Alterations of lymphocyte subpopulations in choroidal melanoma patients undergoing surgery

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Abstract

Purpose: **The alterations of lymphocyte subpopulations assessment after surgery in choroidal melanoma patients compared to cataract patients.**

Material and methods: **12 patients with malignant melanoma of the choroid, 10 patients subjected to surgery due to cataract. Methods – flow cytometric measurement of absolute lymphocyte count, the number of all T cells (CD3+), T helper lymphocytes (CD3+CD4+), T cytotoxic lymphocytes (CD3+CD8+), B lymphocytes (CD19+), NK cells (CD3-CD16+) and T cells (CD3+) cells with γδ TCR, on the day of surgery and two days after it.**

Results: **Comparable numbers of cells were observed in both groups prior to surgery, but the behavior of some populations differed: CD3+, CD3+CD4+ cells increased in melanoma patients whereas they decreased in reference group, the number of T lymphocytes with γδ TCR was significantly higher in melanoma patients before surgery and it did not differ after it.**

Conclusions: **Though there were no significant differences in lymphocyte subpopulations between melanoma patients and the reference group, it seems that the presence of tumour influences the reactivity of the immune system to the trauma (surgery).**

Key words: choroidal melanoma; flow cytometry; lymphocyte subpopulations; T lymphocytes with γδ TCR.

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Introduction

The study was designed to assess the reaction of the organism of melanoma patients to the surgery, in comparison to reaction of relatively healthy controls, undergoing surgery due to cataract. The study was also intended to investigate the numbers of lymphocytes in those patients and to compare them with values described by other investigators.

The results presented in the literature show static values of lymphocyte subpopulations in peripheral blood of uveal melanoma patients. Earlier reports suggested an increase in T helper, T supressor and B lymphocytes [1], but when the data were analyzed in comparison to healthy, age and sex matched controls no differences were found [2]. It is known, however, that both the total number and the proportions of lymphocytes may long stay within normal values independently of the ongoing pathological process. Besides as the lymphocytes in peripheral blood constitute as little as 1% of all present in the organism, the measurements of their absolute numbers or percentages hardly inform about the reaction to local processes. Therefore we decided to investigate the influence of surgery on the above mentioned parameters.

A homogenous group of patients with similar localization of the tumour (exclusively choroidal tumours) was chosen to diminish the known factors influencing the parameters under study. It was previously described that for example involvement of the ciliary body may contribute to the inflammatory response, normally not noticed when only lymphocytes were investigeted [2]. The patients enrolled into the study group presented the same grade as assessed in TNM scale [3]; due to the advanced clinical status they were classified to enucleation. The control group was adjusted in age.

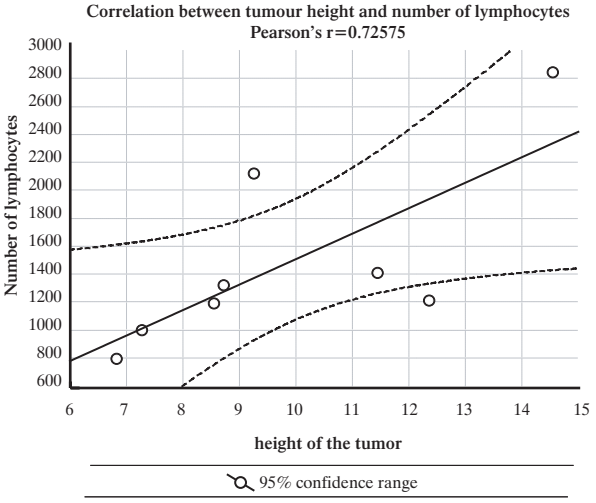
Material and methods

Twelve patients, five women and seven men, aged from 29 to 80 years, mean age 55.8. Tumour localization was assessed to

Table 1. **The medians with quartils of all investigated parameters for the choroidal melanoma patients (n=14) and cataract patients (n=10). Results are expressed as absolute number of cells**

Cells Before surgery After surgery	Melanoma patients n=12		Cataract patients n=10	
	median	quartils	median	quartils
Total lymphocyte count	1465 1726	1212-2121 1153-2026	1574 1480	1096-2022 1254-1861
CD3+ T cells	1021 1386	852-1194 840-1539	1150 1122	707-1383 832-1397
CD3+CD4+	631 889	453-903 439-1134	728 617	522-830 494-924
CD3+CD8+	311 384	252-447 280-541	357 406	278-568 268-618
CD19+ B cells	161 138	105-205 120-262	209 207	103-239 155-321
CD3-CD16+ NK cells	208 235	101-323 154-264	207 156	159-228 125-213
CD4/CD8 ratio	1.8 1.6	1.4-2.4 1.4-2.0	1.7 1.6	1.2-2.0 1.0-2.4
T cells with γδ TCR – %	7.6 2.9	3.1-8.9 2.1-3.3	2.8 2.8	0.9-4.7 1.8-5.2

Figure 1. **The correlation between the height of tumor and the total number of lymphocytes in the choroidal melanoma patients**



be exclusively choroid. In routine clinical examination the size of tumour (height and basis diameter) was assessed. Tumour infiltration towards sclera was found in two patients. All choroidal melanoma patients were subjected to surgery (enucleation). Histopathological investigation allowed to classify all patients as T3 grade acc. to TNM classification. In all patients a sample of peripheral blood was taken on the day of surgery and two days after.

As reference, a group of ten persons, five women and five men, aged from 44 to 80 years, mean age 65, undergoing surgery due to cataract was subjected to similar investigation. In all patients enrolled into the control groups any malignant conditions, inflammatory disorders or immunosuppresive treatment were excluded.

In all blood samples lymphocyte subpopulations were investigated using flow cytometry with a panel of monoclonal antibodies. Following subpopulations were analyzed and expressed in absolute count: the number of all lymphocytes, T lymphocytes (CD3+), T helper lymphocytes (CD3+CD4+), T cytotoxic lymphocytes (CD3+CD8+), B lymphocytes (CD19+), and NK cells (CD3-CD16+), as well as CD4/CD8 ratio. Additionally, the percentage of T lymphocytes (CD3+) with γδ TCR was assessed. All these investigations were performed on flow cytometer (Cytoron) from ORTHO Diagnostic Systems and the analysis was performed using ImmunoCount 2 Software.

The data obtained were analyzed using STATISTICA Software, and the results were expressed as medians because of non-normal distribution. Non-parametric tests were used to assess the significance of differences. The alterations within the groups were tested with Wilcoxon test, whereas the comparison between the groups was estimated using Wald and Wolfowitz test.

Results

Number of lymphocytes was comparable in both groups. A small increase was observed after surgery in the melanoma group and a small decrease in the reference group. The number of CD3+ lymphocytes was lower in melanoma patients and it increased after surgery, whereas no change was observed in reference group. The number of CD4+ cells was similar in both groups before surgery but an increase after surgery was observed in melanoma patients and a decrease in the reference group. In contrast, the number of CD8+ cells increased slightly in both groups. The number of B lymphocytes (CD19+) was lower in melanoma patients and showed a slight decrease in both groups. After surgery an increase in the number of NK cells was shown in melanoma patients and a decrease in the reference group, but the number of cells was similar and the differences were not significant. The percentage of γδ TCR T lymphocytes was within normal values (up to 10% in healthy individuals), but significantly higher in melanoma patients than in control group before surgery (p=0.0352). After surgery it decreased in both groups and the difference was no longer significant. All results (expressed as medians and 25-75% quartils) are given in the *Tab. 1*.

In the choroidal melanoma patients the correlation between all cell numbers and the size of tumour basis as well as the height of tumour were investigated. The statistically significant correlations are shown as *Fig. 1*, *2* and *3*, respectively.

Discussion

The aim of the study was to follow the changes in major lymphocyte subpopulations in patients undergoing enucleation

Figure 2. **The correlation between the height of tumour and the number of CD3-CD16+ NK cells in the choroidal melanoma patients**

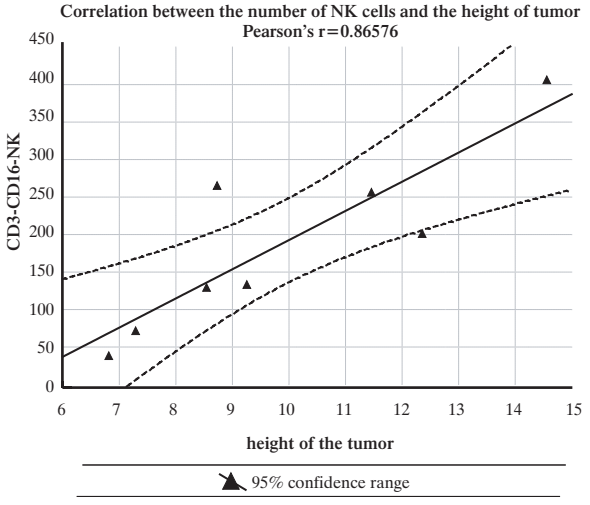
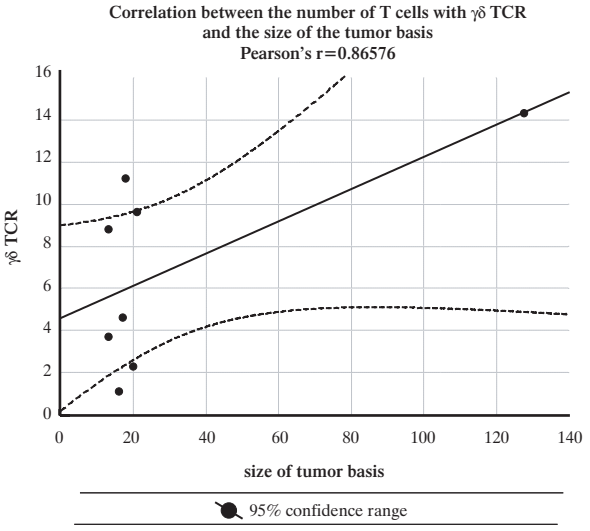


Figure 3. **The correlation between the size of tumour basis and the percentage of T cells with γδ TCR in the choroidal melanoma patients**



due to the presence of tumour. As the growth of melanoma itself may influence the immune status of the patient, similar investigations were performed in a group of patients also undergoing surgery but with no malignant background. This was intended to compare both the values before surgery to see whether the presence of tumour may influence lymphocyte subpopulations, and the alterations occurring in both groups as a result of surgery. Even if the size of tumour (both basis and height) was reliably bound to the number of lymphocytes, number of NK cells and the percentage of γδ TCR T lymphocytes, the melanoma group did not differ from cataract patients, presumably due to large range of results. Nevertheless, the alterations of several investigated parameters were distinct in both investigated groups. It may be concluded that these differences may be due to the influence of tumour on the immune system.

There were reports on the production of cytokines [4-7] in melanoma cells which could be responsible for the differences observed. Our preliminary data showed also changes in acute phase proteins concentrations and glycosylations profiles, both processes mediated by cytokines, probably mainly by interleukin-6 [8]. All these data taken together suggest that the presence of tumour alters the regulatory mechanisms in the cellular immunity.

In earlier reports some data concerning the influence of the tumour localisation on the inflammatory response were presented. It is possible that differences in numbers obtained for particular patients may reflect this influence, especially in case of NK cells. However there was no clear tendency in patients under study which would allow any hipothesis.

The number of T cells with γδ TCR decreased after enucleation in melanoma patients. Such a decrease of cells with γδ TCR was not noticed for the reference group. This could suggest the involvement of this population in the reaction with tumour. Changes in γδ TCR expression may be relevant as the cause or consequence of several diseases. The accumulation of cytotoxic TCR γδ+ cells at the sites of inflammation may suggest their involvement in the local injury process, as it was reported e.g in Behçet disease [10].The presence of of γδ TCR T cells was shown within uveal melanoma in immunohistochemical staining.There were few reports on infliltration of uveal tumours [9]. No characteristic pattern of α/β chains of the TCR was detected but the mortality was associated with advanced stage, patient age and extent of necrosis, whereas survival was increased with evidence of Vγ1 and Vδ1 TCR positive T cells [11]. The data indicate that while tumour infiltrating lymphocytes have a capacity to locate selectively within the tumour they nonetheless comprise a population expressing a diversity of TCR V β genes, showing no clonal expansion. All this is in agreement with the data presented in this paper.

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