The Role of Galectin-3 as a Marker of Cancer and Inflammation in a Stage IV Ovarian Cancer Patient with Underlying Pro-Inflammatory Comorbidities

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Key Words
Galectins · Galectin-3 · Carbohydrate recognition binding domain · Ovarian cancer · Inflammation · Metastasis · Integrative protocol

Abstract
In this case study, I report on a patient with stage IV ovarian cancer and underlying pro-inflammatory comorbidities. Initially, the patient’s inflammatory condition was treated with an intensive integrative anti-inflammatory protocol using a combination of oral and intravenous nutrients and botanicals along with pharmaceutical intervention. This was followed by a standard course of chemotherapy supported by an individualized integrative protocol – with excellent response. Galectin-3 levels as well as other inflammatory and tumor markers were monitored throughout the course of treatment. Correlation with other markers, the clinical course of the disease, and symptomatology are presented. Galectin-3, a novel marker with potential clinical importance in cancer progression as well as inflammation and fibrosis, has been extensively researched in multiple in vitro, in vivo, and epidemiological studies. In this paper, a case in which galectin-3 has been used to assess and monitor patient progress is presented for the first time. This case is of further interest because of its complexity, with the coexistence of acute inflammatory conditions combined with progressing metastatic cancer. Galectin-3 monitoring reflected this complexity; nevertheless, it provided useful information and correlation with other inflammatory markers. The results suggest that monitoring serum galectin-3 as a marker for both inflammatory processes and cancer progression to a higher probability of metastasis may have clinical relevance. Additional clinical research is warranted.

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Introduction

Galectin-3, a unique lectin with a specific affinity for β-galactoside glycoconjugates, is an approximately 30-kDa signaling protein and, like all galectins, contains a carbohydrate recognition domain of approximately 130 amino acids that enable the specific binding of β-galactosides. Galectin-3 is generated intracellularly in both the nucleus and cytoplasm, and at the cell surface. It is also found extracellularly in the general circulation. Little is currently known about the physiological role of circulating galectin-3 in healthy people, but increased expression of galectin-3 has been widely studied [1, 2]. Current research indicates that galectins contribute to diverse physiological and pathological processes via a multitude of complex signaling pathways. Elevated levels of galectin-3 have been shown to play a particularly significant role in promoting inflammation and fibrosis in a wide range of acute as well as chronic conditions, which may share common roots. These include immune and inflammatory responses, neurological degeneration, autoimmune diseases, atherosclerosis and heart failure, diabetes, wound repair, response to infection, lung, kidney and liver disease, and many other conditions [3]. Galectin-3 overexpression has been shown to be a significant promoter of tumor growth, angiogenesis, and metastatic progression [1, 4].

Galectin-3 levels are predictors of all-cause mortality in humans. A landmark study (the PREVEND study) involving 7,968 subjects showed that serum galectin-3 levels were associated with a wide range of risk factors for cardiovascular disease including blood pressure, serum lipids, body mass index, and renal function. After correcting for the classical risk factors (smoking, blood pressure, cholesterol, and diabetes), galectin-3 levels were found to predict all-cause mortality independently [5].

Galectin-3 has been shown to be a useful prognostic biomarker to assist in the detection of early disease and predict prognosis in heart failure patients. In a study by de Boer et al. [6], 592 patients who had been hospitalized for heart failure were followed. The primary endpoint of this 18-month study was all-cause mortality and hospitalization for heart failure. The prognostic value of baseline galectin-3 was compared to other biomarkers. Galectin-3 levels were correlated with higher levels of the inflammatory markers interleukin-6 and C-reactive protein (CRP). The study concluded that galectin-3 is an independent marker for outcome in patients with heart failure. Galectin-3 levels in this population have been shown to correspond to the degree of inflammation, fibrosis, and risk of mortality [7]. Recognition of the importance of galectin-3 as a serum biomarker in high-risk cardiovascular patients with heart failure has resulted in the FDA approval for galectin-3 testing by enzyme-linked immune-sorbent assay (ELISA), with the indication for monitoring heart failure patients.

Galectin-3 levels have been shown to correlate with inflammation and fibrosis in multiple in vivo studies. Tissue galectin-3 levels were consistently elevated in animal models following an induced injury, and the studies included antigen-induced arthritis, acetaminophen-induced liver injury, concanavalin-A-induced hepatitis, and bleomycin-induced lung fibrosis [8–11]. In each of these studies, control animals were compared with knockout mice deficient in galectin-3. Control animals consistently showed significantly elevated galectin-3 tissue levels as well as other markers of inflammation and fibrotic changes. The galectin-3 deficient knockout mice, however, had significantly lower tissue galectin-3 levels as well as reduced histological changes. These results indicate that galectin-3 is not only a biomarker but also a primary promoter of inflammation and fibrosis associated with organ injury. Further studies supporting these conclusions have been conducted in research using galectin-3 binding agents. One study [12] used aldosterone as an inducer of vascular fibrosis. Hypertensive aldosterone-treated rats showed vascular hypertrophy, inflammation, fibrosis, and increased aortic galectin-3 expression. Blocking galectin-3 expression using spironolac-
tone, a known galectin-3 blocker, reversed all of these effects. In wild-type mice, aldosterone increased aortic galectin-3 expression, inflammation, and collagen type I synthesis, whereas no changes occurred in galectin-3 knockout mice, providing further support for a key role of galectin-3 in vascular fibrosis.

Galectin-3 has also been shown to play a significant role in the physiology of tumor progression. In numerous in vitro and in vivo studies, galectin-3 has been found to promote neoplastic progression and metastatic potential via a multitude of pathways [4]. Galectin-3 plays a central role as a signaling molecule regulating many cellular functions including cell growth, adhesion, migration, angiogenesis, apoptosis, and metastatic progression [1, 3]. Studies comparing circulating galectin-3 levels in healthy controls with that in patients with various types of cancer have shown significant increases in circulating galectin-3 in cancer patients. This has been demonstrated in patients with melanoma, lung, breast, colorectal, ovarian, and head and neck cancers as well as non-Hodgkin’s lymphoma [13]. Galectin-3 concentrations were higher in patients with metastatic disease than in sera of patients with localized tumors, suggesting a potential for the use of galectin-3 testing in early-stage cancer as a prognostic marker for metastasis [13]. A recent study [14] compared the serum galectin-3 levels from sera of non-cancer urology patients to those of metastatic prostate cancer (PCa) patients. The galectin-3 levels of patients with metastatic PCa were uniformly higher compared to those of the non-cancer patient controls. The authors suggest that serum galectin-3 levels may be useful complementary markers to the prostate-specific antigen (PSA) blood test for initial evaluation and follow-up monitoring as well as a complimentary diagnostic and prognostic tool for recurrence in PCa patients.

**Case Presentation**

On February 29, 2012, a 51-year-old woman presented to the emergency room for an initial workup of acute left lower quadrant pain. She was otherwise asymptomatic with a negative physical exam for pelvic mass or lymphadenopathy. The patient was in relatively good health prior to the development of her symptoms, reporting mild chronic complaints of gastritis and gastroesophageal reflux disease that had not been thoroughly evaluated or treated. Her prior surgical history included bilateral tubal ligation and cesarean section. The initial CT scan revealed retroperitoneal and mesenteric lymphadenopathy with a mass to the left of the uterine fundus. The subsequent ultrasound on March 12, 2012 showed two hypoechoic masses thought to be leiomyomata with normal-appearing adnexa. A follow-up PET/CT scan on March 14, 2012 was positive for two pathologically hypermetabolic pelvic masses and extensive hypermetabolic lymphadenopathy above and below the diaphragm. The subsequent lymph node biopsy on March 3, 2012 confirmed the presence of metastatic high-grade papillary serous ovarian carcinoma. Chemotherapy with carboplatin/paclitaxel was planned. However, following subclavian port-a-cath insertion, a localized infection developed that was treated with antibiotics, delaying the initiation of chemotherapy.

Shortly after diagnosis, the patient’s initial laboratory evaluation (please refer to table 1 for all laboratory reference ranges) showed a vitamin D deficiency of 16.7 ng/ml and a slightly elevated CRP of 3.65 mg/l. Her carcinoembryonic antigen was within the normal range at 1.8 ng/ml, and her cancer antigen (CA)-125 was elevated at 147 U/ml. She was given oral vitamin D3 at 50,000 international units per day by a nutritionist she saw independently. Her subsequent blood test 3 weeks later showed an elevated 25(OH) vitamin D level of 102.0 ng/ml with CRP continuing to increase to 5.27 mg/l.
The patient was seen at Amitabha Medical Clinic (Santa Rosa, Calif., USA) for a consultation on May 1, 2012, 2 months after her initial workup. She was interested in an integrative program to support her during the upcoming course of chemotherapy. Extensive laboratory workup was initiated. The results revealed a climbing CA-125 of 233.0 U/ml, a spike in CRP to 18.31 mg/l, and a highly elevated 25(OH)vitamin D level of 184.8 ng/ml. Helicobacter pylori IgM, IgG and IgA levels were elevated at >8.0 U/ml, with elevated IgA antibodies (Abs) at 2.35 U/ml. Fibrinogen activity and vascular endothelial growth factor (VEGF) were at the upper end of the normal range at 421 mg/dl and 690 pg/ml, respectively. The patient’s galectin-3 level was 21.8 ng/ml. In recent research, the average value for galectin-3 in the general population was found to be 11.7 ng/ml [5]. In patients with congestive heart failure, abnormal levels for the galectin-3 ELISA test measure >17.8 ng/ml.

A comprehensive integrative program was initiated to address the inflammation and treat the patient’s comorbidities prior to beginning the course of chemotherapy. Treatments were also targeted to address the progressing cancer. The program included an intensive intravenous support protocol, an extensive oral nutrient and botanical regimen, dietary modifications including discontinuation of vitamin D3 supplementation, and other complementary therapies. The treatment of H. pylori was initiated with lansoprazole 30 mg, amoxicillin 1 g, and clarithromycin 500 mg administered together, po bid for 10 days. Intravenous therapies were performed twice per week in a progressively enhanced protocol. Oral supplementation focused on anti-inflammatory, anti-tumor, and immune-enhancing nutrients and botanical combinations. The patient’s inflammatory status was improved with this approach, using multiple therapeutic modalities.

Laboratory values 1 month after treatment initiation showed that galectin-3 decreased to 13.7 ng/ml, 25(OH)vitamin D to 120.0 ng/ml, and H. pylori IgA to 1.76 U/ml. The patient reported an improvement in her general condition and a reduction in pain and discomfort. Markers of cancer progression increased during the same period; CA-125 was 342.0 U/ml, VEGF was elevated at 876 pg/ml, and fibrinogen activity was above the normal range at 433 mg/dl. Chemotherapy was initiated on June 15, 2012 using standard protocols for carboplatin/paclitaxel along with a modified intravenous and oral complementary protocol. The CRP on July 4, 2012 showed a drop to the normal range at 2.29 mg/l. The patient had an excellent response to chemotherapy, with laboratory values of CA-125 reduced to 14.8 U/ml and galectin-3 reaching a low level of 11.9 ng/ml after 6 weeks of treatment.

The patient suddenly developed a widespread erythematous rash thought to be due to an allergic reaction to paclitaxel. This was later determined to be autoimmune dermatomyositis. CRP spiked to 14.31 mg/l, and treatment with cimetidine at 800 mg bid and betamethasone valerate topical cream, which was unsuccessful in resolving the rash, was initiated. Paclitaxel was discontinued. Oral prednisone at 20 mg, 3 tablets once per day, was prescribed as well as topical pimecrolimus 1% and fluocinonide 0.05% – with some improvement.

A restaging CT scan (with oral and IV contrast) of the chest, abdomen, and pelvis on September 4, 2012 showed that the para-aortic and abdominal retroperitoneal lymph nodes were markedly reduced in size. No masses were visualized in the abdomen. The patient completed three more cycles of carboplatin only, with a total course of chemotherapy of 4.5 months. A post-treatment CT scan (with oral contrast) of the chest, abdomen, and pelvis on December 4, 2012 showed complete resolution of all masses without evidence of residual or recurrent metastatic disease. The CA-125 at this time was well within the normal range at 8.0 U/ml. However, galectin-3 was once again elevated at 24.3 ng/ml, with CRP elevated at 8.79 mg/l. The patient had continued on her dermatomyositis treatment regimen, but because of continuing symptoms, she was due to begin a more aggressive treatment with...
methotrexate 2.5 mg, 6 tablets once per week. Repeat galectin-3 on March 19, 2013 showed a highly elevated value at 48.3 ng/ml, with CA-125 remaining stable at 5.0 U/ml. A CT scan was planned to evaluate for cancer reoccurrence, which might not be reflected in serum tumor markers, along with initiation of methotrexate for dermatomyositis. Her CRP, CA-125 and galectin-3 levels will continue to be monitored as evaluation progresses.

Discussion

When the patient was initially evaluated at the Amitabha Medical Clinic, she was in a hyperinflammatory state, which was reflected by elevated CRP and galectin-3 levels. The inflammation could have been triggered by the ongoing metastatic process, the newly diagnosed H. pylori infection, or the port-a-cath infection. During the initial 4 weeks of her integrative protocol prior to initiation of chemotherapy, the patient’s inflammatory markers as well as her symptoms improved significantly, while her tumor and viscosity markers indicated cancer progression. This was postulated to be a reflection of the marked improvement in the patient’s inflammatory status, even in the presence of tumor progression. Galectin-3 levels as well as CRP values normalized as tumor control was achieved with chemotherapy. The patient experienced a sudden onset of skin rash about 6 weeks after the commencement of chemotherapy. CRP, which had been low at 2.29 mg/l, spiked to 14.31 mg/l, while galectin-3 remained low at 11.9 ng/ml. Since galectin-3 is involved in repair mechanisms, elevated levels may occur after a rise in CRP if inflammation is the initiating factor. However, elevation of galectin-3 can also occur prior to inflammatory response if injury occurs first, followed by inflammation as part of the repair process. In the present case, acute inflammation was the initiating factor. As time went on and the dermatomyositis continued, the galectin 3 level became increasingly elevated. There was cancer remission, with the CA-125 remaining well within the normal range while a systemic inflammatory process continued, with galectin-3 continuing on an upward spiral. The prognostic significance of this elevated level is yet to be elucidated, but it was postulated to be an effect of the continuing dermatomyositis. The patient is still followed up and further evaluation is performed.

Conclusion

This case report is the first to demonstrate the clinical use of galectin-3 and its potential ability to reflect changes in both the cancer status and the inflammatory state of the patient. Research in humans supports this conclusion, showing that elevated systemic galectin-3 levels are associated with a wide array of cancer types, with levels corresponding to cancer stage and progression. In addition, research points to a distinct promoter role for galectin-3 in the processes of inflammation, fibrosis, tumor growth, and metastasis. Galectin-3 values have also been shown to be an accurate predictor of all-cause mortality in the general population. The galectin-3 ELISA assay is now specifically indicated as a prognostic marker to monitor progression and mortality risk in chronic heart failure patients. This case study supports the use of galectin-3 monitoring as a marker and potential prognostic indicator in patients with cancer as well as other inflammatory conditions. We are beginning to see research addressing this potential role, as evidenced by a recent study exploring the use of galectin-3 testing as an adjunct to PSA testing in prostate cancer patients [14].
Galectin-3 is also a potential therapeutic target. Clinical and preclinical studies have shown that blocking galectin-3 could have a highly significant impact on inflammation reduction, fibrotic change, tumor progression, and metastatic spread. The galectin-3 ELISA assay is easily available to clinicians.

With clear evidence in the literature of the role galectin-3 plays as both an indicator and promoter of inflammation and cancer progression, additional studies are indicated. This early data should encourage clinicians to incorporate galectin-3 testing into their protocols, which will further the understanding and development of galectin-3 testing as a valuable marker with potential prognostic significance for monitoring inflammation, tumor progression, and treatment response.

Acknowledgement

The author wishes to thank the patient for her support and consent to publish her case.

Disclosure Statement

The author acknowledges his ownership of a dietary supplement company.

References

Table 1. Laboratory reference ranges (Laboratory Corporation of America, Burlington, N.C., USA)

<table>
<thead>
<tr>
<th>Test</th>
<th>Reference Range</th>
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<tbody>
<tr>
<td>CRP (cardiac)</td>
<td>0.00–3.00 mg/l</td>
</tr>
<tr>
<td>Ferritin serum</td>
<td>13–150 ng/ml</td>
</tr>
<tr>
<td>VEGF serum</td>
<td>62–707 pg/ml</td>
</tr>
<tr>
<td>Fibrinogen activity</td>
<td>193–423 mg/dl</td>
</tr>
<tr>
<td>25(OH)itamin D (hydroxy)</td>
<td>30.0–100.0 ng/ml</td>
</tr>
<tr>
<td>CA-125</td>
<td>0.0–34.0 U/ml</td>
</tr>
<tr>
<td>Carcinoembryonic antigen</td>
<td>0.0–4.7 ng/ml</td>
</tr>
<tr>
<td>H. pylori, IgM, IgG, IgA Abs</td>
<td></td>
</tr>
<tr>
<td>H. pylori, IgG Abs</td>
<td>0.0–0.8 U/ml</td>
</tr>
<tr>
<td></td>
<td>negative &lt;0.9, indeterminate 0.9–1.0, positive &gt;1.0</td>
</tr>
<tr>
<td>H. pylori, IgA Abs</td>
<td>0.00–0.88 U/ml</td>
</tr>
<tr>
<td></td>
<td>negative &lt;0.89, equivocal 0.89–0.99, positive &gt;0.99</td>
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<tr>
<td>Galectin-3 &lt;22.2 ng/ml</td>
<td></td>
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<tr>
<td>≤17.8 ng/ml: lower risk of adverse outcomes</td>
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<tr>
<td>≥17.8 ng/ml: higher risk of adverse outcomes</td>
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