Immunological parameters as a new lead in the diagnosis of ovarian cancer

T. BAERT1,2, D. TIMMERMAN1,2, I. VERGOTE1,2, A. COOSEMANS1,2

1UZ Leuven, Gynaecology and Obstetrics, Herestraat 49, 3000 Leuven, Belgium; 2Department of Oncology, Leuven Cancer Institute, KU Leuven, Herestraat 49, 3000 Leuven, Belgium; 3Department of Development and Regeneration, KU Leuven, Herestraat 49, 3000 Leuven, Belgium.

Correspondence at: Thais Baert, KU Leuven, Laboratory of Clinical Immunology, Herestraat 49, CDG, box 811, 3000 Leuven, Belgium. Email: thais.baert@gmail.com

Abstract

Ovarian cancer is the leading cause of pelvic gynecological cancer death in Europe. Prognosis is poor in women diagnosed at stage III to IV or in case disease recurs. Platin-based chemotherapy and radical surgery have already improved prognosis significantly. Novel strategies in the treatment of ovarian cancer are being searched for. The study of the immune system as a valuable parameter in the development of ovarian cancer has been neglected for a long time. Nevertheless, this is a field in full progression and might open new perspectives in the diagnosis and prognosis of ovarian cancer patients and could lead to a more motivated choice of targeted therapies.

Key words: Ovarian cancer, diagnosis, prognostic marker, immunosuppression, regulatory T cell, myeloid derived suppressor cells, tumor-associated macrophages.

Introduction

Ovarian cancer is the second most important type of pelvic tumor in women. Worldwide up to 240 000 women are diagnosed with ovarian cancer each year (WHO, 2014). As signs and symptoms in early ovarian cancer are scarce, up to 63% of patients are diagnosed at stage III or IV, leading to poor prognosis (Vergote et al., 2010). Until now, prognosis is based on the histological type, grade and the staging according to the FIGO classification.

Gynecological ultrasound has proven very useful and accurate in the diagnosis of ovarian cancer, though is subjective. Over the past decade, large progress has been made to standardize and optimize the classification of adnexal masses by the International Ovarian Tumour Analysis (IOTA) group, by means of logistic regression models. Meta-analyses and external validation studies have shown superiority of the IOTA models (Van Calster et al., 2014). In contrast, no serum marker has shown to be specific enough in the diagnosis of adnexal masses as a standalone. However, the most recent model of the IOTA group, the ADNEX model, combines ultrasound characteristic, clinical data and a serum marker, CA125 (Table I). (Van Calster et al., 2014).

Whole body Magnetic Resonance Imaging (MRI) with diffusion weighting is an advantage in the preoperative staging of patients with ovarian cancer, providing important information about retroperitoneal and thoracic nodes (Michielsen et al., 2014). However in the diagnosis of difficult to classify adnexal masses the advantage of MRI compared to transvaginal ultrasound by experts is limited (Kaijser et al., 2014).

So far, research on prognostic markers has focused on genetic markers influencing survival and treatment response in ovarian cancer. In 1996 the first reports were published that suggested BRCA1/2 germline mutation is correlated with improved outcome (Rubin et al., 1996). This was underscored by a recent systematic review (Sun et al., 2014). Recently, a generalized genetic mapping of high-grade serous ovarian cancer was performed by the Cancer Genome Atlas Network (TCGA). They created 4 subtypes based on mutation analysis,
mRNA and miRNA expression and DNA methylation: immunoreactive, differentiated, proliferative and mesenchymal. T-cell chemokine ligands CXCL11 and CXCL10 and the receptor CXCR3 characterized the immunoreactive subtype (The Cancer Genome Atlas Research Network, 2011). This article highlights the importance of the immune system in ovarian cancer. Indeed, once malignant cells arise, the immune system will control their development mainly by the presence of effector T cells (Teff). This stage is called the immune surveillance. If the operation is successful, the malignant cells are eliminated or reside in a dormant state (elimination vs equilibrium). However, cells might also escape the control of the immune system, start proliferating and the tumor becomes clinically apparent (Schreiber et al., 2011). The tumor develops several strategies to evade the immune system. First and most important is the attraction of immunosuppressive cells into the tumor. In the tumor microenvironment there is a rise in immunosuppressive cells and molecules that render the Teff less functional. Most important players seem to be regulatory T cells (Treg), myeloid derived suppressor cells (MDSC) and tumor-associated macrophages (TAM). As a second line of defense the tumor inhibits the infiltration of Teff into the tumor through a decrease in chemotactic molecules and a decreased quality of the tumoral vascularization. Current research in onco-immunology is focusing on methods to overcome this immune-escape.

It is clear that the immune system is an important regulator in the development of cancer. It can be presumed that the determination of these immune cells might give rise to new diagnostic and even prognostic markers for ovarian cancer. Early research in colorectal cancer suggests that the presence of Teff in the tumor might be more important than traditional staging to predict outcome (Galon et al., 2006). In ovarian cancer it has been shown that a high ratio of Teff compared to immunosuppressive Treg in the tumor is associated with better prognosis (Zhang et al., 2003; Curiel et al., 2004). It has become clear that merely the presence of Teff is not enough, the balance between Teff and immunosuppressive cells is important. In a study in metastatic melanoma, Teff were isolated from the tumor microenvironment and analyzed. Immediately after isolation they showed a suboptimal cytokine production and proliferation, however this was normalized when these T cells were expanded ex vivo and stimulatory cytokines were added (Harlin et al., 2006). This underlines the importance of the immunosuppressive tumor microenvironment.

Immunosuppressive cells in general

**Regulatory T cells** (Treg) are a subpopulation of T cells, which maintain tolerance to self-antigens by influencing the activity of other Teff. Their physiological function is to prevent auto-immunity and down regulate undesired T cell responses. We can differentiate between 2 subsets of regulatory T cells. The naturally occurring Treg develop in the thymus and are responsible for central inhibition of auto-immunity. T cells that leave the bone marrow migrate to the thymus to mature. In the thymus autoreactive T cells are deleted. Adaptive Treg develop as a consequence of continued peripheral T cell activation, as is present in chronic inflammation and tumor development. Treg are trafficked to the tumor site through presence of chemokines such as CCL2 and CCL22 in the tumor microenvironment. Expansion of Tregs can be induced by tolerogenic dendritic cells (DCs) through the expression of indoleamine 2,3-dioxygenase (IDO). Transforming epidermal growth factor beta (TGF-β) can convert effector T cells into regulatory T cells leading to further increase immunosuppression (Gajewski et al., 2004).

### Table 1. — Criteria used in the IOTA ADNEX model.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of the patient at examination</td>
<td>Years</td>
</tr>
<tr>
<td>Referral center for Gynecological Oncology</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Maximal diameter of the lesion</td>
<td>mm</td>
</tr>
<tr>
<td>Maximal diameter of the largest solid part</td>
<td>mm</td>
</tr>
<tr>
<td>More than 10 locules</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Number of papillary projections</td>
<td>None/One/Two/Three/More than three</td>
</tr>
<tr>
<td>Acoustic shadow present</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Ascites (outside the pelvis)</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Serum CA-125</td>
<td>U/mL</td>
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Myeloid derived suppressor cells (MDSCs) are a heterogeneous population of early myeloid progenitors at different stages of differentiation. These cells are capable of suppressing the innate (natural killer cells (NK)/non-specific defense) and the adaptive immune system (Teff/specific defense mechanisms). MDSC are influenced by pro-inflammatory cytokines and their presence in the tumor microenvironment could be one of the causes of tumor-associated immunosuppression. The presence of circulating MDSC correlates with poor prognosis, increased metastatic potential and tumor evasion of host immunity. As depicted in Figure 2, the accumulation of MDSC in the tumor microenvironment is stimulated by several factors such as: granulocyte macrophage colony stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), chemokines such as CCL-2 and TGF-β, produced by the tumor and the intratumoral immune cells (Nagaraj et al., 2010; Talmadge et al., 2013). MDSC will recruit and induce Treg through production of IL-10 and TGF-β and the expression of chemotactic molecules on the cell surface of the MDSC. MDSC are myeloid lineage cells and can differentiate into DC when matured; however

Fig. 1. — The role of regulatory T cells (Treg) in the tumor microenvironment: a schematic overview of the most important immunosuppressive mechanisms through which regulatory T cells function and the possibility of feedback-loops. Indoleamine 2,3-dioxygenase (IDO) is an intracellular enzyme that catalyses the oxidative catabolism of tryptophan. IDO suppresses T cell responses and promotes immune tolerance in mammalian pregnancy, tumor resistance, chronic infection, autoimmunity and allergic inflammation. TGF-β: transforming growth factor-β; APC: antigen presenting cell; MHC: major histocompatibility complex; CD: cluster of differentiation.
M1 macrophages and immunosuppressive M2 macrophages. Once attracted to the tumor we often see a switch from M1 to M2 macrophages. Because of this switch, the role of TAM in the tumor microenvironment is complex. The physiological role of M1 macrophages is pathogen clearance and antigen presentation to NK cells and T cells, which leads to activation of the adaptive immune system. On the other hand, M2 macrophages have a low phagocytic capacity and will not properly stimulate NK cells and T cells. They will even counteract the immune response by producing immunosuppressive molecules, leading to poor prognosis with an increased number of TAM.

Intratumoral TAM are in general immunosuppressive and will inhibit an anti-tumoral immune response. Differentiation of monocytes into M1 macrophages is promoted by interferon-γ, lipopolysaccharide (LPS) and other microbial products, leading to killing of bacteria, immunostimulatory functions and anti-tumor cytotoxicity (Sica et al., 2008). The major factors causing the switch from immunostimulatory M1 macrophages to immunosuppressive M2 macrophages in the tumor microenvironment are hypoxia, macrophage colony stimulating factor-1 (CSF-1), TGF-β, IL-4, IL-13 and the presence of apoptosis (Noy et al., 2014). As shown in Figure 3 there are several mechanisms by which intratumoral TAM will alter the tumor microenvironment causing a switch from immunostimulatory to immunosuppressive molecules.
which M2 macrophages cause immunosuppression. M2 macrophages have shown an enhanced expression of IL-10, leading to decreased expression of IL-12 and co-stimulatory molecules by APC (Laoui et al., 2014; Ruffell et al., 2014). M2 macrophages will inhibit Teff through stimulation of IDO and increased secretion of TGF-β (Sica et al., 2008). M2 macrophages will also increase neo-vasculogenesis through increased secretion of VEGF, which conversely will also increase polarization towards M2 (Noy et al., 2014). Furthermore, M2 macrophages will also increase CCL-22, a chemokine that attracts Treg and MDSC to the tumor microenvironment (Curiel et al., 2004). These feedback-loops that strengthen the immunosuppressive microenvironment in the tumor, making it nearly impossible for Teff to significantly reduce tumor volume.

Immunosuppressive cells in ovarian cancer: situation at diagnosis

The prognostic value of immunosuppressive cells in ovarian cancer has been rarely studied. The available literature focuses merely on the presence of immune cells in the tumor rather than on their systemic presence in blood. A recent meta-analysis by Hwang et al has shown a significant survival advantage linked to tumor-infiltrating lymphocytes (TIL), however this biomarker is not yet used in clinical practice, possibly due to differences in definition of TIL (Hwang et al., 2012). Curiel et al. (2004) studied intra-tumoral Treg, which correlated with a reduced survival. PD-L1 expression on tumor cells was shown to be an independent variable correlated with poor survival (Hamanishi et al., 2007). To date no studies have been able to correlate immunosuppressive serum markers, such as TGF-β and IL-10, with diagnosis with prognosis (Mustea et al., 2009; Tas et al., 2014). A recent study correlated VEGF, survivin and second mitochondria-derived activator of caspase (Smac/DIABLO) serum levels to poor prognosis. Survivin and Smac/DIABLO are inhibitors of apoptosis (Dobrzycka et al., 2015).

Future perspectives

The discovery of the importance of the immune system in the development of cancer can help us in the determination of new diagnostic and prognostic parameters. An advantage of the determination of an immunophenotype, compared to genetic differentiation, is bifold. When mapping the immunosuppressive phenotype we do not only take into account the characteristics of the tumor, but also the environment in which the tumor grows. Clinically, this tumor environment is very relevant, as we know that patients with HIV, patients on immunosuppressive drugs against transplant rejection, … are prone to malignancies and have a worse prognosis. A second advantage is the possibility of using serum markers instead of biopsies. This evades the possibility of non-representative biopsies, inter-metastasis heterogeneity and might be able to avoid invasive diagnostic techniques.
Treg, MDSC and TAM are detectable in blood and their secreted compounds are detectable in serum. It might thus be relevant to try to detect these cells in women with suspected ovarian masses. Just like CA125 in the ADNEX model, these cells or their metabolites might be a useful partner with added value to our existing diagnostic tools. And maybe on the long run, it will even be possible to differentiate patients into different subtypes, reflecting a different immunosuppressive signature, representing a possibility to improve outcome through tailored approaches.

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