

Measurement of Glycodelin A in Fluids of Benign Ovarian Cysts, Borderline Tumours and Malignant Ovarian Cancer

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Abstract. *Background:* Glycodelin A levels in fluids of benign ovarian cysts, borderline tumours and ovarian cancer, as well as serum-levels of glycodelin A, were analysed in patients with benign and malignant ovarian tumours. The aim of the study was to investigate if the level of glycodelin A in body fluids of patients with malignant ovarian tumours could be a marker for the disease. Additionally, immunohistochemical investigations of glycodelin A expression in tissue of ovarian cancer were performed. *Materials and Methods:* A total of 158 samples of fluids from benign ovarian cysts, borderline tumours and ovarian cancer were collected during surgery in the Department of Obstetrics and Gynaecology at the University of Rostock, Germany. Additionally, 69 samples of serum from patients with benign ovarian cysts and ovarian cancer on the day before surgery were collected. An ELISA for determination of glycodelin A was used. Immunohistochemical detection of glycodelin A expression was performed on ovarian cancer tissue from 38 patients. *Results:* Malignant cystic fluids showed higher glycodelin A values (mean: 1814.4 ng/ml) compared to fluids of benign ovarian cysts (mean: 784.4 ng/ml). The results of glycodelin A determination were compared using the Mann-Whitney U-test for comparison of the means. There was a statistically significant difference between benign ovarian cysts and malignant ovarian cancer for the fluid ($p < 0.001$). In addition, serum samples of malignant ovarian tumours also showed significantly higher glycodelin A values compared to serum levels of benign tumours ($p < 0.001$). Immunohistochemical staining on ovarian cancer tissue showed a glycodelin A expression in 25-30% of the carcinoma cells. *Conclusion:* High levels of glycodelin A were found in cystic fluids of ovarian cancer. In addition, we also

found higher levels of glycodelin A in the serum of patients with ovarian cancer compared to the serum of patients with benign ovarian cysts. Furthermore, ovarian cancer tissues showed intense staining with a glycodelin A antibody. Further investigations are necessary to show if glycodelin A quantification could help to diagnose ovarian cancer.

Glycodelin, also known as placental protein 14 (1), is a glycoprotein that is synthesized by a variety of tissues and cell types. The glycodelin gene is localized on chromosome 9q13 (2). It contains 180 amino acids, 18 of which correspond to a putative signal peptide (3). Glycodelin is a glycoprotein containing 17.5% carbohydrates (4). The glycosylation of glycodelin is gender-specific (5). For this reason, we distinguish between glycodelin A (isolated from amniotic fluid) and glycodelin S (isolated from seminal plasma). Glycodelin A is rich in mannose (5), while glycodelin S glycans are unusually rich in fructose (6). Glycodelin A is the major glycoprotein synthesized by late secretory endometrium and gestational decidua (7). High levels of glycodelin S are secreted in seminal plasma from the seminal vesicle glandular epithelium (8).

Glycodelin A has potent immunosuppressive (9, 10), contraceptive (11) and morphogenic properties (12, 13). Studies have shown that glycodelin A suppresses both the allogenic mixed lymphocyte reaction and lymphocyte responsiveness to phytohemagglutinin. Glycodelin A suppresses the release of IL-2 and IL-2R from stimulated lymphocytes (14, 15). The cytotoxic activity of NK-cells is inhibited by glycodelin A in the concentration range of 1 to 50 µg/ml (16, 17). During the normal menstrual cycle, glycodelin synthesis in endometrial glands begins 4-5 days after ovulation, whereafter its concentration in tissue and uterine fluid rapidly increases towards the end of the cycle (18, 19). Glycodelin is not present at the time of conception. It has been suggested that these immunosuppressive properties of glycodelin could be important for the fetto-embryonic defence

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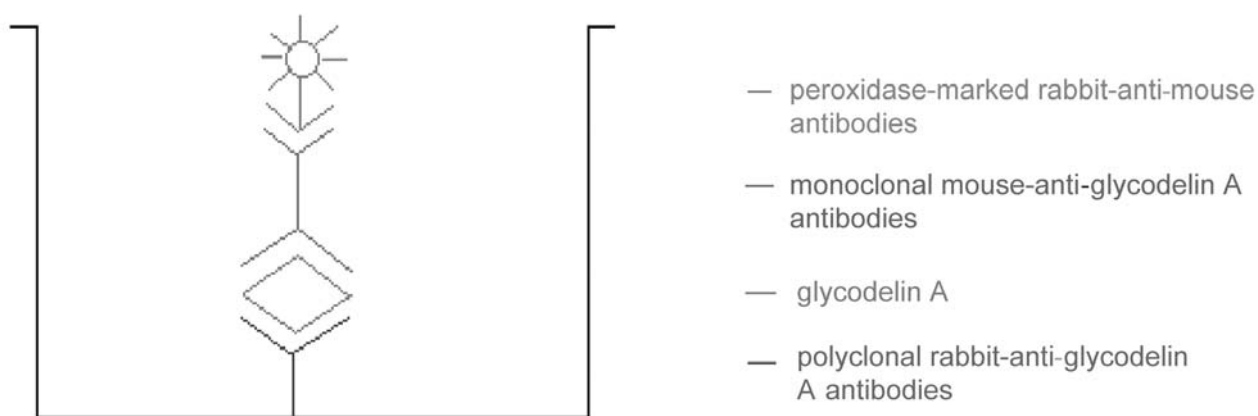


Figure 1. Glycodelin A Sandwich-ELISA.

system (20). *In vivo* studies suggest that the synthesis of glycodelin A may be induced by two hormones secreted by the corpus luteum: progesterone (21) and relaxin (22).

Several studies report on the connection between glycodelin and malignant disease. Glycodelin A was found in the squamous epithelium of both the histologically normal and the neoplastic cervix. These results focus on the possible immunosuppressive effect glycodelin A may have in the cervix (23). There were high levels of glycodelin in the uterine flushings of women with endometrial adenocarcinoma (24) and significant increase in plasma glycodelin levels in endometrial, ovarian and cervical cancer patients when compared to those of controls (25).

In this report, we studied glycodelin A levels in fluids of benign ovarian cysts, borderline tumours and ovarian cancer as well as serum levels of glycodelin A in patients with benign and malignant ovarian tumours. Additionally, we performed immunohistochemistry with rabbit anti-glycodelin A antibodies on ovarian cancer tissue in order to demonstrate if elevated levels of glycodelin A in the serum of cancer patients can be related to glycodelin A expression in tumour tissue.

Materials and Methods

Glycodelin A ELISA. In this report we used a Sandwich-ELISA, developed at the University of Rostock, Department of Obstetrics and Gynaecology. The enzyme-linked immunosorbent assay (ELISA) is based on polyclonal rabbit-anti-glycodelin A antibodies and monoclonal mouse-anti-glycodelin A antibodies (Figure 1). The assay showed an inter-assay variation of 15%, while the intra-assay variation was 7%. The range of the assay was from 15 ng/ml to 3.7 mg/ml glycodelin A.

Fifty μ l coating-buffer (10 μ g/ml polyclonal rabbit-anti-glycodelin A antibodies in phosphate-buffered saline) was added in wells of the ELISA plate (Nunc-Immuno Plate, MaxiSorp F96,

Nunc, Denmark) and incubated mostly overnight at 37°C. The next day, the plates were washed three times and incubated for 90 min at 37°C with 50 μ l of fluid from benign, borderline and malignant ovarian tumours or serum of patients with benign and malignant ovarian tumours. The wells were washed and incubated for 90 min at 37°C with 50 μ l monoclonal mouse-anti-glycodelin A antibodies (1 μ g/ml). The wells were washed again and incubated for 60 min with 50 μ l peroxidase-marked rabbit-anti-mouse antibodies at a dilution of 1:1000. Finally, 50 μ l of substrate-solution (Na_2HPO_4 , citric acid, H_2O_2 and O-phenyldiamine) were added and incubated for 15 min in darkness. The optical density was measured at 450 nm.

Fluid of benign ovarian cysts, borderline tumours and ovarian cancer. We collected 158 samples of fluids from benign ovarian cysts, borderline tumours and ovarian cancer during surgery at the University of Rostock, Department of Obstetrics and Gynaecology. Specifically, there were 128 samples from benign ovarian cysts, 5 samples from borderline tumours and 23 samples from ovarian cancer. The samples were stored at -80°C.

Serum samples from patients with benign ovarian cysts and ovarian cancer. We collected 69 samples of serum from patients with benign ovarian cysts and ovarian cancer on the day before surgery at the University of Rostock, Department of Obstetrics and Gynaecology. Specifically, there were 53 samples from benign ovarian cysts and 16 samples from ovarian cancer. The samples were stored at -80°C.

Immunohistochemistry. For immunohistochemistry on ovarian cancer tissue, we used tissue from 38 patients. Slides were incubated with polyclonal rabbit-anti-glycodelin A antibodies on the automatic NexES ICH (Ventana, Strasbourg, France) immunostainer. Staining was visualized with diaminobenzidine (DAB) (characteristic brown colour).

Statistical analysis. Statistical analysis was performed using the Mann-Whitney *U*-test for comparison of the means. The $p < 0.05$ value was considered statistically significant.

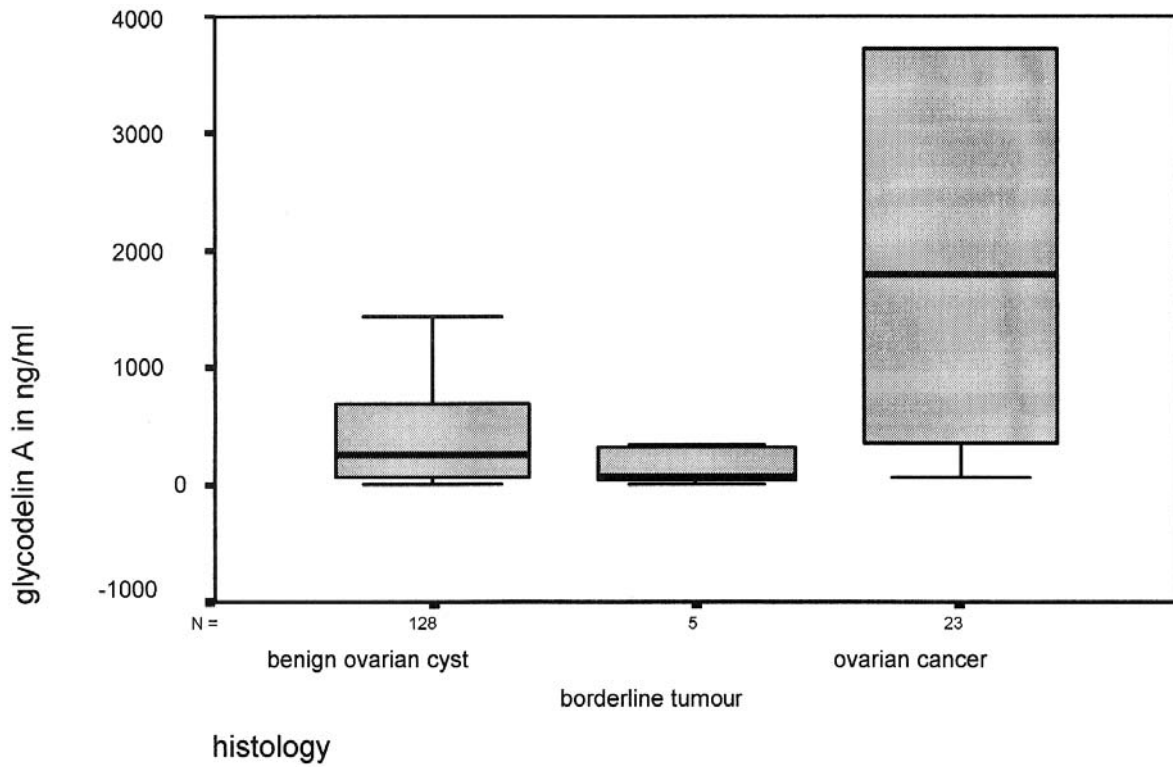


Figure 2. Comparison of glycodelin A level in fluid of benign ovarian cysts, borderline tumours and ovarian cancer.

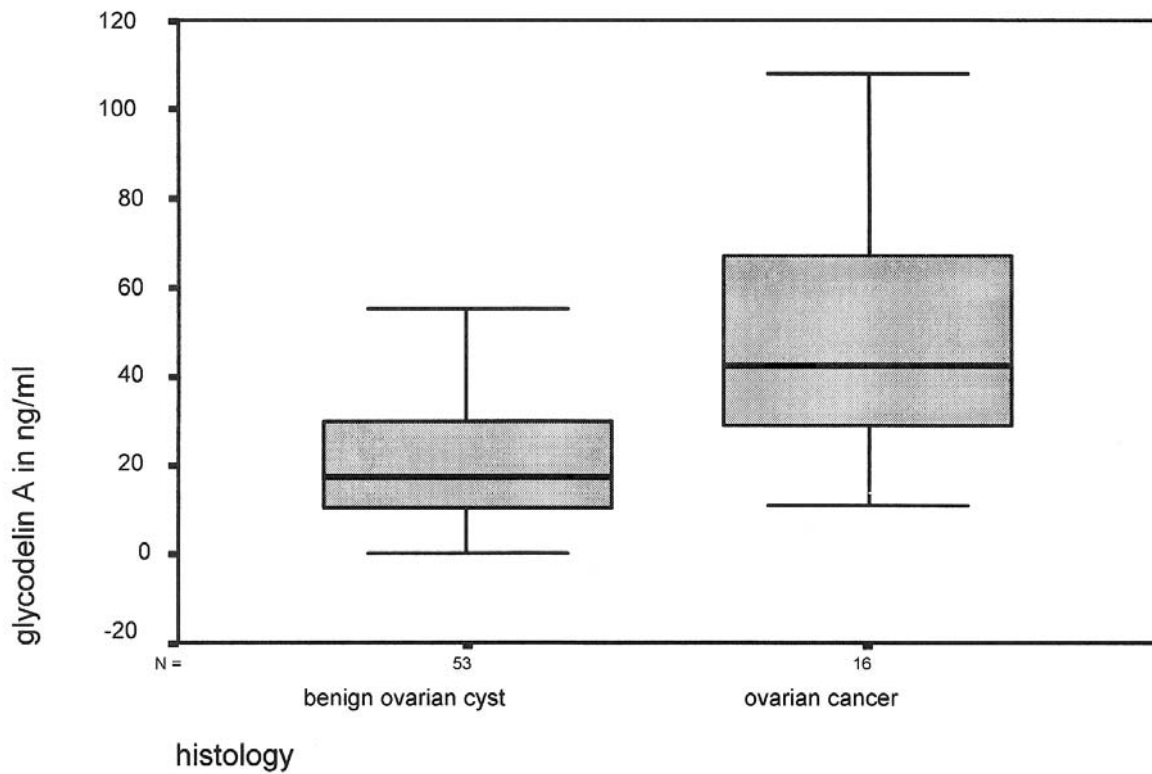


Figure 3. Comparison of glycodelin A level in serum samples of benign ovarian cysts and ovarian cancer.

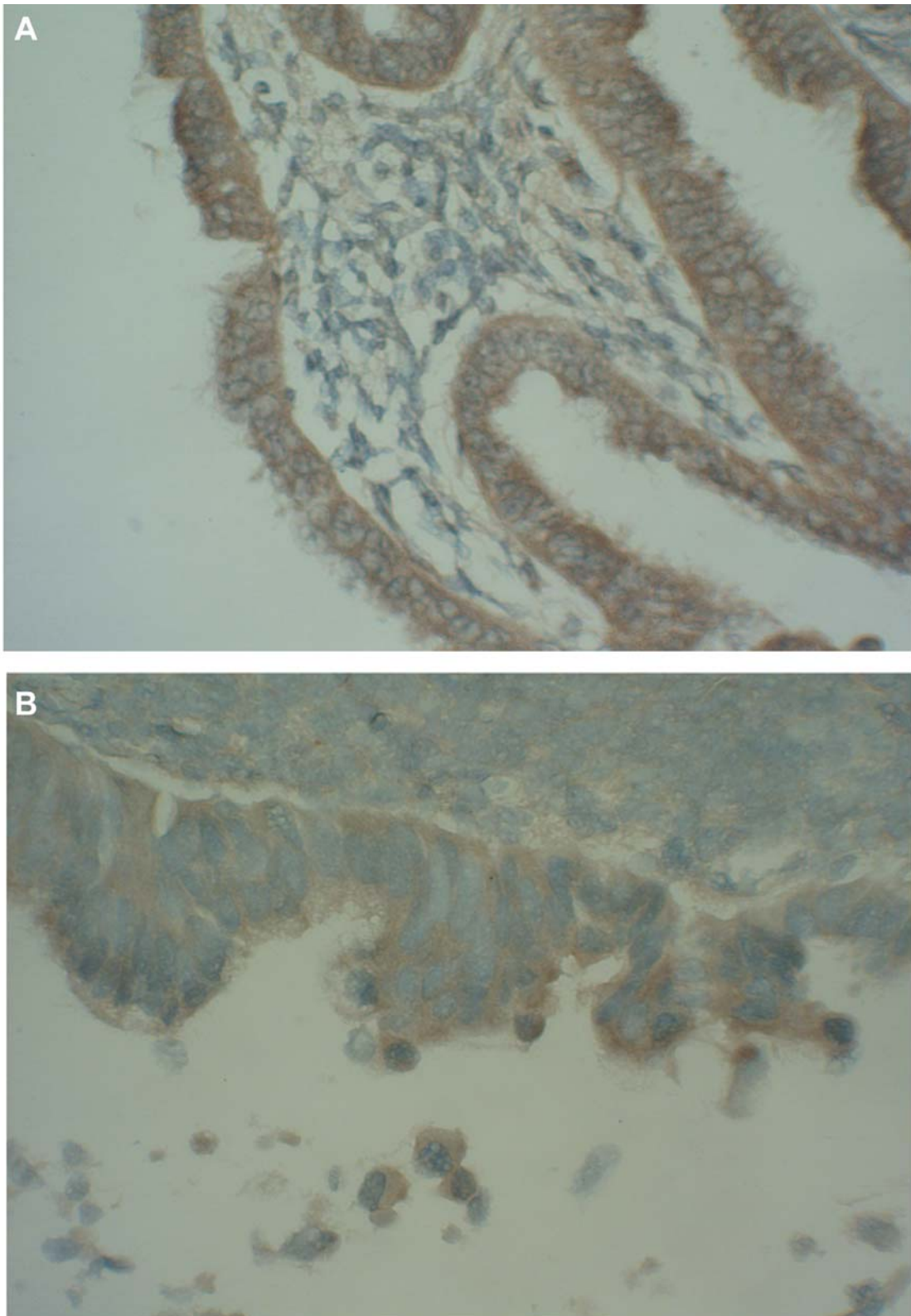


Figure 4. *A. Tumor tissue of ovarian cancer immunostained with rabbit-anti-glycodelin A antibodies, 40x. B. Tumour tissue of ovarian cancer immunostained with rabbit-anti-glycodelin A antibodies, cytoplasmatic positive reaction, 40x.*

Results

Fluids of benign ovarian cysts, borderline tumours and ovarian cancer. A total of 158 collected fluids from benign ovarian cysts, borderline tumours and ovarian cancer were analysed for glycodelin A levels with the Sandwich-ELISA. The results are summarized in Figure 2. Glycodelin A levels showed a statistically significant difference between benign ovarian cysts (mean 784.4 ng/ml) and malignant ovarian cancer (mean 1814.4 ng/ml) ($p < 0.001$).

Serum samples from patients with benign ovarian cysts and ovarian cancer. A total of 69 collected serum samples from benign ovarian cysts and ovarian cancer were analysed for glycodelin A levels with the Sandwich-ELISA. The results are summarized in Figure 3. Glycodelin A levels showed a statistically significant difference between benign ovarian cysts (middle 20.0 ng/ml) and malignant ovarian cancer (middle 47.0 ng/ml) ($p < 0.001$).

Immunohistochemistry. In order to demonstrate whether elevated levels of glycodelin A in fluids and serum samples of ovarian cancer patients are endogenously produced, we looked for the glycodelin A expression in ovarian cancer tissues. A total of 38 tumour tissues were immunostained for glycodelin A with rabbit-anti-glycodelin A antibodies. Ovarian cancer tissues showed intense staining with the glycodelin A antibody (Figure 4A, 4B).

Discussion

The biological function of glycodelin A is still unclear. Glycodelin has immunosuppressive, contraceptive and morphogenic activities. Especially the immunosuppressive effects are of great interest, as they suggest a role of glycodelin in tumour biology. Glycodelin A suppresses the release of IL-2 and IL-2R from stimulated lymphocytes (14, 15). Also, the cytotoxic activity of NK-cells is inhibited by glycodelin A (16, 17). Transfection of glycodelin in MCF-7 breast carcinoma cells inhibits cell proliferation and induces apoptosis as well as differentiation (26)

There were low serum glycodelin levels in pregnant women with absent ovarian function. These observations support the concept that the ovaries might be involved in glycodelin synthesis (27). However, there are reports that show no synthesis of glycodelin from the ovaries (27). On the other hand, studies indicated elevated levels of glycodelin mRNA and protein in the ovarian and endometrial cancer tissue when compared to the normal ovary and endometrium. Increased serum levels of glycodelin have also been found in patients with ovarian cancer (25).

In this report, we collected fluids from benign ovarian cysts and ovarian cancer for glycodelin A quantification.

There were statistically higher glycodelin A levels in fluids of ovarian cancer compared with fluids from benign ovarian cysts ($p < 0.001$). In addition, we measured glycodelin A levels in serum of patients with benign ovarian cysts and ovarian cancer. The serum levels were statistically higher in patients with ovarian cancer.

Immunohistochemistry with polyclonal rabbit-anti-glycodelin A antibodies showed elevated glycodelin A expression in ovarian cancer tissues. The epithelial cells of the ovarian tumour tissue showed intensive staining with glycodelin A antibody.

The differentiation between ovarian cyst and ovarian cancer is still difficult and requires surgery. At the time of diagnosis, 50% of all patients have already developed peritoneal carcinosis. More women die of ovarian cancer than of any other genital tumour, although it makes up only 20% of genital tumours. Since there is no useful tumour marker for the early diagnosis of ovarian cancer, it would be helpful to further study if glycodelin A quantification in serum and/or cystic fluids could be helpful in the early diagnosis of ovarian cancer.

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