Research Institute of Clinical and Experimental Lymphology –
Branch of the Institute of Cytology and Genetics
Siberian Branch of Russian Academy of Sciences

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CYTOKINES - MARKERS OF ONCOGENESIS AND THERAPY EFFICIENCY IN CHEMICALLY INDUCED BREAST CANCER.

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The relevance of research

Breast cancer has a leading position in the incidence rate of cancer among women. Cytokines are regulators of intercellular interactions at the autocrine and paracrine levels, which are produced not only by cells of the immune system, but also by tumor cells. On the one hand, cytokines produced by tumor cells contribute to the evasion of tumor cells from immune surveillance, the progression and metastasis of the tumor, on the other hand, cytokines are mediators of antitumor immunity and biomarkers. A biomarker is defined as a “substance or activity that can be objectively determined and evaluated as an indicator for a normal or pathogenic process, or to monitor pharmacological response to therapeutic intervention” . Cancer biomarkers can be present in blood or tumor tissues and include a wide variety of materials such as enzymes, cytokine, DNA, mRNA, and transcription factors, which are produced either by the tumor itself or by the host in the response to the tumor.

Research purpose

To study the levels of cytokines in blood serum and lymph of the thoracic duct in normal and chemically induced breast cancer in Wistar female rats and during surgical treatment, polychemotherapy and Panagen drug therapy.

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Research objectives

1. To study the levels of lymphatic and blood serum cytokines of Wistar female rats with chemically induced breast cancer (BC);
2. To conduct a comparative study of the levels of lymph and blood serum cytokines of Wistar female rats after surgical removal of chemically induced breast cancer;
3. Compare the levels of lymph and blood serum cytokines of Wistar female rats with chemically induced breast cancer after polychemotherapy;
4. Compare the levels of lymph and blood serum cytokines of Wistar female rats with chemically induced breast cancer after surgery and chemotherapy in combination with Panagen treatment.

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Materials and Methods

- In the experiment, we used **female Wistar rats** of 12 weeks old weighing 250-300 g in 6 groups:
- 1st group - intact rats;
- 2nd group - rats with **breast cancer** (**BC** tumor carriers);
- 3rd group - rats with **breast cancer** after **polychemotherapy** (PCT);
- 4th group - rats after **resection** of breast cancer ,
- 5th group - rats after **resection of breast cancer and polychemotherapy** (PCT),
- 6th group - rats after **resection** of breast cancer in combination with polychemotherapy (PCT) and injection **Panagen** (double-stranded human DNA) .
- The tumor was induced by injection of N-methyl-N-nitrosourea (MHM) into the second mammary gland of Wistar female rats five times (1 injection per week) (Sigma-Aldrich, USA), resulting in the formation of breast cancer after 6 months (morphological type - adenocarcinoma) (Dzhioev, 1984; Volkova, 2014).
- **Surgical treatment was performed** 6 months after the induction of breast cancer. For chemotherapy, the CMF regimen was used (**cyclophosphamide** - at a dose of 3 mg / kg from 1 to 14 days; **Methotrexate** - at a dose of 2.5 mg / kg on days 1 and 8; 5-**Ftorouracil** - at a dose of 15 mg / kg in 1 and 8 days).

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Materials and Methods

The experiment used the substance of the **Panagen** drug (5 mg / kg) based on a **fragmented dsDNA** of 1.7 mg / ml (LSR No. 004429/08 dated 06/09/08) isolated **from human placenta**, which was administered intraperitoneally once within 14 days after 3 hours after administration of cyclophosphamide.

The concentration of cytokines in blood serum and lymph of experimental animals was evaluated by **flow fluorimetry** on a **2-beam laser automated analyzer (Bio-Plex Assay System** (Bio-Rad, USA)) in accordance with the manufacturer's instructions Bio-Plex Pro Rat Cytokine 24-Plex Assay (Bio-Rad, USA).

The results were statistically processed using the Statistica 7.0 software (StatSoft, USA). The significance of differences between the groups was calculated using the nonparametric Mann-Whitney U-test. Differences between groups were considered significant at $p < 0.05$.

Experiment design

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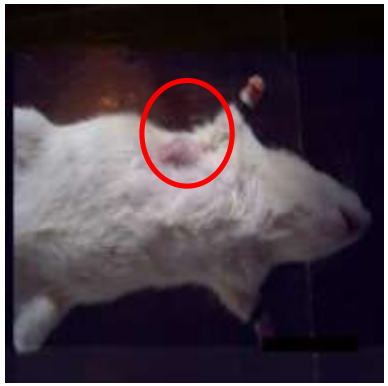
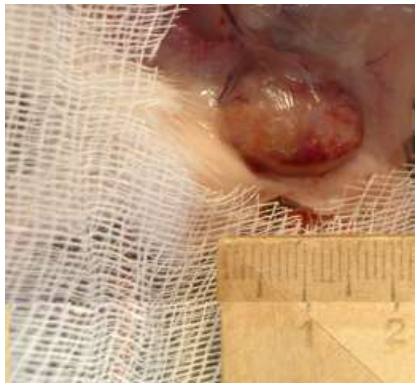
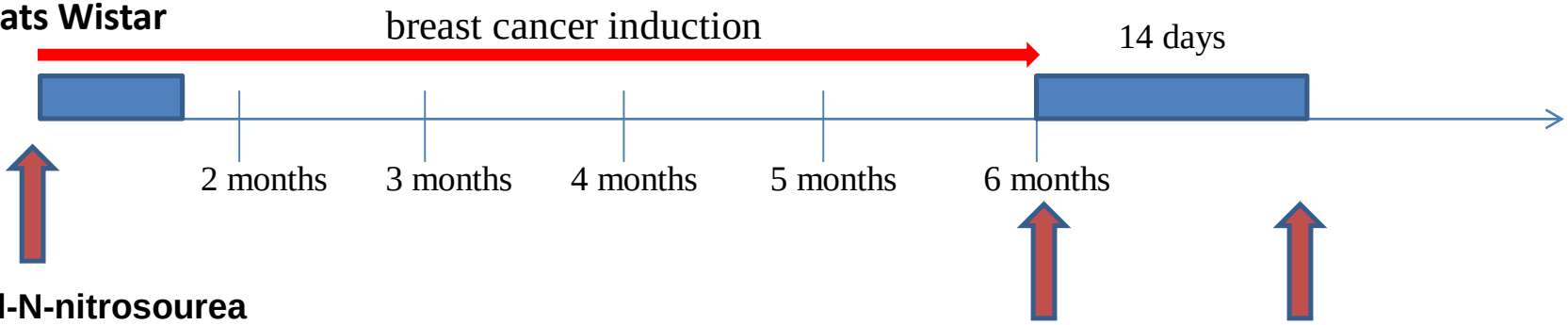




After 6 months of experimental carcinogenesis in its own way breast cancer is a moderately differentiated infiltrative ductal carcinoma

3 month female rats Wistar



N-methyl-N-nitrosourea

Groups of animals:
- **intact animals**
- **BC tumor rats**
- **rats with BC after PCT,**
- **rats after resection BC**
- **rats after resection BC + PCT**
- **rats after resection BC + PCT + "Panagen"**

Treatment
- **poly-chemotherapy (PCT)"CMF":**
  - **Cyclophosphamide (3 mg / kg)**
  - **Methotrexate (2.5 mg / kg)**
  - **Fluorouracil (15 mg / kg)**
- **surgical resection**
- **human dsDNA ("Panagen")**

Material samples:
- **blood serum**
- **lymph**
- **breast tissue**

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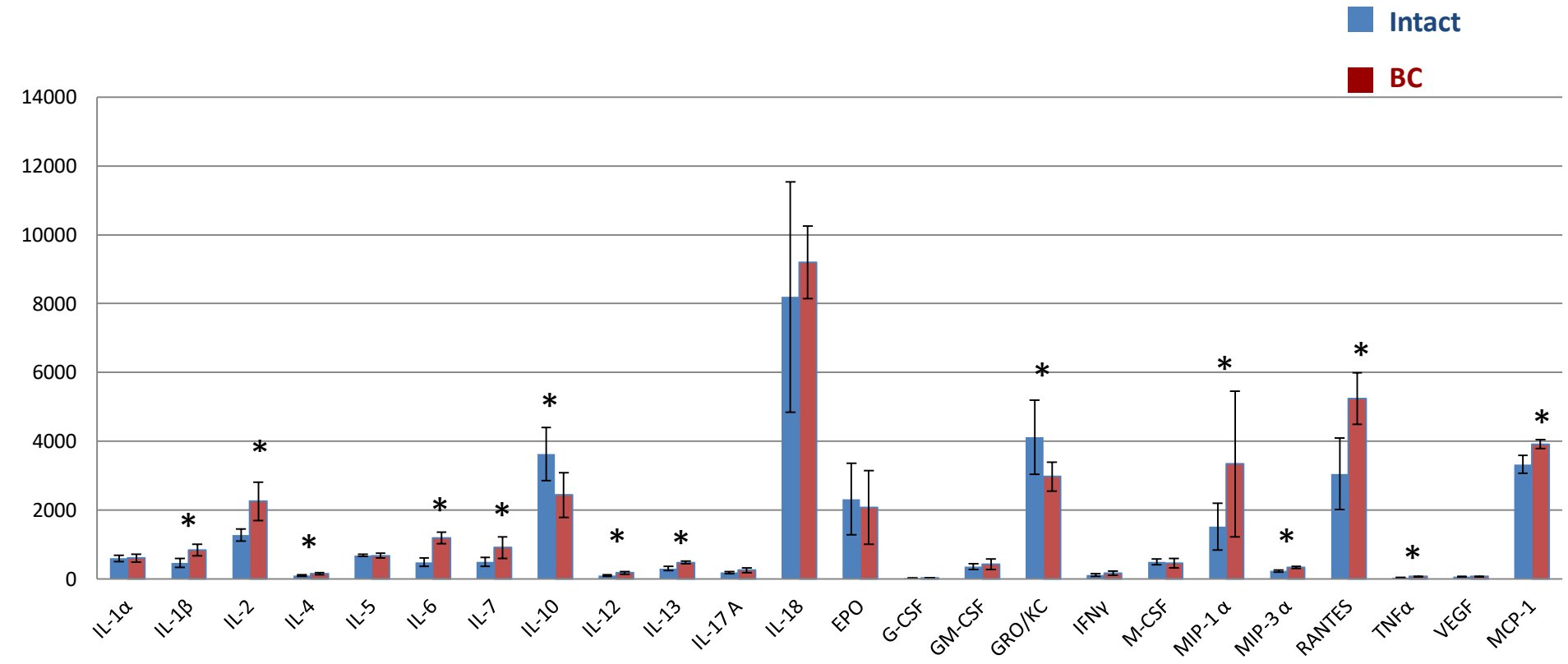
The concentration of cytokines in the blood serum of rat tumor carriers with breast cancer(BC) compared with intact animals (PG / ml)



A significant increase in the levels of cytokines IL-1β, IL-2, IL-4, IL-6, IL-7, IL-12, IL-13, MIP-1α, MIP-3α, RANTES, TNF-α, MCP-1 and decreased levels of GRO / KC and IL-10 in the BC group.

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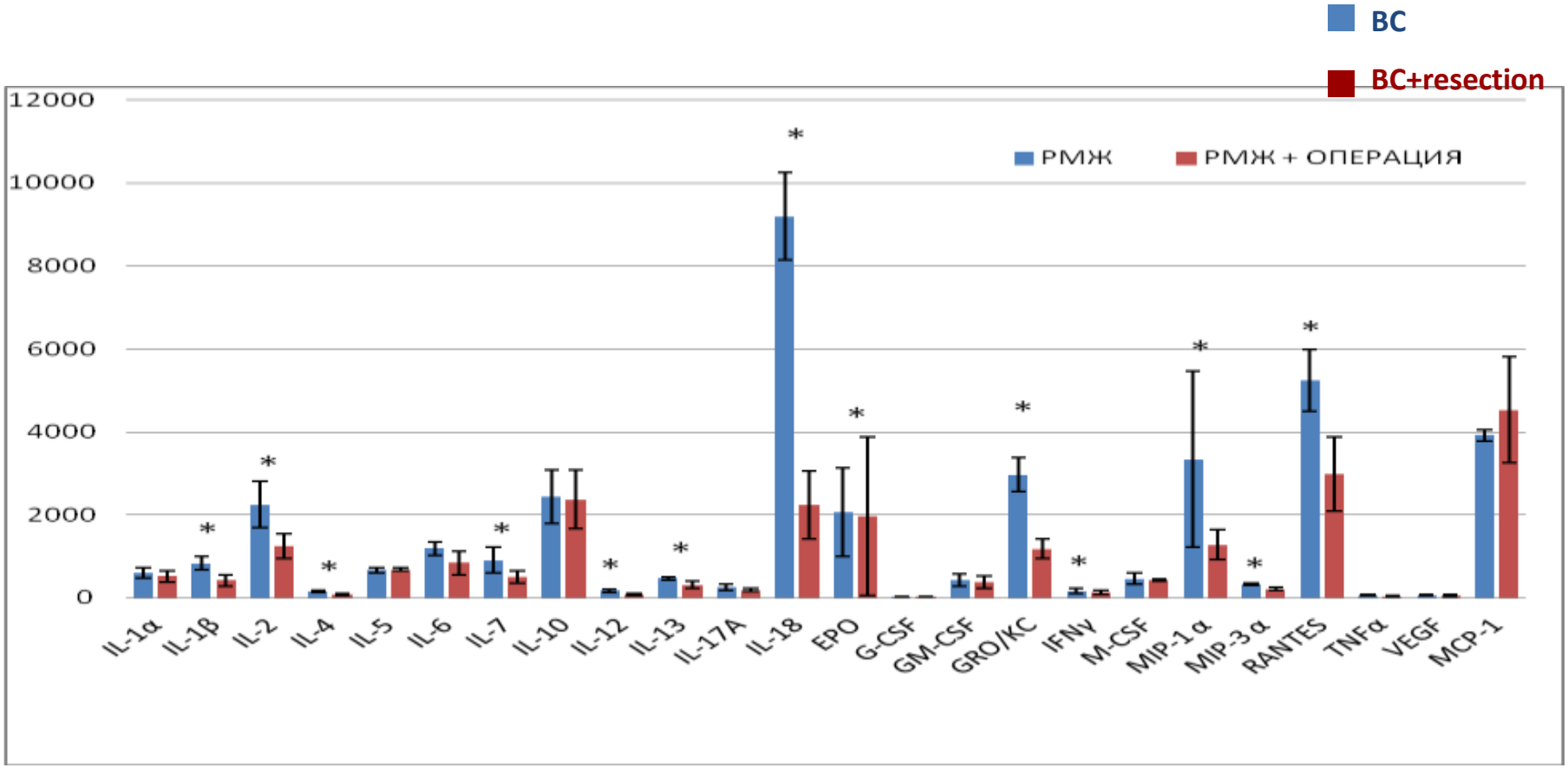
The concentration of cytokines in the blood serum of rat-tumor carriers with breast cancer compared with the animals after resection of BC (pg / ml)



Total resection of the tumor leads to a significant decrease in the level of pro-inflammatory cytokines IL-1β, IL-2, IL-12, IL-18, TNF-α, MIP-1α, MIP-3α, RANTES, GRO / KC, anti-inflammatory cytokines IL -4, IL-13, IL-7 and EPO.

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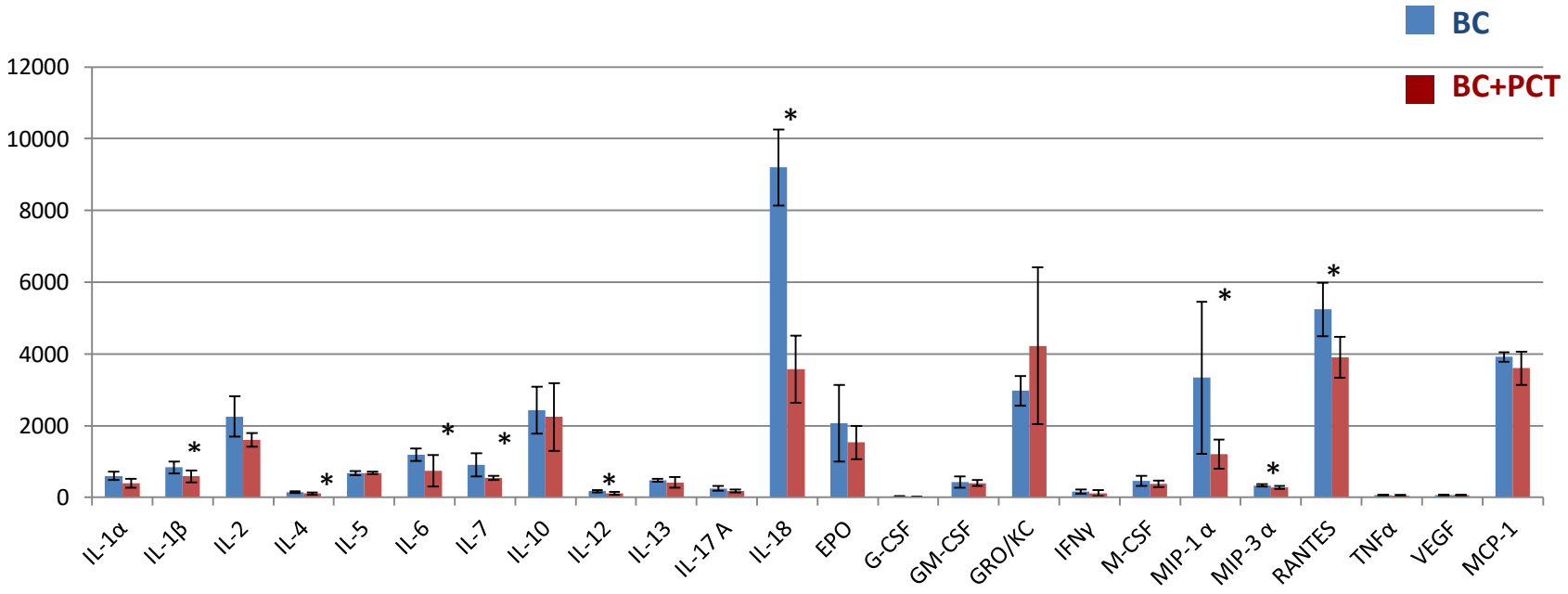
The concentration of cytokines in the blood serum of rats Wistar with BC compared with animals - with BC after polychemotherapy (pg / ml)



A significant decrease in the concentration of IL-1β, IL-4, IL-6, IL-7, IL-12, IL-18, MIP-1α, MIP-3α, RANTES was found in the group of rats with BC who underwent polychemotherapy compared with a group of rats BC without treatment.

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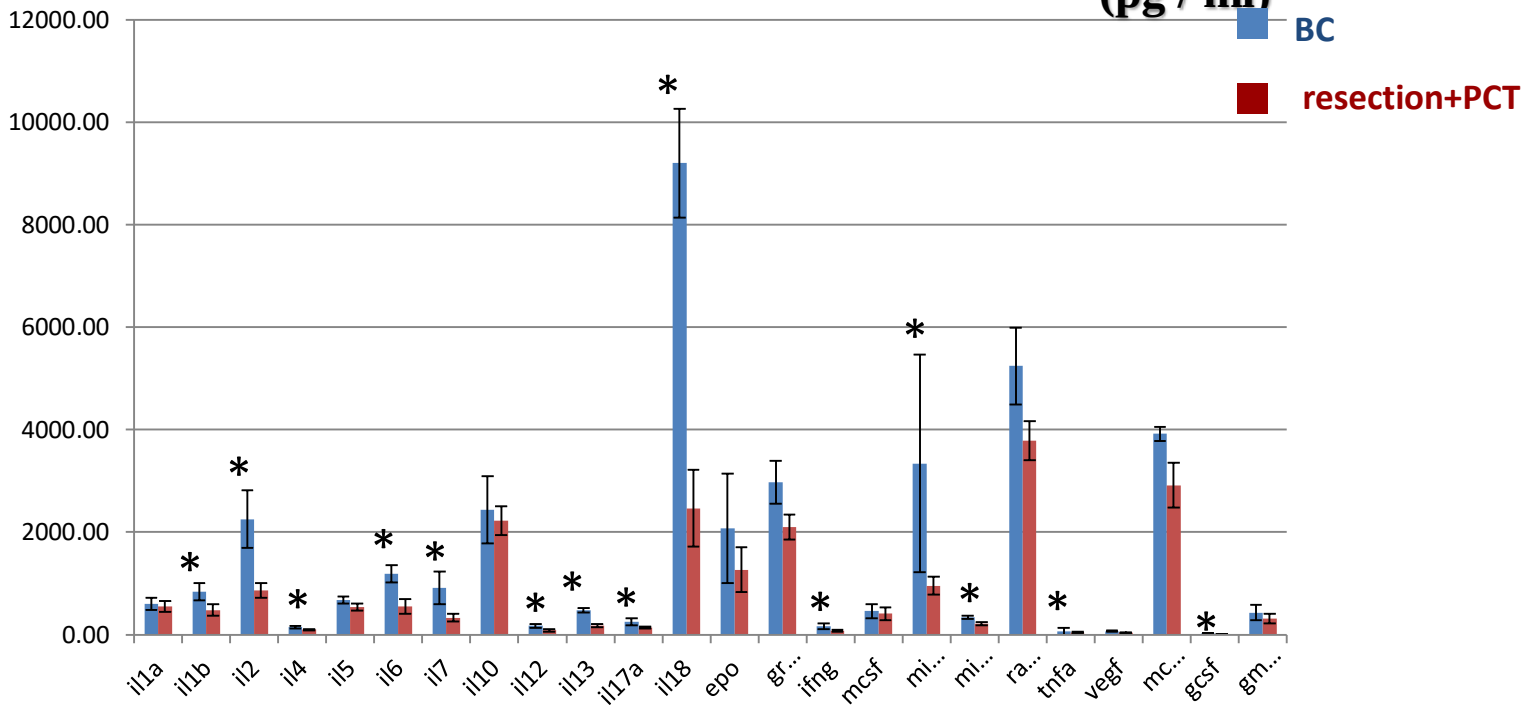
The concentration of cytokines in the blood serum of rat tumor carriers with breast cancer compared with animals after resection of breast cancer in combination with polychemotherapy (pg / ml)



Polychemotherapy after total resection of BC leads to a significant decrease in the concentration of IL-1β, IL-2, IL-4, IL-6, IL-7, IL-12, IL-13, IL-17A, IL-18, IFNγ, MIP -1α, MIP-3α, MCP-1 in rat blood serum compared with the group rats with breast cancer.

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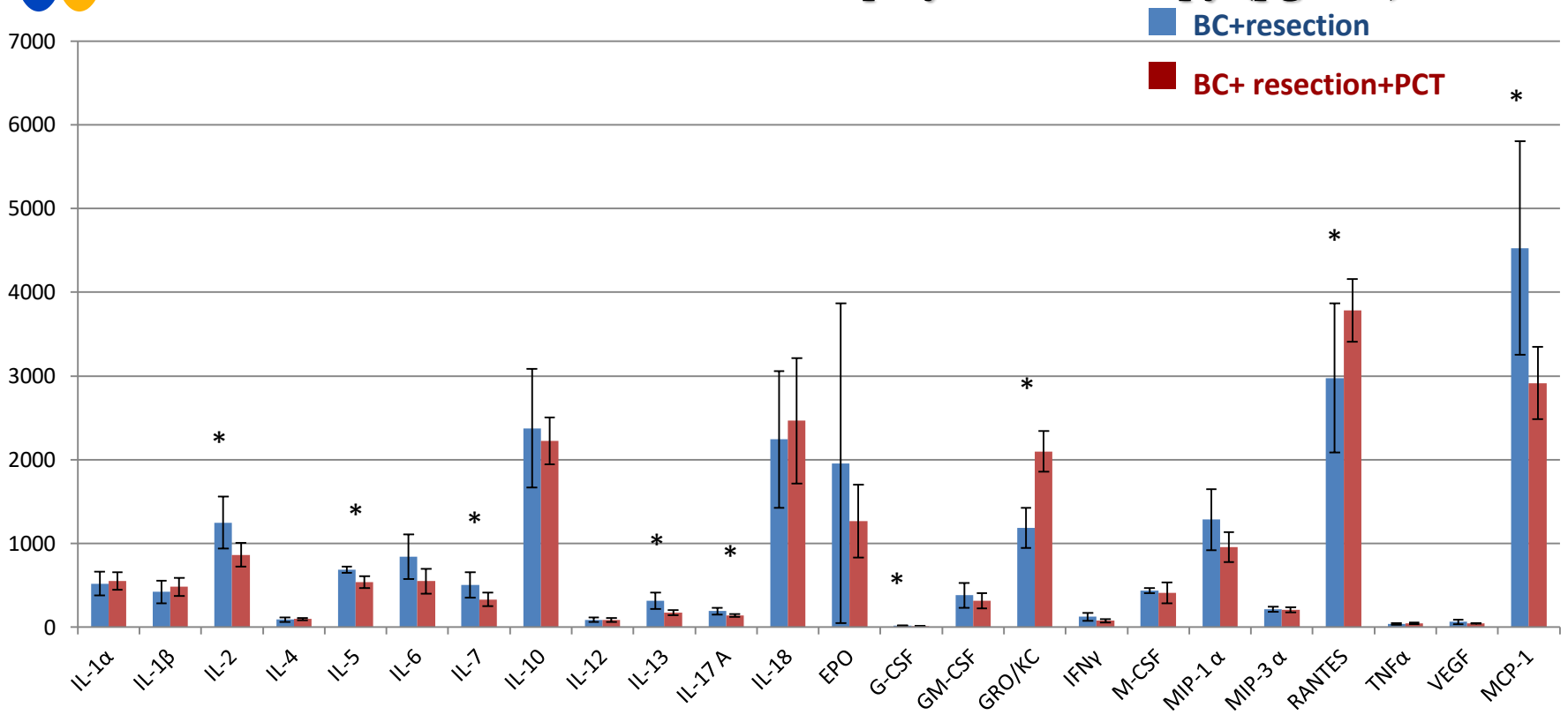
The concentration of cytokines in the blood serum of Wistar rats after BC resection compared with animals after BC resection and polychemotherapy (pg / ml)



In the rat group, after resection BC and polychemotherapy, the concentration of cytokines IL-2, IL-5, IL-7, IL-13, IL-17A, G-CSF, MCP-1 in the blood serum was significantly lower in comparison with the group of operated animals without chemotherapy, but at the same time, the level of production of GRO / KC and RANTES was significantly higher in the rat group after surgery in combination with polychemotherapy.

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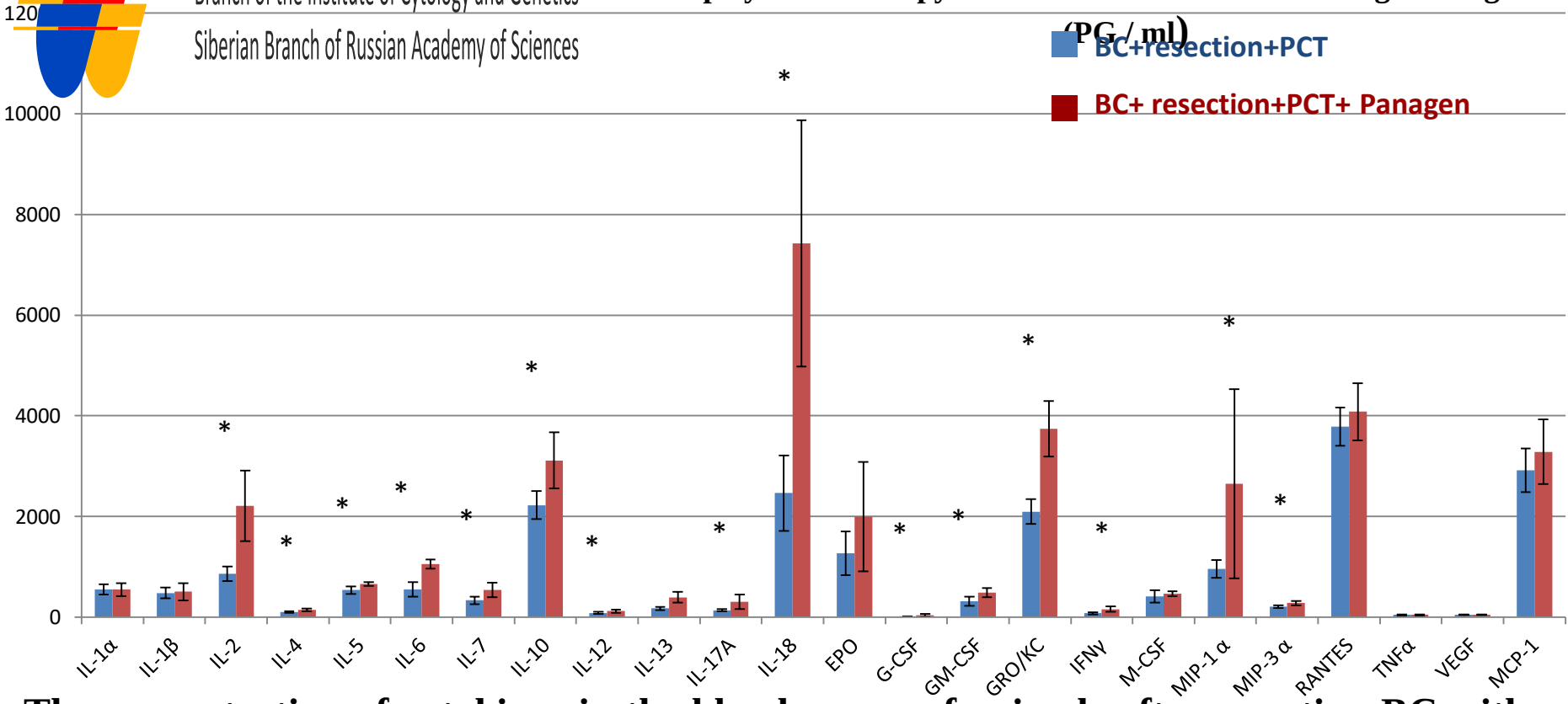
The concentration of cytokines in the blood serum of Wistar rats after resection BC in combination with polychemotherapy compared with animals after resection BC, polychemotherapy and the introduction of the drug "Panagen"



The concentration of cytokines in the blood serum of animals after resection BC with polychemotherapy and injection of the Panagen drug significantly increased compared to the group of rats after resection with BC and polychemotherapy: IL-2, IL-4, IL-5, IL6, IL-7, IL -10, IL-12, IL-17A, IL-18, G-CSF, GM-CSF, GRO / KC, IFN-γ, MIP-1α, MIP-3α.

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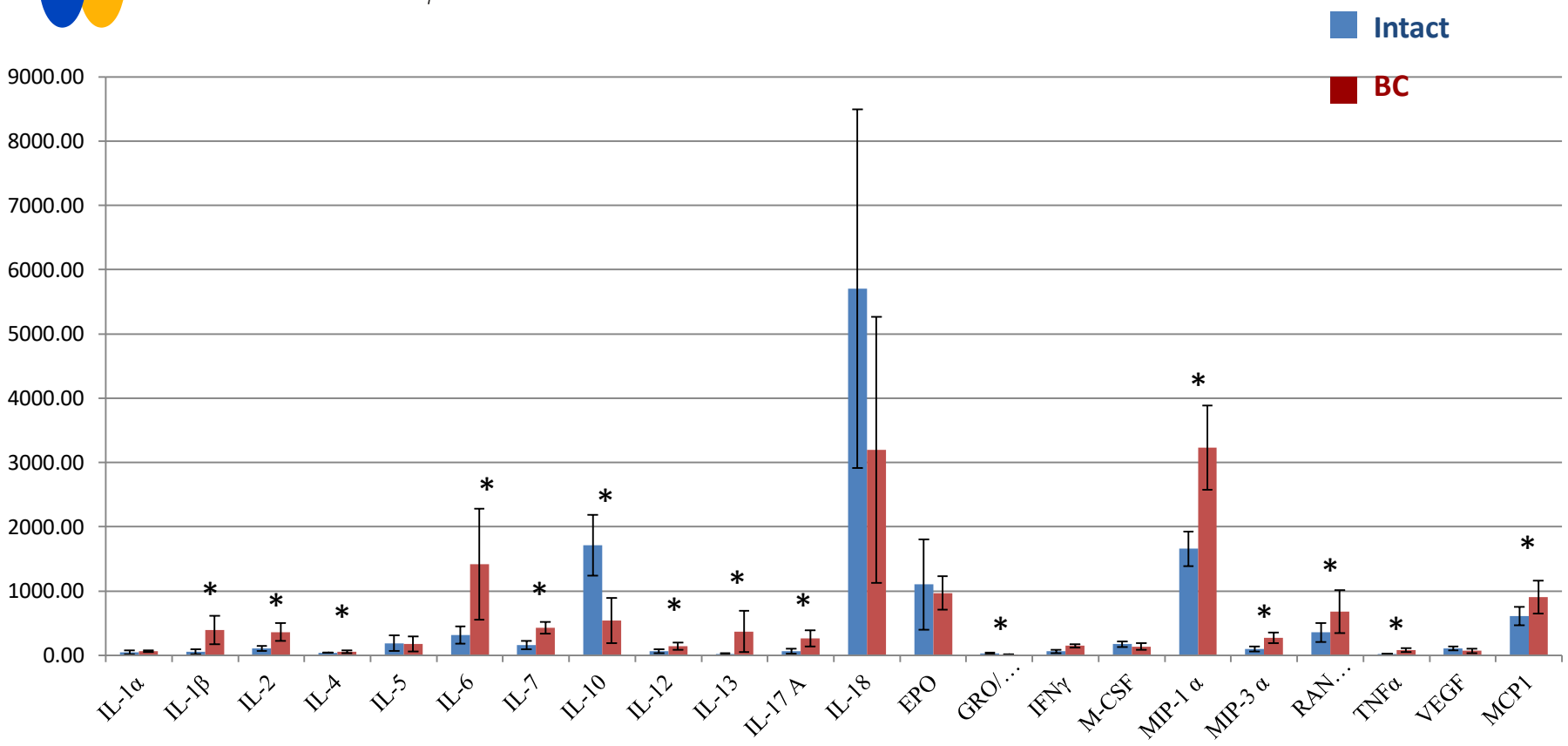
The concentration of cytokines in lymph of rat tumor carriers with breast cancer(BC) compared with intact animals (PG / ml)



Compared with intact animals, the levels of cytokines IL-1β, IL-2, IL-4, IL-6, IL-7, IL-12, IL-13, IL-17A, MIP-1α are significantly higher in the lymph of BC tumor-bearing rats , MIP-3α, RANTES, TNF-α, MCP-1 and significantly lower levels of GRO / KC and IL-10.

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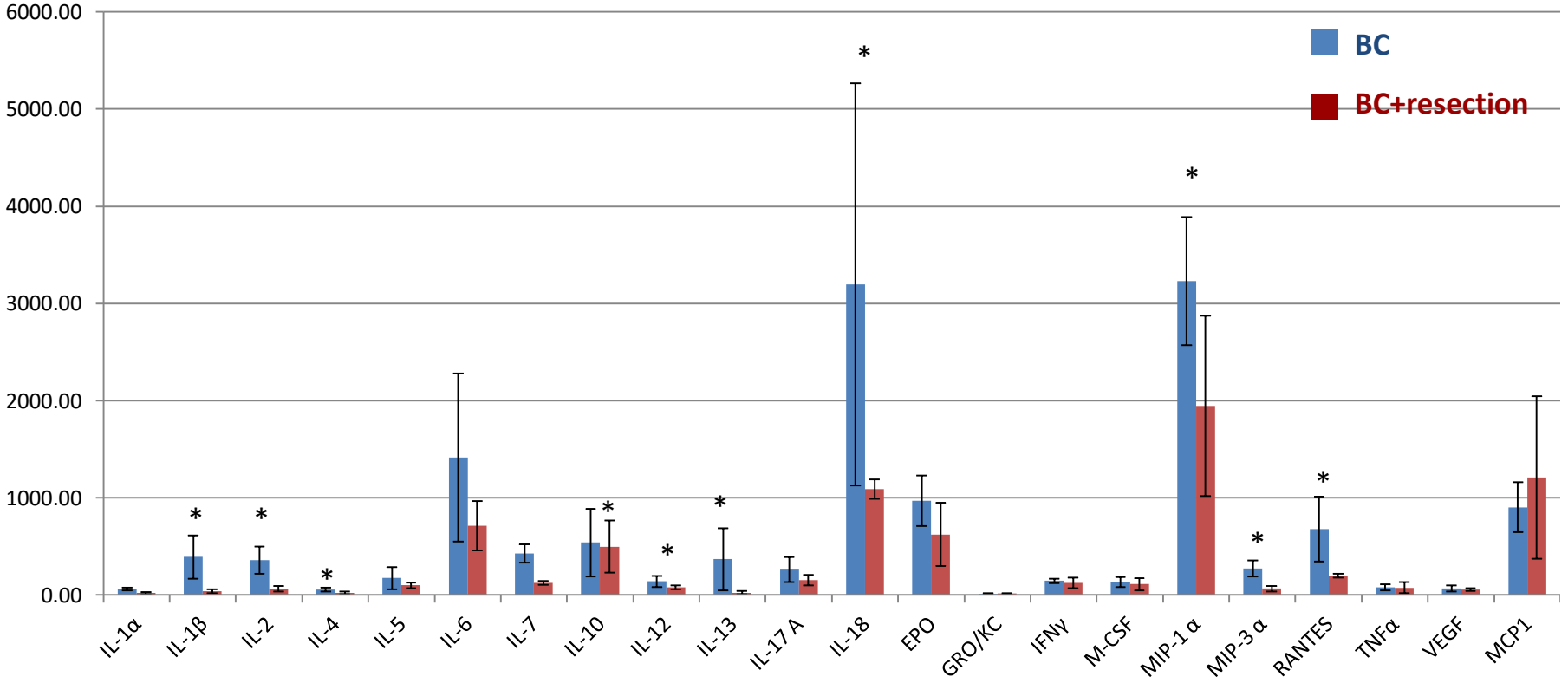
The concentration of cytokines in lymph of rat with breast cancer compared with the animals after resection of BC (pg / ml)



Resection BC leads to a significant decrease in the concentration of cytokines in lymph such as: IL-1β, IL-2, IL-4, IL-7, IL-12, IL-13, IL-18, MIP-1α, MIP-3α, RANTES compared to animals with breast cancer.

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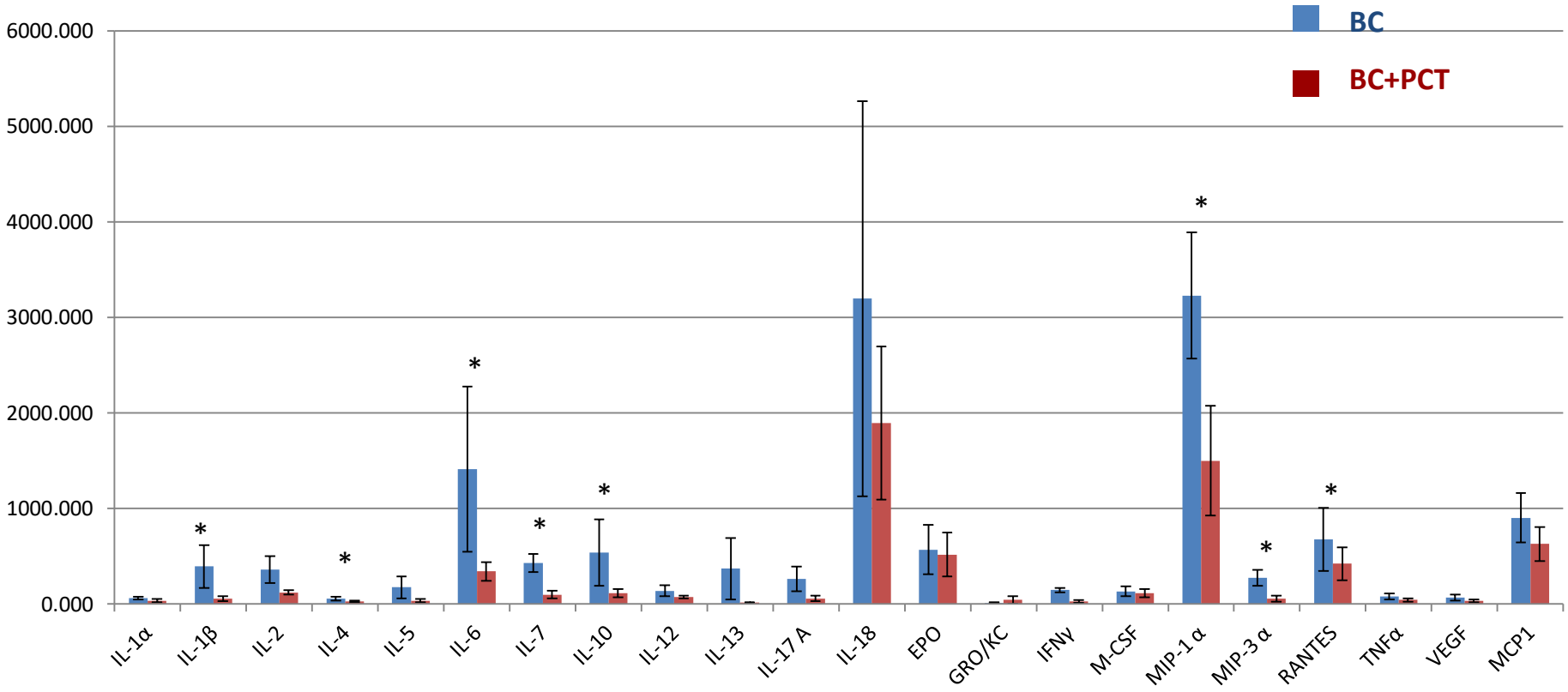
Concentrations of cytokines in the lymph of Wistar female rats with breast cancer compared with BC tumor-bearing animals after polychemotherapy



Polychemotherapy led to a significant decrease in the concentration of IL-1β, IL-4, IL-6, IL-7, IL-10, MIP-1α, MIP-3α, RANTES in the lymph of female rats with breast cancer compared with the group of animals - BC tumor carriers without polychemotherapy.

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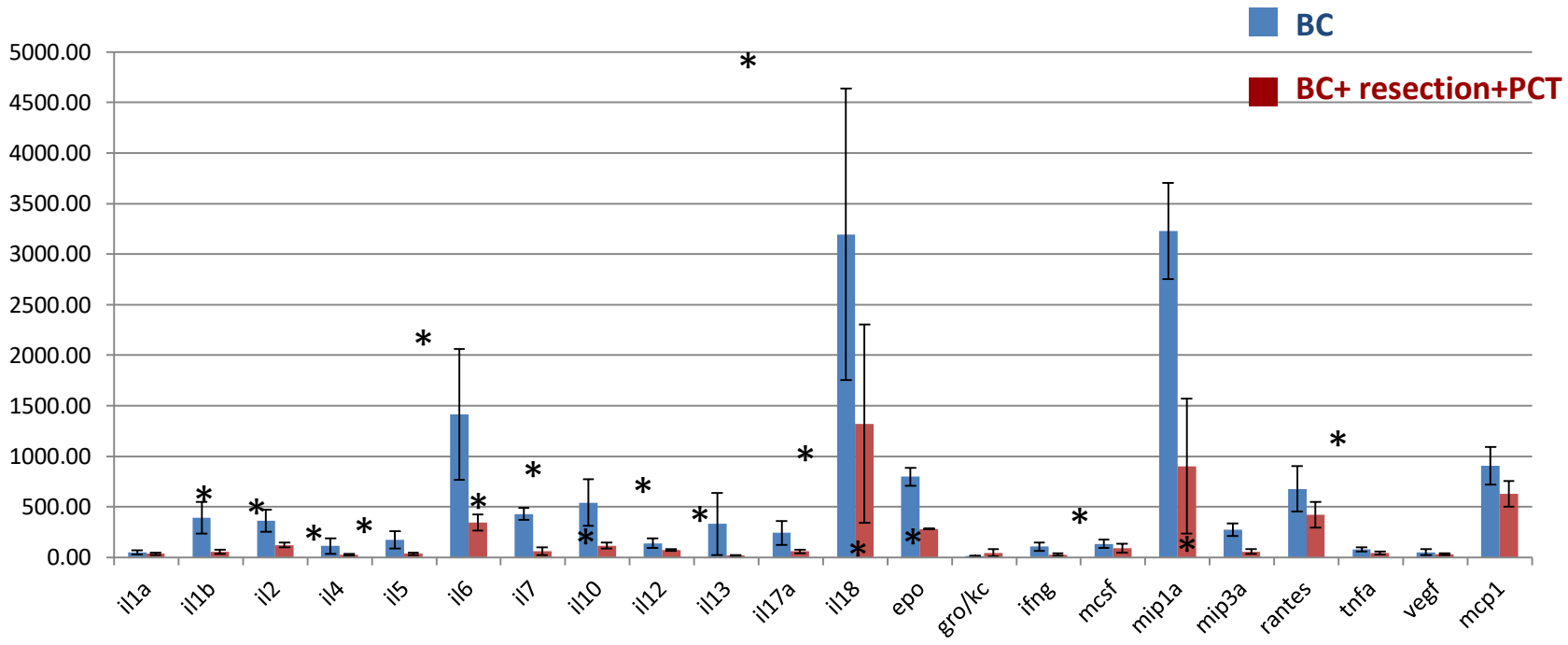
The concentration of cytokines in limph of rat tumor carriers with breast cancer compared with animals after resection of breast cancer in combination with polychemotherapy (pg / ml)



Polychemotherapy after breast cancer resection causes a decrease in the concentration of such cytokines as: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL -17A, EPO, GRO / KC, IFNγ, MIP-1α, RANTES, MCP-1 in rat lymph, compared with animals with breast cancer

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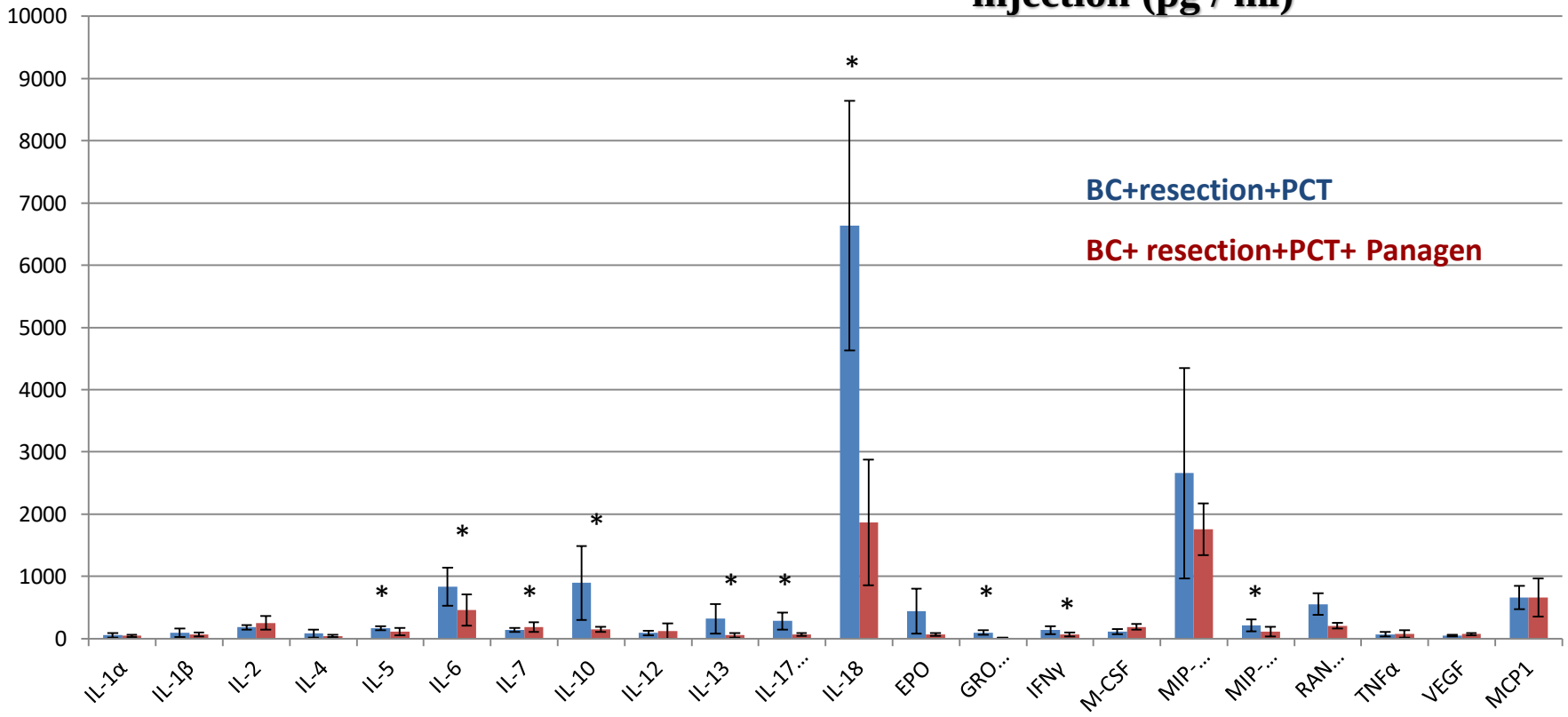
Concentrations of cytokines in the lymph of Wistar female rats after breast cancer resection and polychemotherapy as compared with animals after resection BC, polychemotherapy and Panagen drug injection (pg / ml)



The concentration of cytokines in limph of animals after resection BC with polychemotherapy and injection of the Panagen drug significantly increased compared to the group of rats after resection with BC and polychemotherapy: IL-5, IL-6, IL-7, IL-10, IL-13, IL-17A, IL-18, GRO/KC, IFNγ, MIP-3α

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CONCLUSIONS

1. **Concentrations of cytokines such as: IL-1β, IL-2, IL-4, IL-6, IL-7, IL-12, IL-13, IL-17A, IFN-γ, MIP-1α, MIP-3α, RANTES , TNF-α, MCP-1 in the blood serum and lymph of Wistar female rats with experimental breast cancer is significantly higher than in intact animals, which allows these cytokines to be considered biomarkers of breast cancer.**
2. **Breast cancer resection causes a decrease in the concentration of proinflammatory cytokines such as IL-1β, IL-2, IL-12, IL-18, TNF-α, chemokines MIP-1α, MIP-3α, RANTES, as well as anti-inflammatory cytokines IL-4 , IL-13 in blood serum and lymph, which confirms the opinion that these cytokines are produced by breast cancer cells.**
3. **The decrease in the concentration of IL-1β, IL-4, IL-6, IL-7, IL-12, MIP-1α, MIP-3α, RANTES in the blood serum and lymph of rats with breast cancer after polychemotherapy reflects the antiproliferative effect not only on lymphoid cells, but also on tumor cells;**
4. **A comparative study of the concentration of cytokines in the lymph and blood serum of animals after resection of breast cancer, polychemotherapy and injection of the Panagen drug showed that most cytokines such as: IL-5, IL-6, IL-7, IL-10, IL-13, IL-17A, IL-18, GRO / KC, IFNγ, MIP-3α in blood serum and lymph are higher after administration of the Panagen. This suggests that "Panagen" not only determines the antitumor effect, but also stimulates the lymphoid cells of the immune system;**
5. **Cytokines of lymph and blood serum in experimental breast cancer are biomarkers of tumor growth, and their concentration depends on the type of treatment.**