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Original Article

# Hyperglobulinemia as a marker in the development of immune deficiency and complications in inflammatory breast cancer patients with type 2 diabetes mellitus

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## Abstract

Dynamic monitoring of cryoglobulinemia throughout the postoperative period in patients with inflammatory breast cancer may indicate changes in their concentration in blood serum depending on the time of observation. The study aimed to determine cryoglobulin levels and plasma glucose levels as influencing factors in the development of immune system deficiency in inflammatory breast cancer, which could lead to different types of postoperative complications. In all patients, blood serum cryoglobulinemia conditions and plasma glucose levels were observed on the third, fifth and seventh day after the procedure and the content of lymphocyte subpopulations in the early postoperative period. Twenty patients with inflammatory breast cancer were divided into two groups: the first included 10 patients with normal levels of cryoglobulins in blood serum 60–80 mg/l (less than 108 mg)/l, corresponding to level 0-I of cryoglobulinemia and blood glucose range 4.4–6.6 mmol/l. The second group included 10 patients with primary hypercryoglobulinemia 298.6±2.5 mg/l; 1.3±0.08% (more than 280 mg/l), corresponding to level II-III of cryoglobulinemia and blood glucose range over 10.0 mmol/l. In both groups of patients, there was an increase in the concentration of cryoglobulins and plasma glucose levels on the third day after surgery. Thus, studies have shown that patients with cryoglobulinemia and type 2 diabetes mellitus operated for tumor-induced inflammatory breast cancer have formed a secondary immune deficiency. This is determined by abnormalities in the cellular and humoral immune system and as a consequence of developing postoperative inflammatory complications in patients with diabetes mellitus.

**Keywords:** hypercryoglobulinemia, inflammatory breast cancer, plasma glucose level, immune deficiency, type 2 diabetes mellitus, complications.

## Introduction

Several years ago, patients with inflammatory breast cancer have been shown to be immunocompetent, as demonstrated by their ability to develop complications. Thus, a couple of studies' number of patients was needed to establish an immune profile unique to IBC patients and to determine whether the immune system plays a role in the pathogenesis of the disease and its currents [1].

Numerous conceivable molecular mechanisms have been proposed to clarify the causal relationship between T2DM and many types of cancer, such as breast cancer. Inflammation-related conditions may be of primary significance in clarifying the connection; therefore, something would encourage interconnected

states. It will build up the multi-faceted, bidirectional, and double relationship between diabetes, its treatment and breast cancer subtype-specific rate, prognosis, and treatment [2].

Breast carcinoma is one of the most malignant tumors; the latest epidemiological and clinical studies have shown that breast tumors and inflammation have decisive relationships with each other. Inflammation is an essential component of the tumor microenvironment, and the change of inflammatory cells might influence the collapse of antitumor immunity. Peripheral blood tests, at the time of diagnosis and treatment, may reflect inflammatory conditions within the neoplasm and at an early stage after surgical removal of the tumor.

Evaluation of peripheral blood parameters including the level of cryoglobulins, lymphocyte and monocyte counts, which are indicators of a systematic inflammatory response, have been proposed as prognostic factors for many malignancies [3].

Diabetes is one of the foremost vital inveterate conditions worldwide and breast cancer is the foremost predominant cancer in women worldwide. A few sorts of investigations have been conducted to find out the interface between diabetes and its potential for expanding the chance of breast cancer [4].

An intensive study of the relationship between markers of inflammation in peripheral blood and the treatment or prognosis of breast cancer will be of critical clinical significance and application prospects and also can provide useful information for clinicians [5]. Research progress in the prognostic role of the peripheral blood markers in breast cancer provides a new method for prognosis and further treatment of inflammatory breast cancer [6].

Chronic hyperglycemia, in collaboration with the other metabolic distortions in patients with diabetes mellitus, can cause harm to different organ frameworks, leading to the advancement of debilitating and life-threatening wellbeing complications, most conspicuous of which are microvascular and macrovascular complications leading to a 2-fold to 4-fold expanded hazard of cardiovascular infections. This study describes the pathogenesis, identification, clinical introduction and delivery standards for diabetes [7].

The absence of protocols in the oncology community governing the treatment of inflammatory breast cancer leads to significant differences in the choice and controlled tactics [8].

However, no less important is the question of the development of complications and prolongation of the disease after neoadjuvant chemotherapy and follow-on operation for inflammatory breast cancer [9].

Of all the variety of disorders after radical mastectomy, most often, we are thinking about recurrences (7.9%), metastasis (15.5%), a different type of inflammatory complications (34%), even during the first year after the beginning of the treatment [10].

According to various authors, it may be related to the level of the human organism's general and local immunological reaction upon initial detection of inflammatory breast cancer and other types of cancers [11].

The aim is to determine cryoglobulin levels and plasma glucose levels, as an influencing factor in the development of the immune system deficiency in inflammatory breast cancer, which could lead to different types of postoperative complications.

## Material and methods

For a more in-depth study of patients, the study lasted from 2015 to 2020 and directly analyzed the treatment outcomes of 20 patients with inflammatory breast cancer. The patients were split into two groups: the first included 10 patients with a blood glucose range of 4.4–6.6 mmol/l, with normal levels of cryoglobulins in blood serum 60–80 mg/l (less than 108 mg/l), corresponding to 0-I levels of cryoglobulinemia and the second group included 10 patients with a blood glucose range over 10.0 mmol/l, with primary hypercryoglobulinemia 298.6±2.5 mg/l; 1.3±0.08% (more than 280 mg/l), corresponding to II-III levels of cryoglobulinemia. All patients performed surgical intervention in the volume of radical mastectomy by Madden. According to TNM, they corresponded to the T4b-dN0-2M0. Patient distribution depends on the stage – T2-3 N0 M0 – 17.5%, T2-3 N1-2M0 – 73.2%, T4 N1-2M0 – 9%., histologically confirmed adenocarcinoma GIII-GIV, Her – 2 negatives, Lum B, ECOG 0-2 status. Patients of both groups are represented by age (average 68 years old and range from 57 to 79 years old). The number of postoperative complications was studied, depending on changes in the immunological status of patients. All patients who had postoperative complications (postoperative laceration, inflammatory infiltration, seroma) and non-surgical complications compared to patients who did not have them, showed conditions of cryoglobulinemia and plasma glucose levels on the third, fifth, and seventh day after operative intrusion and the content of lymphocyte subpopulations in patients with inflammatory breast cancer in the early postoperative


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period. Preliminary data did not reveal a significant difference between groups with intraoperative complications, p≥0.05 and no significant difference was found in groups without intraoperative complications. All patients from both groups were radically operated on (R0 -intrusion) after neoadjuvant endocrinological and chemotherapy treatment depending on immunohistochemically status. Patients of both primary groups had signs of inflammatory breast cancer like edema (more than 30% square of the breast), skin thickening, and redness (Figure 1).

In the immediate postoperative period, the level of cryoglobulins in the blood serum was detected, and the high-level coordination with the development of inflammatory complications was revealed.

Determination of cryoglobulins, i.e., serum immunoglobulins, which are reverse precipitated at temperatures below 37°C. The method of A.E. Kalovidoris with modifications isolation of cryoglobulins from blood serum. The concentration of cryoglobulins was evaluated spectrophotometrically on a spectrophotometer SF-46 in the dynamics on the 3rd, 5th, 7th days after surgery. The plasma glucose level in a blood sample is taken after an 8-hour overnight fasting.

Evaluation of the cell composition (immunophenotyping) of human blood lymphocytes, the main component in the assessment of immune status, is performed by flow cytofluorimetry. Immunophenotyping is a characterization of cells using monoclonal antibodies or any other probes that allow judging their type and functional state by the presence of a set of cellular markers. Markers of immunocompetent cells are part of the tumor microenvironment: CD3 Marker of TAK lymphocytes DAKO, polyclonal CD8 Marker of T-cytotoxic lymphocytes DAKO, clone C8/144B CD138 Marker of plasma cells (syndecan-1, clone MI15), CD68 Marker of macrophages (mainly M1-type CD16, D3 macrophages) M2-type macrophages Cell Marque, clone MRQ-26.

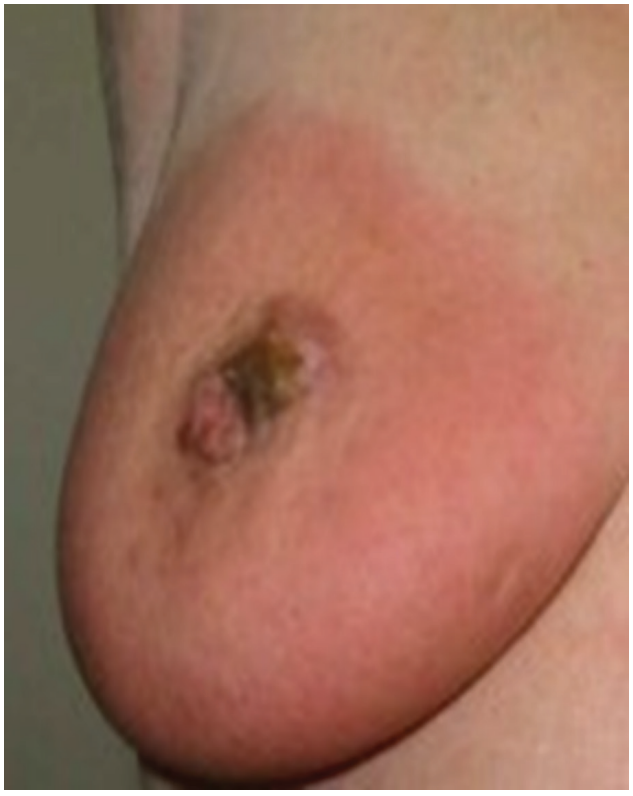

Figure 1: Primary inflammatory breast cancer.

Table 1: The structure of postoperative complications with type 2 diabetes mellitus.

| Complications | Absolute number | % |
|---|---|---|
| **Postoperative laceration** | 2 | 10 |
| **Inflammatory infiltration** | 4 | 20 |
| **Seroma** | 2 | 10 |
| **Partial necrosis of the edges of the wound** | 2 | 10 |
| **All** | 10 | 50 |

Note: Univariate Analysis, Complication Severity (HR=0.47, 95% CI: 0.22–0.53, p=0.05).

## Results and discussion

At the start of the postoperative period, it was noted that the number of complications in the group with primary hypercryoglobulinemia, the percentage of complications tends to be 100 (Table 1 and Figure 2).

In most patients, the content of cryoglobulins was average (298.6±2.5 mg/l; 1.3±0.08%) – 11 (55%), which corresponds to type II cryoglobulinemia and plasma

Figure 2: Inflammatory infiltration with seroma of the postoperative wound with type 2 diabetes mellitus.

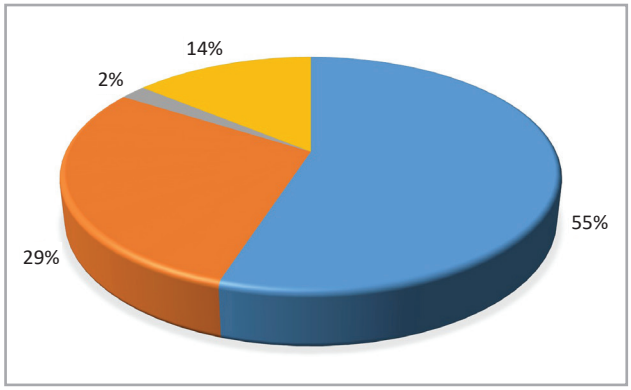


Figure 3: Patients with inflammatory breast cancer with different content of cryoglobulins (n=20).

glucose level 8.0–10.0 mmol/l; with suspected cryoglobulinemia (79.4±1.01 mg/l) was 6 (29%), plasma glucose level 4.4–6.6 mmol/l; high content (477.3±48 mg/l; 3.4±0.2%) was registered in (14%), which indicated type III cryoglobulinemia and plasma glucose level above 10.0 mmol/l [Hazard Ratio (HR)=0.71, 95%, Confidence Interval (CI): 0.22–0.83, p=0.005] (Figure 3).

The study found that all patients had a significant increase in cryoglobulin levels by an average of 106.5% and hyperglycemia on average 20% higher than the initial blood glucose indicator on the first day after the initial detection of the disease and corresponds Napodano C. and colleagues [12] – the immune system is constantly confronted with endogenous and external antigens; nonetheless, even when impaired, it will strive to retain tolerance to self. In this scenario, cryoglobulins creation might indicate an abnormal adaptive immune system response to varying degrees of dysfunction.

Dynamic monitoring of cryoglobulinemia throughout the postoperative period in patients with inflammatory breast cancer revealed the inadequacy of changes in their concentration in the serum depending on the time of observation. In both groups of patients, there was an increase in the concentration of cryoglobulins on the 3rd day after surgery. On the 5th day, there was a decrease in the concentration of cryoproteins in the blood of patients from 158.3±28.6 mg/l

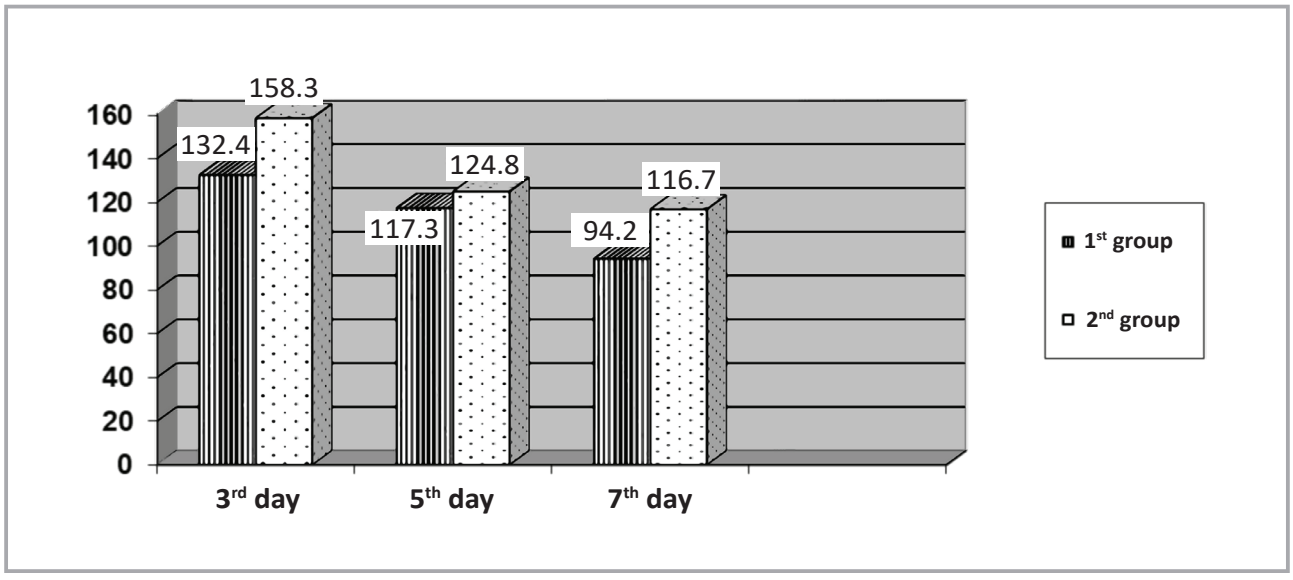


Figure 4: Dynamics of immunoglobulin content in cryoprecipitates of patients with inflammatory breast cancer.

Table 2: The content of lymphocyte subpopulations in patients with inflammatory breast cancer in the early postoperative period.

| Indicators | Normal range | First group (n=10) | Second group (n=10) |
|---|---|---|---|
| | | 3 days after operative intrusion | 3 days after operative intrusion |
| **CD 3+** | 66.2+0.5 | 47.12+0.91 * | 50.93+0.83 * |
| **CD 4+** | 43.9+0.8 | 29.12+0.61 * | 30.77+0.6 * |
| **CD 8+** | 27.0+0.9 | 15.21+0.35 * | 16.2+0.3 * |

Note: * – p<0.05 relative to normal range.


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to 124.8±10.8 mg/l in the second group of patients with gastric cancer from 132.4±82.2 mg/l to 117.3±63.9 mg/l in group I. (HR=0.54, 95% CI: 0.22–0.73, p<0.05) (Figure 4), which practically correlates with Hiam-Galvez study [13] – the findings revealed that efficacy, particularly in terms of sustained immune response, was comparable in patients with mild illness to that found in individuals with moderate/severe disease. Initial parameters of immune status in patients of the main and control groups before surgery and on the first day after surgery revealed (p>0.05) significant differences.

There was a substantial increase in the relative number of CD3 + cells in the peripheral blood of patients with hypercryoglobulinemia who were operated on for inflammatory breast cancer (66.2 0.5%, p=0.05). The results of the study of subpopulations of lymphocytes are presented in Table 2, and supports by [14] – these immunological features were associated with clinical outcomes including pathological response, a positive correlation between plasma CD3 + cells and classical monocytes in HER2+ BC patients under NAC, a trend between increased CD3 + cells and classical monocytes levels and lower rates of pathologic complete response at the end of NAC.

The level of CD 4+ and CD 8+ was also significantly reduced compared to the normal range, HR=0.44, 95% CI: 0.28–0.52, p<0.05 [15].

This confirms that against the background of hyperglobulinemia, the level of inflammatory reaction is much higher than in its absence. Any remaining chronic inflammation leads to cancer or, in our case, the development of recurrences and metastasis of the disease. Also, there was a significant increase in the plasma glucose level above 10.0 mmol/l, which may be associated with dysfunction of the antitumor immune threshold between healthy tissue and tumor.

## Conclusions

Thus, studies have shown that patients with cryoglobulinemia and type 2 diabetes mellitus operated for inflammatory breast cancer of tumor origin formed secondary immune deficiency. This is determined by abnormalities in the cellular and humoral immune system and as a consequence of developing postoperative inflammatory complications in patients with diabetes mellitus. Any remaining chronic inflammation leads to cancer or, in the case of cancer, a disease like diabetes mellitus developing recurrences and metastases of the disease.

## Conflict of interest

The authors declare no conflict of interest.

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