RESEARCH ARTICLE

Role of Galectin-3 Combined with Multi- Detector Contrast Enhanced Computed Tomography in Predicting Disease Recurrence in Patients with Ovarian Cancer

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Abstract

Galectin-3 (Gal-3) is an endogenous β-galactoside-binding lectin, playing an important role in the pathogenesis of multiple malignancies. Aim of the study was to evaluate in a group of patients treated for ovarian cancer (EOC), the role of Gal-3 combined with multi-detector contrast-enhanced computed tomography (MDCT), as predictor of recurrence disease. Seventeen follow-up patients with recurrent ovarian cancer and 13 follow-up patients with stable ovarian disease, who performed MDCT at one-year follow-up after cytoreductive treatment, were enrolled. Serum Gal-3 concentrations were determined by using ELISA method. Twenty healthy controls were included in the analysis. Two radiologist blinded to patients status, reviewed MDCT exams, recording the following signs of disease recurrence: local tumor spread, enlarged lymph-nodes, carcinomatosis implants and metastases. We calculated the respective threshold values of Gal- 3 identified by ROC curve analysis for each imaging findings related to disease recurrence : lymphoadenopathies 92.45 ng/ml (AUC: 0.81, Se=91% Spe=73%), carcinomatosis 85.95 ng/ml (AUC:0.93 Se= 93.7%, Spe=92.8%), local tumor spread 99.05 (AUC:0.90, Se=100%, Spe=73%) and metastasis 99.05ng/ml (AUC :0,78, Se=100% , Spe=70%). A significant correlation between high Gal-3 serum levels and presence of local tumor spread (n=11/17, p:0.001), carcinomatosis (n=16/17, p:0.00), lymphoadenopathies (n=15/17, p:0.00) and metastasis (n=11/17, p:0.003) related with recurrence disease was observed. Patients with recurrence of ovarian cancer presents higher Gal-3 values compared to women with stable diseases. Gal-3 combined to CECT should be used to improve the monitoring of EOC patients.

Keywords: Galectin-3- multi-detector contrast-enhanced computed tomography- Ovarian cancer

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Introduction

Epithelial Ovarian Cancer (EOC) represents the seventh most common cancer in women worldwide and is one of the most aggressive gynaecologic malignancy, with a poor prognosis in advanced stage (Haruta et al., 2011). However, because there is no effective screening test for this tumor and its symptoms are vague, approximately 70–80% of patients are diagnosed at advanced stage of the disease (stage III and IV, in accordance with the International Federation of Gynecology and Obstetrics (FIGO)) and the survival rate is low. Despite continuing advances in surgical and oncological therapies, EOC has a high probability to relapse and to develop distant metastases (Jaison et al., 2012). Actually, more than 70% of stage III–IV patients have a relapse of the disease, and even in stage I or II, the relapse rate is 20–25% (30–75%). The poor prognosis and the frequent disease relapses requires to establish periodic surveillance programs, in order to promptly introduce further therapeutic approaches thus reducing the morbidity and mortality rate.

Several studies have been conducted to assess factors involved in ovarian carcinogenesis, especially aimed to identify possible therapeutic targets and potential biological markers to be used as early prognostic indicators of disease relapse, improving the follow-up surveillance (Tagawa et al., 2012; Dutta et al., 2010; Moore et al., 2010, Papa et al., 2016).

Recently, it has been a great interest on a group of carbohydrate-binding proteins involved in tumor genesis and disease progression: Galectins. Galectins are a family of proteins playing different roles in various biological process. To date, fifteen members of the galectin family have been identified and classified into three subgroups,

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based on their structure and numbers of the carbohydrate recognition-binding domain (CRD): prototype, chimera type and tandem repeat type (Barondes et al., 1994). Gal-3 is unique among the galectins in possessing a long proline- and glycine-rich NH2-terminal domain in addition to the CRD (More et al., 2016). Previous studies have shown a correlation between high expression of Gal-3 in tumor cells and the malignant properties of several types of cancer (Iurisci et al., 2000; Ahmed et al., 2015; Cardoso et al., 2016; Danguy et al., 2002; Fukumori et al., 2007).

Gal-3 has different functions, depending on the site of its expression. Overexpression of intracellular Gal-3 inhibits apoptosis while extracellular Gal-3 enhances tumor cell adhesion to extracellular matrix (ECM) and promotes the escape of tumor cells from the primary tumor sites leading to cancer progression (Danguy et al., 2002). Previous investigations suggested the role of Gal-3 in EOC carcinogenesis (Lu et al., 2016; Mirandola et al., 2014; Kim et al., 2011) but to our knowledge no study has been yet conducted to evaluate its role as a marker of disease progression in patients with EOC.

There is still no consensus in literature regarding how to best follow up women with EOC cancers. Currently, multi- detector contrast enhanced computed tomography (MDCT) performed after the administration of intravenous contrast medium, is the imaging modality of choice for staging ovarian cancer but also for treatment follow-up, ensuring reproducibility of the results for future comparison in a short examination time with a reported accuracy of about 92%-97% (Fischerova et al., 2014; Bharwani et al., 2011).

Purpose of this study is to assess the role of Gal-3 combined with MDCT as early prognostic indicator of disease progression in a group of follow-up patients treated for ovarian cancer.

Materials and Methods

Patients

Seventeen follow-up patients with recurrent ovarian cancer (the average time to relapse was 9 months) and 13 follow-up patients with stable disease (median age of 64 y, age range: 35 –82 years) diagnosed and referred to the Department of Radiological and Oncological Sciences of Policlinico Umberto I Hospital, Rome, Italy, between December 2012 and December 2015 were consecutively enrolled in the study.

At the baseline the mean value of CA125 was 382 U/mL for patients with recurrent ovarian cancer and 20 U/mL for patients with stable disease. Of the total 30 women, 24 were in a postmenopausal state and 6 patients were in a premenopausal state. Twenty-four out of the 30 (80%) women were affected by papillary serous carcinoma and 6 out of 30 (20%) were affected by papillary serous carcinoma 'poorly' differentiated and undifferentiated of grade G3/4.

We selected patients at the 12-13 months of follow-up after cytoreductive treatment (surgery and chemotherapy) for EOC who performed a MDCT examination body scan and were then subjected to blood sampling for analysis of Gal-3 levels. The median interval elapsed between MDCT examination and blood sample was 5 days.

All patients had histological diagnosis of EOC confirmed and defined on the surgical specimen obtained after surgery according to the International Federation of Gynecology and Obstetrics (new revised FIGO, 2014) (Prat 2014; Pereira et al., 2015)

In addition 20 healthy women (mean age of 60-years,age range :22- 80 years) with no personal /family history of EOC and negative trans-vaginal US examination were included in the analysis.

Informed consent was obtained from each patient to participate in the study.

Methods

Gal-3 assay

Patient sera were acquired following a standard collection protocol. Samples were obtained by venous puncture, collected in a Red Top Vacutainer, centrifuged for 10 min at 1,300 × g. and stored at -80 °C until analysis. We used BMG Gale-3 Assay, an in vitro diagnostic device that quantitatively measures Gal -3 in serum or plasma by enzyme linked immunoassorbent assay (ELISA) on a microtiter plate form.

MDCT imaging protocol

All patients performed a contrast-enhanced high-resolution multi-detector row computed tomography (MDCT) (Somatom Sensation 64; Siemens Medical System, Erlangen, Germany) by using the following parameters: $0.6\times64\text{-mm}^2$ collimation, 3-mm section thick-ness, 250 effective mA s, 120 kVp, and 0.8-1.5-mm reconstruction interval of coronal and sagittal images. An intravenous injection of 20 mg of butylscopolamine (Buscopan, Boehringer) was administered in all patients to relax the bowel wall and reduce peristaltic bowel movement before MDCT examinations.

In MDCT bowel opacification is crucial for the differentiation of bowel loops from peritoneal implants and facilitates lymph node detection. For this purpose at least 1,000 ml of water was administered orally within 1 h prior to the MDCT study. The MDCT scans were acquired in basal conditions and after administration of contrast medium, cranio-caudally, from the dome of the diaphragm to the pelvis. The images were post- processed with Multiplanar Reconstruction (MPR) on sagittal and coronal plane sections with a 1-mm interval to improve the anatomical analysis, particularly of the surface lesions. A dual-phase protocol, arterial and venous phases, after intravenous administration of contrast medium was performed in order to assess local tumor extent. Non-ionic iodinated contrast medium (350 mg I/mL, Iomeron, Bracco, Milan, Italy) was administered intravenously utilizing an automatic injector (Stellant DCT, Medrad, Indianola, PA) at a rate of infusion of 3- 3.5 mL/s for a total volume of 90- 120 mL.

Imaging Analysis

Two radiologists with 7 and 20 years of experience in genitourinary imaging retrospectively analyzed the MDCT images in consensus, blinded to patients clinical status and to the Gal-3 blood levels.

The following imaging findings considered as signs of disease activity were recorded:

a) Local tumor recurrence: including the presence of solid tissue near the vaginal vault, the parametria, the bladder, the ureters, bowel or pelvic side-wall invasion.

b) Peritoneal carcinomatosis: single or multiple sites of micro-nodular implants (<1 cm) or diffuse macronodular implants (1.5 cm) above or below the mesocolon and omental involvement with marked thickening (omental cake).

c) Lymphoadenophaties: The onset of lymph node >1 cm and/or the 20 % size increase of pre-existing lymph nodes.

d) Distant Metastasis.

e) Presence/Absence of ascites.

Stable ovarian cancer was diagnosed when the disease did not follow any of the parameters described above.

Statistical evaluation

The ROC analysis was used to assess the different cut-off values of Gal-3 for each imaging findings of disease relapse. Results were presented with 95% of Confidence Intervals (CI 95%).

We performed the Fisher's exact probability test for univariate analyses and, where possible, the χ^2 test with continuity correction to assess the statistical significance of difference in Gal-3 levels and MDCT imaging findings between patients affected by recurrent disease and patients with stable disease.

Statistical analysis was performed using SPSS 19.0 for Windows®. Statistical significance was set at $p<0.05$.

Results

The mean serum levels of Gal-3 were 29.8 ng/ ml in healthy controls (range:14- 75 ng/dl), 22.2 ng/ml (range :18.5- 28 ng/dl) in patients with stable disease and 103.2 ng/ml (82- 150 ng /dl) in patients with recurrent disease.

Serum levels of Gal-3 in patients with disease progression were significantly higher than those in stable disease patients ($p<0.001$) and those in healthy controls (P: 0.004), but no differences were found between stable disease patients and healthy controls (p:0.7).The Gal-3 values distribution of follow-up patients is shown in Figure 1.

For each imaging findings we calculated the respective threshold values of Gal- 3 identified by ROC curve analysis, related with the disease recurrence. Considering lymphoadenopathies the cut-off value was 92.45 ng/ml (AUC :0.81,CI:0.67-0.97, Se=91% Spe=73%) while for metastasis was 99.05ng/ml (AUC :0,78, CI:0.60-0.94 Se=100% , Spe=70%) (Figure 2).

Considering local tumor spread and carcinomatosis values identified by ROC curves were respectively 99.05 (AUC:0.90 ,CI:076-1.02, Se=100% , Spe=73%) and 85.95 ng/ml (AUC :0.93,CI:0.80-1.06, Se = 93.7%, Sp=92.8%) (Figure 3).

The different MDCT signs of disease relapse in patient with recurrent disease are shown in Table1.

Elevated serum Gal-3 levels were significantly correlated with imaging findings of disease recurrence and particularly with the presence of local recurrence (n=11/17, p:0.001), carcinomatosis (n=16/17, p:0.00), lymphoadenopathies (n=15/17, p:0.00) and distant

Table 1. Imaging Findings in Patients with Disease Relapse

	A	*LTS*	*C*	*L*	*M*
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
4	+	+	+	+	+
5	–	+	–	+	+
6	–	+	+	+	+
7	+	–	+	+	–
8	–	+	+	–	–
9	+	+	+	+	+
10	–	+	+	+	+
11	+	–	+	+	–
12	+	–	+	+	–
13	+	+	+	–	+
14	–	–	+	+	–
15	–	+	+	+	+
16	+	–	+	+	–
17	+	–	+	+	+

A, Ascites ; LTS, Local Tumor Spread; C, Carcinomatosis; L, Lymphoadenopathyes; M, Metastasis)

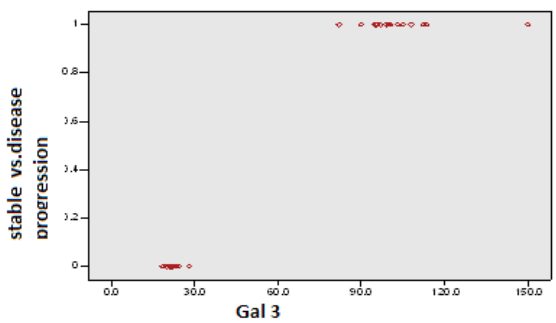

Figure 1. Frequency Distribution of Gal 3 Values in Patients with Stable Disease and in Those with Disease Progression

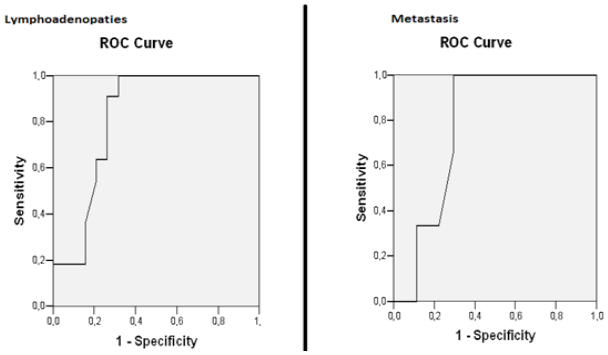

Figure 2. ROC Curve Analysis for Determining the Threshold Value of Gal-3 for Lymphoadenopathies (92.45 ng/ml, AUC :0.81, CI:0.67-0.97) and for Metastasis (99.05ng/ml, AUC :0,78, CI:0.60-0.94)

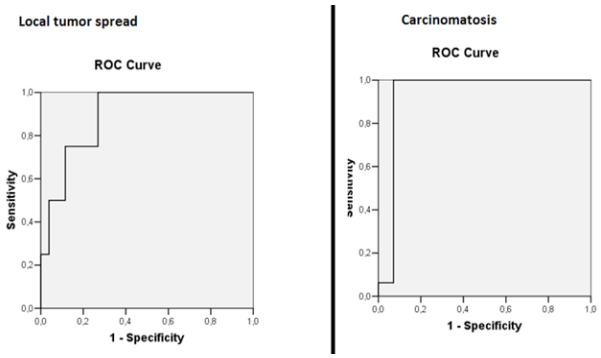

Figure 3. ROC Curve Analysis for Determining the Threshold Value of Gal-3 for Local Tumor Spread (99.05, AUC:0.90, CI:076-1.02) and Carcinomatosis (85.95 ng/ml, AUC :0.93, CI:0.80-1,06).

metastasis (n=11/17, p:0.003) .

Considering distant metastasis the most frequente site was liver (n=5), followed by lung (n=4) and pleura (n=2)

However we found that Gal-3 expression was not related to Patients age (p: 0.4) and with the presence of ascites (p:0.006).

Discussion

Gal-3, a beta-galactoside-binding lectin, is a member of the galectin family implicated in several biological events (Wang et al., 2016). A series of experimental evidences assessed the role of Gal-3 expressions in tumorigenesis. Indeed, extracellular Gal-3 seems to be associated with cell migration, adhesion, and cellular interactions while intracellular Gal-3 inhibits apoptosis and is highly related in neoplastic progression and metastasis spreading (Yoshii et al., 2016; Newlaczyl et al ., 2011; Liu et al ., 2005).

The functional significance of Gal-3 overexpression in EOC is not yet well known. The most important biological characteristics of EOC include tumor infiltration into the surrounding tissues and the implantation of metastasis. At the time of diagnosis, most (75%) epithelial ovarian cancers have progressed to stage III or IV and the majority of patients with advanced or high-grade ovarian cancer relapse and die of disease (Armstrong et al ., 2002; Herzog et al., 2004). For that reason great attention has been paid to find biological markers to be used as early predictor of disease relapse (Rossi et al., 2016; Musella et al., 2017; Marchetti et al., 2014).

In the last few years, the combination of biomarkers has been utilized to improve specificity in advanced ovarian cancer. Currently the biological markers accepted and used in the detection of disease recurrence and lymph node dissemination are Ca125 and HE4 (Freydank et al., 2012; Zhen et al ., 2014;Anastasi et al ., 2014; Manganaro et al ., 2013; Midulla et al ., 2012).

Previous studies have suggested that Gal-3 can be used as disease marker in ovarian cancer diagnosis. Gal-3 seems to mediate drug resistance, promote motility and metastatic invasion thus supporting the importance of Gal-3 as a potential therapeutic target in EOC. Balasubramanian et al., (2009) examined urinary Gal-3 levels to stage ovarian cancer and found a strong correlation between the stage of disease at diagnosis with Gal-3 concentrations. Kim et al., (2011) in their series, showed that high Gal-3 expression in EOC correlated with shorter progression-free survival of patients .

To the best of our knowledge this study is the first one evaluating the role of Gal-3 combined with MDCT as a potential prognostic factor in predicting disease recurrence.

We found that Gal-3 protein expression in patients with recurrent disease was significantly up-regulated compared to patients with stable disease ($p<0.001$) and those in healthy controls ($p< 0.004$).

The most innovative aspect of our study was to assess the combination of Gal-3 with the different signs of disease relapse on MDTC which is the standard imaging method for the post- operative surveillance of women with ovarian cancer according the European Society of Urogenital Radiology and the American College of Radiology guidelines for ovarian cancer staging and follow up (Forstener et al ., 2010; Mitchell et al., 2013). Multi-detector contrast-enhanced Computed Tomography is an available and accurate imaging method that allows a proper assessment of the disease extension in a short examination time. Recurrent ovarian malignancy usually appears as a pelvic mass at the site of surgery or as diffuse peritoneal carcinomatosis, pleuro-pulmonary lesions, lymph node, or liver metastases (Diaz-Gil et al., 2016).

We found a statistic correlation between high levels of Gal-3 and presence of lymphoadenopaties ($p<0.001$). No previous studies related metastatic lymph-nodes in EOC with the circulating levels of Gal-3.

However Miyazaki et al showed that Gal-3 expression was observed significantly stronger in metastatic lymph nodes than in the primary gastric cancers (Miyazaki et al., 2002) , while Salajegheh et al found that overexpression of galectin-1 and 3 proteins were higher in patients with papillary thyroid carcinoma with lymph node metastases (Salajegheh et al ., 2014).

In addition we found a strong correlation between high levels of Gal- 3 in patients presenting local tumor recurrence ($p<0.001$) and metastasis ($p<0.003$)

This is probably related to the enhanced cell adhesion to extracellular matrix components, cell motility and invasiveness due to Gal-3 over-expression, activating molecules related to the adhesion of ovarian cancer cells, such as β1 integrins and E-cadherin. This results are in agreement with a previous study of Lu H et al., (2016) demonstrating that Galectin-3 regulates metastatic capabilities and chemotherapy sensitivity in epithelial ovarian carcinoma via NF-κB pathway. This could have important implications also in the therapeutic management of EOC. Mirandola et al., (2014) suggested that the Gal-3 inhibitor could inhibit the invasive and migratory capabilities of ovarian cancer cell lines and primary cancer cells.

Finally in our study we found higher levels of Gal 3 levels in patients with carcinomatosis ($p<0.00$) compared to the healthy controls. Carcinomatosis is often the most common manifestation of disease recurrence in EOC

patients. Yang et al., (2006) found that the expression of Gal- 3 in gastric cancer lesions can be used as a biological marker of peritoneal metastasis from gastric cancer.

In our study we used MDCT to detect recurrence of disease. The choice of using MDCT examination is due to its availability, to low radiation dose in comparison with Computed Tomography- Positron Emission Tomography (CT-PET) and to the costs. Several studies validated the effectiveness of Fluorodeoxyglucose (FDG) CT-PET in the assessment of patients with recurrent ovarian cancer (Brunetti et al., 2006; Grant et al ., 2014). Actually FDG CT-PET has a reported sensitivity of 80%- 100% for the detection of recurrent ovarian cancer, But a critical factor is represented by its spatial resolution (approximately 6-10 mm); therefore, its sensitivity for depicting lesions smaller than 1 cm is lower than that for larger lesions. Sub-centimeter lesions of omental carcinomatosis may not demonstrate sufficient radiotracer uptake, even if infiltrative changes indicative of the presence of disease are seen at MDCT.

Conflict of interest

The Authors declare that there are not conflicts of interest.

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