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Precision immunotherapy; dynamics in the cellular profile of pleural effusions in malignant mesothelioma patients

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ABSTRACT

Objectives: Clinical studies have proven the potential of immunotherapy in malignancies. To increase efficacy, a prerequisite is that treatment is tailored, so precision immune-oncology is the logical next step. In order to tailor treatment, characterization of the patient's tumor environment is key. Pleural effusion (PE) often accompanies malignant pleural mesothelioma (MPM) and is an important part of the MPM environment. Furthermore, the composition of PE is used as surrogate for the tumor. In this study, we provide an insight in the dynamics of the MPM environment through characterization of PE composition over time and show that the immunological characteristics of PE do not necessarily mirror those of the tumor.

Materials and methods: From 5 MPM patients, PE and tumor biopsies were acquired at the same time point. From one of these patients multiple PEs were obtained. PEs were acquired performing thoracocenteses and total cell amounts were determined. Immunohistochemistry was performed to quantify immune cell composition (T cells, macrophages) and tumor cells in PE derived cytospins and tumor biopsies.

Results: The PE amount and (immune) cellular composition varied considerably over time between multiple (n = 10) thoracocenteses. These dynamics could in part be attributed to the treatment regimen consisting of standard chemotherapy and dendritic cell (DC)-based immunotherapy. In addition, the presence of T cells and macrophages in PE did not necessarily mirror the infiltration of these immune cells within tumor biopsies in 4 out of 5 patients.

Conclusions: In this proof-of-concept study with limited sample size, we demonstrate that the composition of PE is dynamic and influenced by treatment. Furthermore, the immune cell composition of PE does not automatically reflect the properties of tumor tissue. This has major consequences when applying precision immunotherapy based on PE findings in patients. Furthermore, it implies a regulated trafficking of immune regulating cells within the tumor environment.

1. Introduction

Malignant pleural mesothelioma (MPM) is a highly aggressive tumor mainly caused by the inhalation of asbestos fibers. MPM can develop from both the visceral pleura and the parietal pleura. The occurrence of pleural effusion is associated with approximately 70% of the MPM patients, especially in MPM of the epithelioid subtype [1]. Pleural effusion accumulates in the pleural space when influx of effusion outweighs efflux. Increased production occurs due to excessive plasma leakage through hyperpermeable intratumoral vessels. In addition, blockade of the pleuropulmonary lymphatics by tumor cells results in reduced absorption [2]. Build-up of pleural effusion can result in profound shortness of breath and deterioration of quality of life. Although effusion cytology for diagnostic purposes in MPM is controversial and not recommended in the ESMO Clinical Practice Guidelines for malignant pleural mesothelioma, drainage is commonly performed to relief symptoms [3]. Pleural effusion of mesothelioma patients can comprise different cell types and soluble factors, which can be derived directly from the tumor, its environment and/or from the vasculature. Immune cells like T cell subsets (e.g. CD8 T cells and regulatory T cells)

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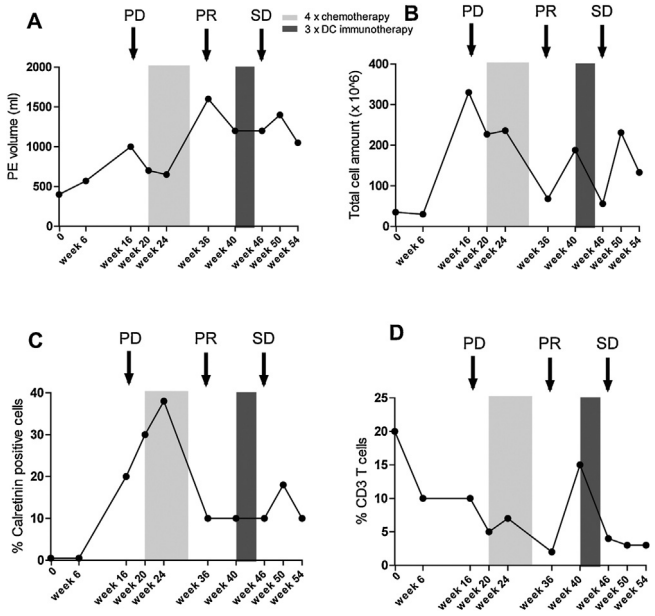

Fig. 1. Dynamics in pleural effusion amount and cellular composition in an MPM patient over one year from diagnosis onwards. *Abbreviations*: PE = pleural effusion, PD = progression of disease, PR = partial response, SD = stable disease. CT-scan evaluated disease states are depicted above the graphs. The grey bars depict the period during which the patient received 4 courses of chemotherapy (cisplatin/pemetrexed, light grey bar) and 3 courses of DC immunotherapy (dark grey bar). Ten subsequent thoracocenteses were performed in order to relieve dyspnea. Panel A shows the variation in pleural effusion amount. Panel B shows the variation in total cellular amount. Panel C shows the percentage of calretinin positive tumor cells. Panel D shows the variation in relative amount of CD3 positive T cells over time.

and tumor-associated macrophage subsets (anti-tumor M1 or pro-tumor M2 TAMs) are present in most malignant pleural effusions [4–7].

As immunotherapy is gaining ground in many different tumor types, various immunotherapeutic approaches are also being investigated in clinical studies in mesothelioma patients [8–12]. In order to optimize the efficacy of immunotherapy in mesothelioma, a tailor-made approach is warranted.

Given the close proximity of pleural effusion to the pleural tumor, it is an important part of the mesothelioma environment and often used as a surrogate marker for the tumor tissue. Furthermore, the pleural cavity can be an attractive site to locally administer (immuno)therapies. In this study, we investigate the robustness of the pleural effusion composition and whether it reflects the pleural tumor in mesothelioma.

2. Materials and methods

2.1. Patient material

Five MPM patients were selected from whom tumor biopsies and pleural effusion cytospins were derived at the same time point. The biopsies and effusions were acquired during VATS surgical biopsy procedures, prior to any treatment. All patients were diagnosed with MPM of the epithelioid subtype by the Dutch National Mesothelioma Panel. Pleural effusion was collected in sterile tubes or bags without anticoagulant. Pleural cells were pelleted from pleural effusions using centrifugation at 400*g* for 10 min. Ficoll density gradient centrifugation was applied to pleural effusions with evident blood contamination to separate the red blood cells from the leucocytes. Tumor biopsies were embedded in Tissue-Tek II OCT-compound (Miles, Naperville, IL, USA), snap frozen in liquid nitrogen and stored at −80 °C. Tissue sections (6 μm) were cut using a HM-560 cryostat (Microm, Heidelberg, Germany). Cytospin preparations were made of an optimally diluted PE cell suspension (Shandon Cytospin 4, Thermo Electron Corporation, Massachusetts, United States). This study is a retrospective analysis of data, all patient materials were acquired between 2010 and 2013.

2.2. Immunohistochemistry

Cytospins and tumor biopsies were stained with CD8 (Dako, Glostrup, Denmark) and the commonly used M2 macrophage marker CD163 (eBioscience, San Diego, USA). The cytospin series of the patient followed in time were stained for calretinin, CD68 and CD3 (Dako). Antibodies were incubated for 1 h and detected using the RAM – APAAP method (Dako). Naphtol-AS-MX-phosphate (0.30 mg/ml, Sigma-Aldrich Chemie B.V., Zwijndrecht, the Netherlands) and new fuchsine (160 mg/ml in 2 M HCl; Chroma-Gesellschaft, Köngen, Germany) were used as substrate. An isotype-matched antibody was used as control. The percentage of CD8- or CD163- positive cells in representative areas of the tumor biopsies was determined as described earlier [13,14]. In the cytospins, in three representative areas the amount of positively stained cells among a total of 100 cells was counted and we defined the average value of three counts as the percentage of positive cells in the cytospin. Tumor slides and cytospins were independently evaluated by L.L. and K.B.

3. Results

3.1. Longitudinal pleural effusion follow-up

One patient underwent 10 thoracocenteses to relieve dyspnea symptoms in the course of one year. The maximum pleural effusion amount was drained until the flow through the drain stopped. During this year the patient was treated with four courses of cisplatin and pemetrexed and as a maintenance treatment with three courses of dendritic cell-based immunotherapy and pleurodesis attempts with talc [15]. Treatment responses were evaluated according to the Modified RECIST criteria [16,17]. Since no measurable lesion was present when the diagnosis was made, initiation of

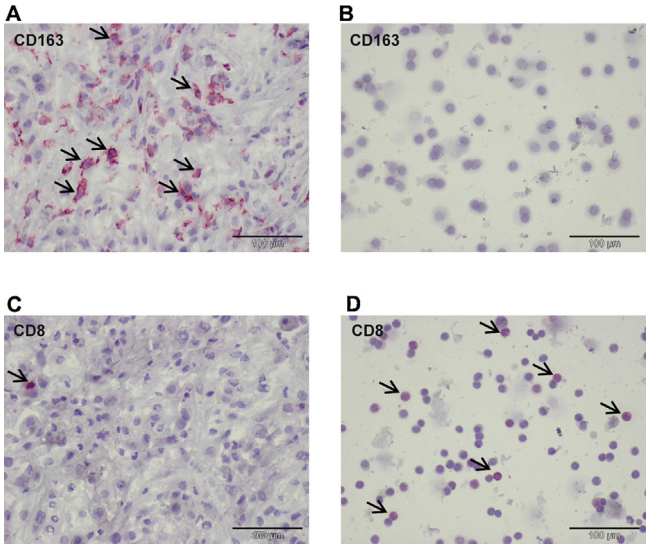

Fig. 2. Immunohistochemical staining of MPM biopsy and pleural effusion cytospin of patient number 5. Arrows indicate cells positive for either CD163 (Panels A and B), or CD8 (Panels C and D). Panels A and B show staining of M2 macrophage marker CD163 in the tumor biopsy (Panel A) and pleural effusion cytospin (Panel B). Panels C and D show staining of CD8 in the tumor biopsy and pleural effusion cytospin respectively.