Bactericidal capacity of platelets in gastric cancer patients

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The aim of this study was to evaluate bactericidal capacity of platelets in patients suffering from gastric cancer. Number of platelets and their bactericidal activity were measured in 32 cancer patients (divided into 2 groups: I – resectable cancer, II – non-resectable one) and 32 normal donors. In group I the number of platelets was 259.136 ± 84.459 x $10^3/\mu$l. It was increased comparing to the normal donors 193.219 ± 55.493 x $10^3/\mu$l. After the surgery increase in platelet number was observed (472.05 ± 111.772 x $10^3/\mu$l). In group II an increased number of platelets was observed (265.1 ± 81.813 x $10^3/\mu$l) and it was maintained in a post-operative period: 234.2 ± 54.141 x $10^3/\mu$l. In group I bactericidal capacity of platelets was $2.25 \pm 7.33\%$, whereas it increased significantly after the surgery – $4.7 \pm 7.46\%$. In group II, it was $8.6 \pm 17.61\%$ before and $4.72 \pm 4.76\%$ after the surgery. In normal donors this ability was 21.66 ± 16.66. In gastric cancer patients increased platelet number was observed. Significant increase in platelets number occured after a radical tumor removal. Decreased bactericidal activity of platelets was noticed in gastric cancer patients. After surgical removal of the tumor, platelets partly reclaimed bactericidal capacity. In patients presenting disseminated gastric cancer, bactericidal capacity of platelets could be permanently impaired.

Key words: gastric cancer, blood platelets, bactericidal capacity of platelets

One of the less known platelets function is their ability to phagocyte and kill bacteria. Megakaryopoesis is inhibited after acute infection with viruses or bacteria. In contrast, chronic inflammation is often associated with reactive thrombocytosis [10]. The ability of phagocytosis has a single platelet as well as its agregates [3, 9]. Activated platelet changes its shape into irregular, containing plenty of pseudopods. Moreover it intensifies its intracellular energetic proceses and increases protein production [1]. An organell centralization connected with vacuolization and releasing reaction is brought. Platelet granules contain: peroxidase, acid phosphatase, cationic proteins and proteolytic enzymes. The substances above represent high activity for killing bacteria [15, 17, 18]. What is more, platelets exert cytotoxic effects on tumor cells [7], what was assessed in oncological patients [2]. The level of platelet lytic activity appears to be dependent on the stage of tumor. It increases twice in the early illness stage, whereas in the terminal period it decreases twice. Platelets adhere to cancer cells and join to the antigenic determinants. Then characteristic structural changes, such as Golgi apparatus hypertrophy, increase in secretory granulations and discplacing them towards contact zone with a neoplastic cell are observed. Platelet cytotoxity ensues from their ability to product and release lytic mediators. They can penetrate to the aimed cell by diffusion through the cell membrane or by endocytose [2]. Additionally, platelets play an important role in tumor metastasis. Platelet-derivered proteolytic enzymes facilitate the release and migration of tumor cells across the vessel wall. Superficial sialic acid of tumor cells promotes formation of platelet-tumor cell aggregates, what facilitates their survival in vascular system, in spite of natural immunity [12]. Furthermore, activated platelets release substancies which increase vascular permeability, factors stimulating myocytes proliferation, platelet activating factor, prostaglandins, histamine and serotonine. The substances above facilitate an implantation and growth of metastatic tumor [5].

Material and methods

Bactericidal capacity of platelets was assessed in 32 patients suffering from gastric cancer and in 32 normal donors. Due to the stage of tumor growth, patients were divided into

two groups. The first one included patients after a radical surgical tumor removal, whereas the second one ranged patients with non-resectable gastric cancer. The third group was comprised of normal donors. The first group consisted of 22 patients, 15 women and 7 men aged between 50 and 79 (mean 67.1 years). 2 women were diagnosed with early cancer T1 NOMO of a malignancy level G2. All the others presented advanced cancer. Among them the tumor size according to TNM staging system was: pT2 revealed in one, pT3 in 14 and pT4 in 5 patients. From the macroscopic point of view, there was 1 patient classified as Borrmann type II, 13 as Borrmann type III and 6 as Borrmann type IV. Malignancy of G2 in 7 and G3 in 13 patients was observed. In 14 patients gastrectomy with oesophagoenterostomy of double track type was performed. In other patients, subtotal gastic resection was performed: by the Rydygier's method in 7 patients and by the Billroth II method in 1. At the same time lymphadenectomy was performed (between 2 and 52 lymphatic nodes were removed). In 9 patients the removed lymphatic nodes were not affected by metastasis, whereas in the group of 13 patients metastases in the nodes were found. Microscopic examination confirmed the presence of the metastases in 1 to 30 lymphatic nodes. 5 patients had just single metastasis (in up to 3 lymphatic nodes), whereas 8 patients had 6 and more nodes affected. According to the Lauren's histologic type, in 13 patients intestinal cancer and in 9 patients disseminated one was affirmed. According to the Ming's classification, 2 women suffered from an expansive gastric cancer, whereupon others had an infiltrating cancer. During the surgery, every patient had a polyuretan tube lead in through the nose to the small intestine under the entero-enterostomy. It enabled implementing of enteral nutrition 12 hours after the operation. Enteral nutritional supplements were delivered by the peristaltic mechanic pump.

The second group were patients with non-resectable gastric cancer of T4 N3 M1, 4 women and 6 men aged between 35 and 82 (mean 59.8 years). Only 3 of them had paliative operations performed, whereas the others underwent only exploratory laparotomy.

The number of platelets and bactericidal capacity of platelets and plasma were examined in each patient. The tests were repeated in 12th or 14th day after the surgery. The blood for analysis was sampled from the veins to heparine (concentration 50 IU/ml) in the ratio 9:1. ACD in the amount of 0.2 ml/ml of blood was added to the probes to lower plasma pH and decrease physiological platelets ability to coagulate. Additionally, ACD prevents platelets secretion. The number of platelets (in thousands/μl) was assessed with the use of hematological analyzator Technicon H-1 System by the GRONER and TYCKO method [4]. We used *Staphylococcus aureus* ATCC 6538 P in accordance to the Mantur's method as a substrate to estimate bactericidal capacity of platelets [11]. The results were analyzed statistically with the Mann-Whitney and Kolmogorov-Smirnov tests.

Results

In patients suffering from resectable gastric cancer, the average number of platelets was 259.136 ± 84.459 x $10^3/\mu$l (Tab. 1). It was significantly higher comparing to the normal donors 193.219 ± 55.493 x $10^3/\mu$l (Tab. 1 and 3). After the surgery increase in platelet number was observed, in the average of 472.05 ± 111.772 x $10^3/\mu$l (Tab. 1).

In patients with non-resectable gastric cancer, in comparison with the control group, an increased number of platelets was observed. An average one was 265.1 ± 81.813 x $10^3/\mu$l (Tab. 2). Similar platelets number was maintained in a post-operative period: 234.2 ± 54.141 x $10^3/\mu$l (Tab. 2).

Before the surgery no significant differences in the number of platelets in patients suffering from resectable (group I) and non-resectable (group II) were observed (Tab. 3). In group I, after the gastric cancer removal, the number of platelets increased significantly, whereas in group II no significant differences in these field were observed.

Table 1. The number of platelets in patients suffering from resectable gastric cancer before (group Ia) and after (group Ib) the surgery and in normal donors (group III).

Number of platelets	Average (x$10^3/\mu$l)	Standard deviation ($\pm$)	Minimum (x$10^3/\mu$l)	Maximum (x$10^3/\mu$l)
Group Ia	259.136	84.459	10.0	419.0
Group Ib	472.05	111.772	258.0	690.0
Group III	193.219	55.493	110.0	310.0

Table 2. The number of platelets in patients suffering from non-resectable gastric cancr before (group IIa) and after (group IIb) the surgery and in normal donors (group III).

Number of platelets	Average (x$10^3/\mu$l)	Standard deviation ($\pm$)	Minimum (x$10^3/\mu$l)	Maximum (x$10^3/\mu$l)
Group IIa	265.1	81.813	165.0	453.0
Group IIb	235.2	54.141	191.1	329.0
GroupIII	193.219	55.493	110.0	310.0

Table 3. Statistical analysis of the collected platelets in particular groups examined with the Mann-Whitney and Kolmogorov-Smirnov tests; the level of differences with p<0.005

Groups	Mann-Withney test	Kolmogorov-Smirnov test
Ia–IIa	p=0.96	p=0.99
Ia–Ib	p<0.000001	p<0.000002
Ia–III	p=0.003	p=0.0017
Ib–III	p<0.0000001	p=0.0
IIa–IIb	p=0.43	p=0.38
IIa–III	p=0.013	p=0.03
IIb–III	p=0.53	p=0.13

Table 4. Bactericidal capacity of platelets in patients suffering from resectable gastric cancer before (group Ia) and after (group Ib) the surgery and in normal donors (group III)

Bactericidal capacity of platelets	Average (%)	Standard deviation (±)	Minimum (%)	Maximum (%)
Group Ia	2.2	57.33	0.0	32.1
Group Ib	4.7	8.46	0.0	29.6
Group III	21.66	16.66	2.5	74.5

Table 5. Bactericidal capacity of platelets in patients suffering from a non-resectable gastric cancer before (group IIa) and after (group IIb) the surgery and in normal donors (group III)

Bactericidal capacity of platelets	Average (%)	Standard deviation (±)	Minimum (%)	Maximum (%)
Group IIa	8.61	7.61	0.0	42.3
Group IIb	4.72	4.76	0.0	12.6
Group III	21.66	16.66	2.5	74.5

Table 6. Statistical analysis of the bactericidal capacity of platelets in particular groups examined with the Mann-Whitney and Kolmogorov-Smirnov tests; the level of differences with $p<0.05$

Groups	Mann-Withney test	Kolmogorov-Smirnov test
Ia–IIa	p=0.53	p<0.001
Ia–Ib	p=0.024	p<0.0001
Ia–III	p<0.0000001	p=0.0
Ib–III	p<0.00001	p<0.00001
IIa–IIb	p=0.18	p=0.08
IIa–III	p=0.003	p=0.0001
IIb–III	p=0.005	p=0.03

Additionally, the increase in bacteriocidal capacity of platelets in patients suffering from gastric cancer was noticed. It was low in both groups of patients, with resectable (Tab. 4) and non-resectable (Tab. 5) tumors.

Before the surgery, platelets in only 5 patients from group I demonstrated bacteriocidal activity. At the same time in 17 patients (77.3%) from this group, platelets did not reveal any bactericidal activity. In a post-operative period, significant increase in the bactericidal capacity of platelets was observed (Tab. 4 and 6) in 11 patients (50%). Among the patients, whose platelets were not able to kill bacteria before the surgery, it has changed after the surgery in 9 cases (52.9%). In 8 patients (47.1%) platelets did not acquire the ability to kill bacteria even after the tumor removal. At the same time, in 3 patients (13.6%) a decrease in bactericidal capacity of platelets was observed.

On the other hand, in the group of patients with disseminated cancer, the bactericidal capacity of platelets was revealed in 3 patients. In the other 7 (70%), platelets did not acquire the ability to kill bacteria (Tab. 5).

After the surgery in 5 patients (50%) bactericidal activity was not revealed, in 3 (30%) a moderate increase and in 2 (20%) the decrease of this activity was observed. Statistical analysis of the bactericidal capacity of blood platelets before and after the surgery did not demonstrate any significant differences (Tab. 6).

Discussion

Within the tumor development and its metastasis, activation of the clotting cascade and angiogenesis process increases and proinflammatory cytokines level raises. The role of platelets in neoplasmatic processes, as well as in metastatic tumor formation and growth, is partly discovered. The formation of metastatic tumours could be inhibited by heparin, a vitamin K antagonist, and inhibitors of platelet aggregation, what potentially could decrease the number of metastases and prolong experimental animals as well as cancer patients survival [6]. The commencement of the neoplastic process progresses with an increase in platelets number. In comparison with normal donors, our results proved significantly greater number of platelets in patients suffering from gastric cancer. We observed the thrombocytosis in operable as well as in non-operable cancer patients. Their preoperative values were entirely similar in both tested groups. Significantly increased platelets level was held up in post-operative patients, who underwent a radical tumor removal. On the other hand, in patients with disseminated gastric cancer, no significant differences were noticed in pre-operative nor in post-operative period. The hypercoagulable state is found in most cancer patients [13]. In patients suffering from renal carcinoma, platelets counts remained independent prognostic factors, whereas platelet counts were significantly lower in men with medication, and decreased with the age particularly in women [8]. Activated platelets may also be a source of vascular endothelial growth factor (VEGF). In patients after surgical treatment of esophageal cancer platelets contributed to VEGF levels in plasma as well as in serum [14]. Within platelet-released P-selectin and platelet GPIIb/IIIa and alphavbeta3 integrins, these cells play a significant role in cancer metastasis [16, 19].

The aim of this study was to evaluate bactericidal capacity of platelets in patients suffering from gastric cancer. Declined bactericidal activity was revealed in both tested groups. The differences between operable and inoperable gastric cancer were not statistically important. Significant dissimilarities were demonstrated only in the Kolmogorov-Smirnov's test.

A valuable information was the detection of the increase in bactericidal capacity of platelets in patients after a tumor removal, which was, on the other hand, still lower than in healthy population. Perhaps the radical surgery is respon-

sible for this efficiency return and even nutritional treatment might be responsible for that process. The supply of enteral nutritional diets with the use of peristaltic pump by the naso-intestinal tube in the first post-operative hours is a safe and effective therapeutic method. In patients with non-resectable gastric cancer significant decrease in platelet bactericidal activity was maintained after the surgery. There are still few informations about the bactericidal capacity of platelets in patients suffering from cancer. The above research has been conducted in the aim to evaluate bactericidal capacity of platelets in patients suffering from gastric cancer and to stimulate further research of this type.

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