified as low grade tumours and FIGO grade 3 tumours are considered high- grade tumours, we still found that most of our tumours demonstrated low morphological tumour grades [33, 34]. Most of our PD-L1 positive cases showed loss of MLH1/PMS2 while none demonstrated loss of MSH2/MSH6, which is consistent with findings by Amarin *et al.* [26] The study by Zong *et al.* [27] did not specify which of the mismatch repair markers were deficient in their study and as such we could not compare results of our PD-L1 positive, specific mismatch repair deficient cases with their results. Four of our cases showed solitary loss of MLH1 but none showed isolated loss of PMS2. This contrasts with Amarin *et al.*'s [26] findings of isolated PMS2 loss, but no cases of solitary MLH1 loss. Isolated MLH1 loss is not common, but has been documented by Hashmi *et al.* [35] and more recently, has been identified in germline polymorphisms of *MLH1* [35, 36]. The absence of cases demonstrating MSH2/MSH6 may be explained by the possible occurrence of variations in the incidence of various mutations in different

TABLE 3. Concordance between IHC and overall mutation status (MMR deficient or MSI) with PD-L1 expression.

MMR IHC				Overall abnormality (MMR deficient and/or microsatellite unstable tumours)			
Agreement	Kappa	Std. Error	*p*-Value	Agreement	Kappa	Std. Error	*p*-Value
68.3% (60.6–75.9%)	0.066	0.078	0.2232	67.6% (59.9–75.3%)	0.112	0.077	0.2659
Age <60 yr							
64.6% (50.6–78.6%)	−0.03	0.103	0.1767	62.5% (48.3–76.7%)	−0.039	0.099	0.160
Age ≥60 yr							
70.1% (60.8–79.4%)	0.120	0.105	0.329	70.1% (60.8–79.4%)	0.188	0.102	0.390
Tumour Grade 1							
75.0% (61.7–88.3%)	0.123	0.169	0.465	68.2% (53.9–82.5%)	−0.055	0.1352	0.212
Tumour Grade 2							
55.6% (42.9–68.1%)	0.000	0.089	0.178	68.3% (56.4–80.1%)	0.196	0.1074	0.072
Tumour Grade 3							
81.6% (68.7–94.5%)	0.257	0.208	0.678	65.8% (49.9–81.6%)	0.127	0.1543	0.415
Tumour Grade 1 & Age <60							
80.0% (57.1–100%)	−0.098	0.0730	0.059	73.3% (48.0–98.7%)	−0.111	0.092	0.086
Tumour Grade 2 & Age <60							
47.6% (24.3–70.9%)	−0.009	0.127	0.255	61.9% (39.3–84.6%)	0.056	0.165	0.401
Tumour Grade 3 & Age <60							
75.0% (46.3–100%)	−0.125	0.095	0.084	50.0% (16.8–83.2%)	−0.161	0.154	0.177
Tumour Grade 1 & Age ≥60							
72.4% (55.1–89.7%)	0.166	0.206	0.588	65.5% (47.1–83.9%)	−0.043	0.175	0.316
Tumour Grade 2 & Age ≥60							
59.5% (44.0–75.0%)	0.011	0.121	0.500	71.4% (57.2–85.7%)	0.270	0.137	0.547
Tumour Grade 3 & Age ≥60							
84.6% (69.8–99.5%)	0.416	0.241	0.912	73.1% (54.8–91.4%)	0.295	0.205	0.717
Binary Tumour FIGO Grade: Low grade							
63.6% (54.3–72.8%)	0.031	0.082	0.705	68.2% (59.3–77.2%)	0.105	0.090	0.247
Binary Tumour FIGO Grade: High grade							
5.3% (0–12.7%)	0.033	0.029	0.276	65.8% (50.0–81.6%)	0.127	0.154	0.415

MMR: mismatch repair; FIGO: International Federation of Gynecology and Obstetrics; IHC: Immunohistochemistry.

TABLE 4. Association between PD-L1 and IHC with and without adjustment.

	OR (95% CI)	AUC	Adjusted OR*	AUC*
IHC				
Proficient	Ref		Ref	
Deficient	1.58 (0.57; 4.34)	0.549	1.67 (0.59–4.66)	0.620

**Adjusted for patient age and tumour grade. AUC: area under the curve; OR: Odds ratio; CI: Confidence interval; IHC: Immunohistochemistry.*

ethnic groups [29]. In addition, it is postulated that there may be different pathogenetic mechanisms responsible for isolated MSH6 loss and furthermore, use of different antibody clones may yield different results [26].

Our study interestingly illustrated that 52.6% of PD-L1 positive cases were microsatellite stable and 63.2% were MMR proficient. When we included MSI-Low tumours in the category of microsatellite stable tumours, the number of PD-L1 positive microsatellite stable cases increased to 84.2%. It has been shown that PD-L1 positivity may be detected in up to 20% of endometrial cancers that retain MMR staining, but our cohort of cases shows more than 2-fold increase of such cases [12]. Whilst technical factors such as PD-L1 antibody clone, methodology of testing, whole tissue sections versus tissue microarray sections may account for variability in positive PD-L1 staining, it is possible that other complex molecular

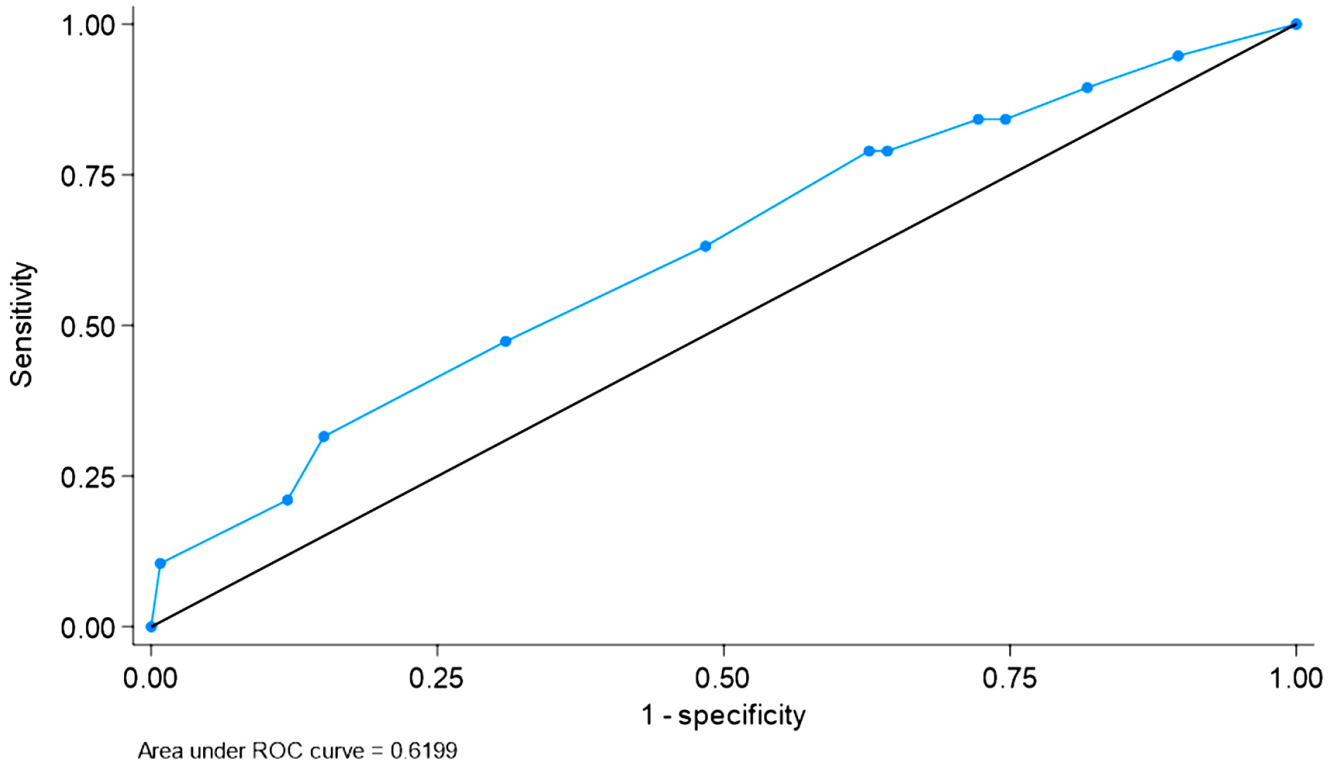


FIGURE 3. The area under the Receiver Operating Characteristic (ROC) curve illustrates the relationship between MMR IHC independently for PD-L1 expression. The area under the curve (AUC) of ROC = 0.6199. Thus, the IHC model discriminates between PD-L1 positive and negative patients in about 62% of cases.

mechanisms may be at play, which stimulate a robust immune response culminating in detectable PD-L1 reactivity [12, 37]. Further research into possible interactions is needed in this regard. In addition, these findings suggest that in our patient population PD-L1 staining should be considered in all cases of endometrial carcinomas to identify cases that may derive benefit from immune checkpoint inhibitors.

Despite 80% of our PD-L1cases demonstrating methylation, there was no significant association. Pasanen *et al.* [38] similarly did not show significant immunological differences in their MMR deficient methylated cases. Kir *et al.* [29] however, showed that *MHL1* hypermethylated tumours had a greater incidence of PD-L1 positivity than unmethylated cases.

While our study showed that there was high agreement between results from IHC and PD-L1, in addition to a high degree of agreement between PD-L1 and overall MMR deficiency and/or microsatellite instability, there was a low concordance and no statistically significant result (Table 3). This is in contrast to findings by Siraj *et al.* [39] who showed that there were higher levels of PD-L1 positivity in cases showing MMR loss. Following an adjustment for patient age and tumour grade, our study demonstrated a fair, but not statistically significant predictive ability of MMR IHC independently for PD-L1 positivity.

A comparison between our cohort and that of Amarin *et al.*'s [26] and Zong *et al.*'s [27], showed a statistically significant difference in PD-L1 staining with our cases being much lower than those noted in the Jordanian and the Chinese studies. There are several limitations and thus possible explanations for the numbers of cases that stained PD-L1 positive in our cohort, such as our small sample size. The Department of Anatomical Pathology at the University of the Witwatersrand/National Health Laboratory Service provides a pathology service to state hospitals in the southern Gauteng area. While a large number of clinics and hospitals have their specimens analysed by the Department of Anatomical Pathology, this study only assessed cases of endometrial carcinomas from this region, not the entire province or country. It is envisioned that with time and greater funding opportunities, larger studies involving Anatomical Pathology departments from around the country may be included to allow for a larger sample size and to increase the demographic diversity of the population assessed. Another possible factor accounting for our PD-L1 staining results may be the PD-L1 antibody clone that we used (22C3), which binds to PD-L1's extracellular domain while other studies have used alternate clones [17, 32]. It is known that different antibodies have varying affinities to binding domains which may account for variable incidence rates [40]. In addition, the age of tissue blocks in our cohort may have resulted in reduced PD-L1 affinity as some of our cases were more than 5 years old. Our tissue blocks are however, stored at room temperature in departmental archives and fresh tissue cuts were made prior to PD-L1 testing to ensure that the integrity of antigenicity was preserved. Furthermore, these cases were subjected to PCR for MSI assessment and methylation analysis which required that DNA was quantified using a Nanodrop

1000 Spectrophotometer. However, there is a possibility that the older cases may have had changes in binding domains due to older age. While not all studies have documented the age of their tissue block, Kir *et al.* [21] had used tissue blocks that were less than 2 years of age to reduce staining variability in their study. Although the International Association for the Study of Lung Cancer has stipulated that PD-L1 assessment of lung malignancies should be on tissue blocks that are less than 3 years of age, and the Dako PD-L1 IHC 22C3 pharmDx information for use document states that lung biopsy tissue blocks undergoing PD-L1 assessment should be under 5 years of age, the same is not necessarily true for other malignancies and there have been studies on PD-L1 testing on older tissue blocks [23, 26]. Furthermore, our study assessed PD-L1 staining on a single tissue tumour section while Kir *et al.* [29] had used up to five tissue sections for testing, thus increasing the likelihood of detecting PD-L1 reactivity.

A strength of our study includes the fact that whole tissue sections were used instead of tissue microarrays (TMA), thereby increasing sensitivity and likelihood of PD-L1 detection which may otherwise may have been missed on TMA due to intratumoural heterogeneity [32]. In addition, our study is, to the best of our knowledge, the first to assess PD-L1 staining on endometrial carcinomas in South Africa and on the African continent.

5. Conclusions

While the numbers of PD-L1 positive endometrial carcinomas in our cohort were not high, the treatment options for patients with such findings is significant and raises the possibility of reduced morbidity and mortality, and improved longevity. Furthermore, identification of PD-L1 positive cases in more than half of our endometrial carcinoma cases implies that we should not restrict PD-L1 testing to only our mismatch deficient cases, but that all our endometrial carcinomas should undergo such testing, as such patients may have a good response to immune checkpoint inhibitors. In conclusion, it is envisioned that this study may provide the impetus for future possible immune therapies for endometrial cancer patients in the state sector of South Africa. Additionally, our study provides data from a developing country which adds to current global data. This allows for provision of a greater knowledge pool from which gynaecologic oncologists, medical oncologists, radiation oncologists and anatomical pathologists may draw upon, with the goal of improved patient care.

AVAILABILITY OF DATA AND MATERIALS

The data are contained within this article.

AUTHOR CONTRIBUTIONS

RW—contributed to the conception and design of the study; and contributed to the drafting of the manuscript and critically reviewed the intellectual content therein. IM—contributed to the statistical analyses and drafting of the manuscript. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was performed in accordance with the Declaration of Helsinki with relevant consent and ethical approval by the Human Research Ethics Committee (Medical), clearance certificate number: M2102100.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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