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Immunohistochemical evaluation of two antibodies against PD-L1 and prognostic significance of PD-L1 expression in epithelioid peritoneal malignant mesothelioma: A RENAPE study

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Abstract

Background: Epithelioid peritoneal malignant mesothelioma (EPMM) is the most common subtype of this aggressive tumor. We compared two antibodies against PD-L1, a recent theranostic biomarker, and evaluated the prognostic value of PD-L1 expression by mesothelial and immune cells in EPMM.
Methods: Immunohistochemistry was performed on 45 EPMM. Clinical and pathological data were extracted from the RENAPE database. Using E1L3N and SP142 clones, inter-observer agreement, PD-L1 expression by mesothelial and immune cells and inter-antibody agreement were evaluated. The prognostic relevance of PD-L1 expression was evaluated in 39 EPMM by univariate and multivariate analysis of overall survival (OS) and progression-free survival (PFS).
Results: Inter-observer agreement on E1L3N immunostaining was moderate for mesothelial and immune cells, and fair for mesothelial and poor for immune cells using SP142. Using E1L3N, 31.1% of mesothelial and 15.6% of immune cells expressed PD-L1, and 22.2% of mesothelial and 26.7% of immune cells using SP142. Inter-antibody agreement was moderate. In most positive cases, 1–5% of tumor cells were positive. Using E1L3N, PD-L1 expression by lymphocytes was associated with better OS and PFS by both univariate and multivariate

Abbreviations: EPMM, epithelioid peritoneal malignant mesothelioma; PD-L1, programmed death-ligand 1; PD-1, programmed death 1; CRS + HIPEC, cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy; HE, hematoxylin and eosin; TIL, tumor-infiltrating lymphocytes; PTL, peritumoral lymphocytes; FFPE, formalin-fixed paraffin-embedded; TMA, tissue microarray.

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analysis. Cytoreductive surgery with hyperthermic intraperitoneal chemotherapy predicted better prognosis than other treatments. Solid subtype was an independent prognostic factor for worse OS.

Conclusion: E1L3N appeared easier to use than SP142 to evaluate PD-L1 expression. A minority of EPMM expressed PD-L1, and only a few cells were positive. PD-L1 expression by immune cells evaluated with E1L3N was an independent prognostic factor in EPMM.

Keywords: Peritoneal mesothelioma; Epithelioid subtype; PD-L1; Immunohistochemistry; Survival

Introduction

Peritoneal malignant mesothelioma (PMM) arises from peritoneal serosal surfaces and is a rare and highly aggressive disease. Asbestos exposure may cause PMM. Other risk factors are suspected, but the pathogenesis remains largely unknown.[1] The current estimated annual incidence of PMM is 2500 cases worldwide.[2]

The histological classification of malignant mesothelioma remains the same in the 2015 WHO classification as it was in the 2004 classification. The morphology of peritoneal malignant mesothelioma (PMM) exhibits a variety of major histological types, divided into epithelioid, sarcomatoid and biphasic (epitheloid and sarcomatoid) categories.[3,4] The epithelioid type is the most common. Epithelioid peritoneal malignant mesotheliomas (EPMM) generally present a tubulopapillary, papillary, micropapillary, trabecular, solid or pleomorphic pattern.[5] The histological type of PMM has been reported to be of prognostic significance. The epithelioid type is the least aggressive of the three main types.[6] On the other hand, a solid growth pattern in this type is associated with aggressive clinical behavior and poor prognosis.[7] The mitotic index in tumors with epithelioid histology is also considered to be of prognostic significance, but other histopathological parameters, such as nuclear grade, depth of invasion, and stromal desmoplasia do not appear to be strong predictors of outcome.[7–10] The prognosis for EPMM is somber. Classic surgical treatment or systemic chemotherapy have been shown to yield a median overall survival (OS) of 6–12 months.[11] Cytoreductive surgery (CRS) combined with hyperthermic intraperitoneal chemotherapy (HIPEC) appears to be the gold standard of treatment because of significantly improved outcomes and reports of long-term survival.[12,13] Despite these favorable results, the combined therapy carries significant perioperative morbidity for more than half of patients. Other prognostic factors are therefore needed. Several studies have sought a strong histopathological prognostic factor. Kusamara et al. showed that Ki-67 is a powerful predictor that yields good OS in patients with PMM.[14] On a molecular level, the combination of homozygous CDKN2A deletion and hemizygous NF2 loss in peritoneal mesotheliomas is an independent prognostic factor for shorter PFS and OS. In contrast, loss of BAP1 activity was not associated with changes in clinical outcome.[9,15] The immune system is regulated by numerous receptor-ligand checkpoints which prevent primed effector T cells from reacting to specific antigens and avoid autoimmune reaction. Tumor cells express neoantigens that are recognizable by the host immune system as nonself. To avoid elimination, the tumor cells interfere with these immune regulation mechanisms. For example, the interaction of programmed death 1 (PD-1) expressed by T lymphocytes and programmed death-ligand 1 (PD-L1) on antigen-presenting cells is one of the immune inhibitory checkpoints. The PD-L1 protein is a recent theranostic biomarker that has been evaluated in most cancers.[16–18] Blockade of PD-1/PD-L1 interaction by therapeutic monoclonal antibodies against either PD-1 (nivolumab, pembrolizumab) or PD-L1 (atezolizumab, durvalumab, avelumab) seems an innovative and promising therapeutic approach.[19] In some cases and in some countries, immunohistochemistry evaluating PD-L1 expression may be a legal requirement before treatment with anti-PD-L1. Antibodies against PD-L1 are numerous but none have shown marked superiority for screening tumors before treatment.

In this study, our main objective was to compare immunostaining with two anti-PD-L1 antibodies (E1L3N clone and SP142 clone) in EPMM, and our secondary objective was to evaluate the prognostic value of PD-L1 expression by tumor cells and inflammatory infiltrates.

Patients and methods

Patients and clinical samples

We retrospectively examined 45 diagnostic samples from patients presenting with EPMM from 1999 to 2015 within the RENAPE network. Clinical data were extracted from the RENAPE observational registry.[20] All patients gave their informed consent before formalin-fixed paraffin-embedded (FFPE) tumoral samples were stored. No supplementary material was sampled for this study.

Microscopic examination was performed by seven experienced pathologists on entire slides stained with hematoxylin and eosin (HE). They assessed the histological subtype (solid or not solid) using the current WHO 2015 classification. The localization of the lymphocytic reaction was evaluated (tumor-infiltrating lymphocytes, TIL, and peritumoral lymphocytes, PTL) and its intensity was scored as 0, absent; 1, mild; 2, moderate; or 3, intense. Tissue microarrays (TMAs) were produced using FFPE tumoral

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