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Article

Perforin and Granulysin-Mediated Cytotoxicity in Colorectal Cancer Patients

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Abstract

Background and Objectives: Incidence of colorectal cancer (CRC) in developed Western countries is constantly growing. CRC represents the third most common cancer and the second leading cancer-related cause of death worldwide. Innate and adaptive immunity plays a pivotal role in the tumor response, but many of these interactions are still not well understood. Granulysin (GNLY) is an effector, cytolytic molecule, present in human cytotoxic granules of different lymphocyte subpopulations, mainly in cytotoxic T cells and NK cells. Pore-forming proteins GNLY, perforin and granzymes, play a key role in cell-mediated immune responses against tumors and infections. *Materials and Methods:* We aimed to analyze perforin and GNLY-mediated cytotoxicity in the peripheral blood of patients with CRC by flow cytometry. Simultaneously, the cells were labelled with monoclonal antibodies against perforin, GNLY and different surface antigens (CD3, CD4, CD8 and CD56). Phenotypes of lymphocyte subpopulation and expression of perforin and GNLY were analyzed using intracellular and surface immunofluorescence. *Results:* Total perforin and GNLY expressions in peripheral blood mononuclear cells (PBMC) were significantly lower than in the control group. Statistically significant differences were observed in the distribution of perforin and GNLY expression in different stages of tumors classified according to Dukes, indicating that the percentage of total perforin and GNLY were significantly diminished in accordance with tumor progression. Perforin and GNLY expression was significantly reduced in NK and NKT cells, accompanied by reduced cytolytic potential in patients with CRC and a consequent reduction in their ability to eliminate tumor and infected cells. *Conclusions*: The determination of cytotoxic potential may provide a valuable assessment of a patient's immune status and represent a novel therapeutic target. Patients with CRC exhibit markedly impaired perforin- and GNLY-mediated cytotoxicity that correlates with disease progression. Assessment and restoration of cytolytic potential may therefore

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serve as indicators of immune competence and promising therapeutic strategies to improve perioperative and oncologic outcomes.

Keywords: colorectal cancer; cytotoxicity; granulysin; immunological status; perforin

1. Introduction

The incidence of colorectal cancer (CRC) in developed Western countries is constantly increasing, and research is focused on finding possible ways of earlier diagnosis, as well as potential new targets for therapy. The incidence of CRC is very high, currently ranking third in frequency of occurrence and second in mortality. In 2018 approximately 1,8 million new diagnosed cases were reported, with about 881 000 lethal outcomes [1]. Traditional, classical epidemiology investigates many risk factors such as nutrition, lifestyle, obesity, environmental and genetic factors that may lead to increasing incidence. Genetic factors include positive family anamnesis and hereditary CRC syndromes [2]. Positive family history is present in 10-20% in all cases with CRC [3]. Hereditary CRC syndromes include non-polyposis and polyposis syndromes [4]. Non-polyposis syndromes represent Lynch syndrome and familial CRC. The polyposis syndromes are more frequent and easily diagnosed, but systematic molecular analysis is necessary for proper diagnosis [5]. Environmental lifestyle risk factors are modifiable and include types of nutrition (red meat, processed meat, low intake of fruits and vegetables, deficiency of vitamins B and D) [6,7], obesity [8], reduced physical activities [9–11], smoking [12,13], alcohol intake [14] etc. In etiopathogenesis of CRC very important roles have inflammatory bowel disease (IBD) [15,16], changes in ferroptosis, necroptosis, and pyroptosis [17], diabetes mellitus, type 2 [18], menopausal hormone therapy, the use of different drugs (aspirin, statin, non-steroid anti-inflammatory drugs - NSAID). Potential protective factors should also be considered: adequate physical activity and specific nutrition (tree nuts, vitamins and calcium supplements, dietary fiber, whole grains, dairy products, fish intake) [5]. Innate and acquired immune response represents a pivotal role in the tumor response, but many of these interactions are still not well understood. Granulysin is an effector, cytolytic molecule, presents in human cytotoxic granules of NK and cytotoxic T cells. Together with perforin and granzymes, who also form pore, plays a key role in cell-mediated immune responses against tumors and infections [19]. GNLY induces cell death by mitochondrial damage, accompanied with releasing of cytochrome c, as well as apoptosis-inducing factors. However, these molecular mechanisms are not well defined. Cell death induced by GNLY is apoptotic, followed by the release of phosphatidylserine, which breaks the cell membrane and activates caspase 3. The apoptotic process begins with an intracellular Ca2+ increase and formation of mitochondrial ROS [20]. Its recombinant 9 kDa form has tumoricidal and antimicrobial activity, killing gram-positive and gram-negative bacteria, yeast, fungi and parasites, emphasizing that the killing of intracellular pathogens depends on perforin. After activation, GNLY is released, not only from NK cells, but also from both subpopulations of T cells (CD4+ and CD8+ cells). GNLY can also activate monocytes and dendritic cells [21]. In addition to the activation of GNLY in tumor and infectious diseases, its important role was also observed in the etiopathogenesis of autoimmune [22], skin [23], reproductive disorders [24], and in transplantation [25,26]. Exocytoxic release of perforin, granzymes and death receptor pathways represent a key mechanism of NK-mediated apoptotic tumor cell death [27]. Perforin initiates cell membrane invagination, granzymes delivery and via NK cells may control tumor development and metastasis [28,29]. Moreover, NK cells, with stimulation of death receptors, may remove target cells and induce the releasing of cytotoxic granules which contain perforin and GNLY, as well as granzymes too. Changes in these cytotoxic and apoptotic mechanisms represent a basic problem in all tumor types [30]. Immune escape of tumor cells may be induced by different mechanisms which include hinder NK cell-mediated antitumor activities with cancer cells' secretion of soluble factors or recruitment of suppressor cells [31]. Although numerous studies have been conducted on changes in immune status during colon cancer, the complexity of these etiopathogenetic mechanisms has still not yet been fully

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elucidated. In this work, we examined the co-expression of perforin and GNLY in patients with different stages of CRC, attempting to contribute to the elucidation of NK-mediated antitumor activity.

2. Materials and Methods

2.1. Study Design, Patients and Healthy Volunteers

A cross-sectional study involved 50 patients with newly diagnosed CRC and 40 healthy volunteers as a control group. The peripheral blood samples of CRC patients were acquired form 50 patients (ages 38–89; mean: 71 years) who underwent CRC surgical procedure in the Clinic of Abdominal Surgery, University Hospital Mostar, Bosnia and Herzegovina (No 46/26, 21 June 2017). The control group was constituted of 40 age-matched subjects (ages 20–90; mean: 65 years). Blood samples were collected in accordance with the International Health Guidelines in the Declaration of Helsinki "Ethical principles for medical research involving human subjects". The study was approved by the Ethics Committee of the University Hospital Centre Mostar, Bosnia and Herzegovina. All subjects included in the study signed informed consent. The exclusion criteria were subjects younger than 18 years, presence of any acute or chronic inflammatory disease, immunological disease, autoimmune disorders, patients who were treated with biological, radiation or immunosuppressive therapy, or blood transfusions, as well as patients who underwent some other major surgical procedures.

2.2. Isolation of Peripheral Blood Mononuclear Cells

Venous peripheral blood samples of CRC patients and healthy volunteers were collected by venipuncture into "vacutainer" (Becton Dickinson, Franklin Lakes, NY) and peripheral blood mononuclear cells (PBMC) were isolated on the density gradient by Lymphoprep (Nycomed Pharma AS, Oslo, Norway) and centrifugate for 20 min, at 600 G. After centrifugation, MNLC were collected and washed twice in medium (Roswell Park Memorial Institute -RPMI 1640 medium), (Invitrogen, Auckland, NZ, USA). PBMC were resuspended with final concentration of 1 × 106 per sample in fluorescent-activated cell sorting (FACS) buffer. Before intracellular and cell surface antigens detection, the viability of the cells was checked with propidium iodide 0.5 lg/ml per 106 cells (Sigma–Aldrich, St. Louis, MO, USA) and by flow cytometric analysis (FACSCalibur, Becton Dickinson, San Jose, CA, USA) and was always >95% [32,33].

2.3. Simultaneous Detection of Cell Surface Antigens and Intracellular Antigen (Perforin) by Flow Cytometry

PBMC were aliquoted (106 per aliquot), washed in FACS buffer and fixed in 100 μL PBS enriched with 4% paraformaldehyde, at pH 7,4 for 10 minutes at room temperature.

The cells were washed twice in FACS buffer and permeabilized for 20 min at room temperature with saponin buffer (0.1% saponin) (Sigma, Poole, Dorset), blocking the non-specific Fc receptor binding and fixated [34–36]. Cells were labelled intracellular with anti-P monoclonal antibody (Department of Physiology, Immunology and Pathophysiology, Medical Faculty, University of Rijeka, Croatia). Simultaneously, surface markers: CD3/ CD4, CD3/ CD8, CD3/ CD56 were labelled with phycoerythrin-5 (Cy-PE5)-conjugated anti-CD3 (mouse UCHT1, IgG1), phycoerythrin (PE)-conjugated anti-CD4 mAb (mouse RPA-T4, IgG1), PE-conjugated anti-CD8 (mouse RPA-T8, IgG1), and PE conjugated anti-CD56 (mouse B159, IgG1) (BD Biosciences, Erembodegem, Belgium). As negative controls were used isotype-matched mouse antibodies, conjugated with FITC, PE or CY-PE5 by direct immunofluoresce method. All blood samples were collected and analyzed using CellQuestPro software on FACSCalibur (Becton Dickinson, San Jose, CA, USA). A minimum of 104 cells were analyzed.

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Expression of intracellular molecule perforin was investigated in all lymphocyte subpopulations: CD3+CD56- (T lymphocytes), CD3-CD56+ (NK cells) and CD3+CD56+ (NKT cells).

2.4. Simultaneous Detection of Cell Surface and Intracellular Antigen (GNLY) by Flow Cytometry

The preparation of PBMC for examination the expression of cytotoxic molecule of GNLY was identical as for the perforin expression investigation, except that intracellular staining was done with GNLY. In this examination, mouse IgG1 anti-human GNLY (cat. no. D-185-3; RC8 clone; MBL International) was added as primary antibodies and isotype-matched IgG1 (cat. no. bd554121, MOPC-21 clone; BD Pharmingen) as controls. After similar procedures like in perforin detection, surface antibody staining was performed in the same manner as double staining for perforin determination [37–39].

2.5. Statistical Analysis

The results were analyzed by program STATISTICA 12.0 (StatSoft Inc., Tulsa, OK, USA) and were presented as median (25th–75th percentile). The differences between groups were examined using Kruskal–Wallis non-parametric test and differences considered statistically significant at a p level of <0.05.

To investigate within-group differences, Mann–Whitney U-test was performed. The level of significance was adjusted to account for the number of mutual comparisons. Correlation between the percentage of total perforin and total GNLY was established using Spearman's rank correlation coefficient.

3. Results

3.1. Total P and GNLY Expression in (PBL) Lymphocytes

Expression of P and GNLY within gated peripheral blood lymphocytes (PBL) were analysed by flow cytometry (Figure 1A and B). The percentage of P+ cells in control group was 27,46 (22,58-32,26), while in CRC patients P+ cells was 13,33 (3,45-25,95). The expression of total perforin in CRC patients was significantly lower than in controls (Figure 1A). A non-parametric test for independent samples (Mann-Whitney U Test) was used. There is a statistically significant difference in the value of total perforin between patients with CRC and the control group (p<0,05).

A statistically significant difference is also present in the expression of total GNLY (Figure 1B) between patients with CRC, where the total GNLY+ cells were 19,5 (12,36-25,85) and controls have 30,21% GNLY+ cells (25,67-32.2) and is lower in patients with CRC in comparison with controls with statistically significant differences p<0,05.

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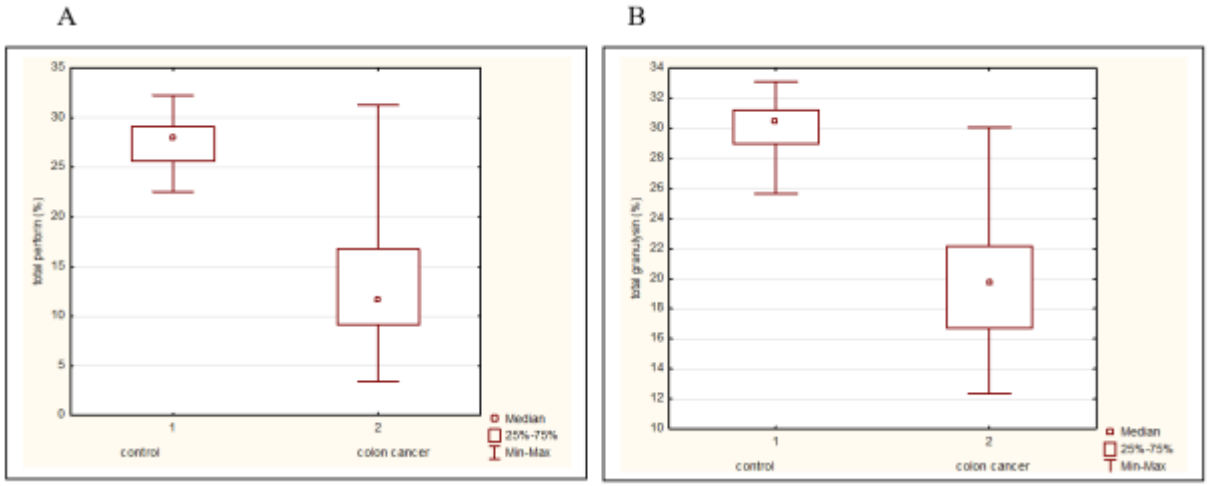

Figure 1. Total perforin and granulysin (GNLY) expression in peripheral blood lymphocytes (PBL). 1A. Total perforin expression in PBL in patients with colorectal cancer and in healthy volunteers as control ($p < 0,05$). 1B. Total GNLY expression in PBL in patients with colorectal cancer and in healthy volunteers as control ($p < 0,05$). The non-parametric test for independent samples (Mann-Whitney U Test) was used and a statistically significant difference in the value of total P and total GNLY were found between patients with colorectal cancer and the control group ($p<0,05$). Abbreviations: P - perforin; GNLY - granulysin; PBL - peripheral blood lymphocytes.

3.2. P Expression in Different Lymphocyte Subpopulations in Peripheral Blood Lymphocytes

The percentage of P+cells was examined in different lymphocyte subpopulations: in T lymphocytes (CD3+CD56-), then in T lymphocyte subsets: T heplers (CD3+CD4+), T cytotoxic cells (CD3+CD8+), NK cells CD3-CD56+) and in NKT cells (CD3+CD56+) by flow cytometry. The percentage of P+ cells in peripheral blood T lymphocytes, T helpers and T cytotoxic cells did not differ significantly among the investigated groups (data not shown). However, the percentage of peripheral blood P+ cells in NK cells: CD3-CD56+P+ (Figure 2A) significantly decreased in CRC patients in comparison with controls. The similar results were in NKT cells (CD3+CD56+P+) with significantly lower percentage in CRC patients compared with controls (Figure 2B). Again, the non-parametric test for independent samples (Mann-Whitney U Test) was used and a statistically significant difference in the value of P+ cells were found between patients with CRC and the control group ($p<0,05$).

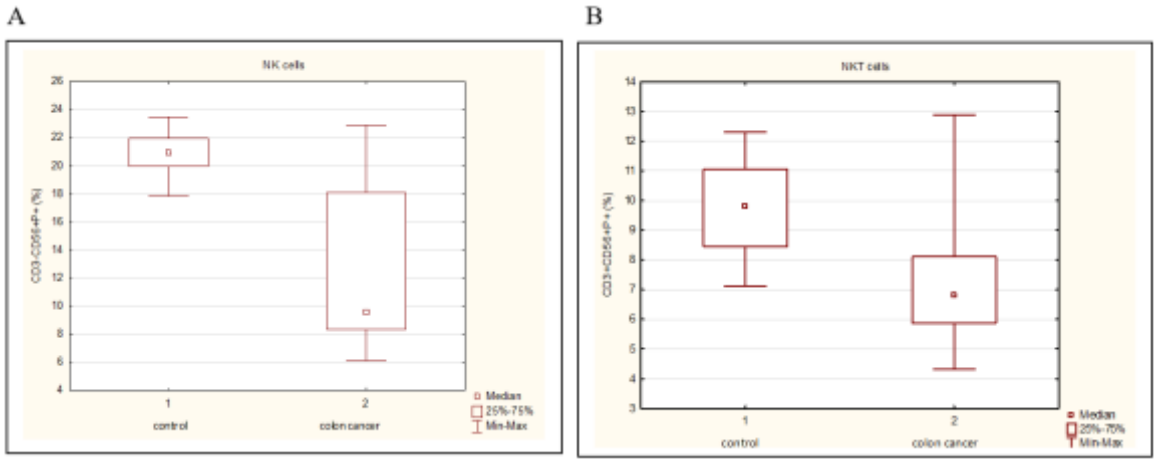

Figure 2. Proportion of P+ cells in the peripheral blood subpopulations. 2A. Frequency of P+ cells in NK cells (CD3-CD56+) ($p < 0,05$). 2B. Frequency of P+ cells in NKT cells (CD3+CD56+) ($p < 0,05$). The non-parametric test for independent samples (Mann-Whitney U Test) was used and a statistically significant difference in the value of P+ cells were found between patients with colorectal cancer and the control group ($p<0,05$). Abbreviations: P - perforin.

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3.3. GNLY Expression in Different Lymphocyte Subpopulations in Peripheral Blood Lymphocytes

GNLY expression was analyzed in different subpopulations of lymphocytes. Neither in T lymphocytes (CD3+CD56-), nor in helper and cytotoxic T subsets, the percentage of GNLY did not change significantly compared with controls (data not shown).

Figure 3 illustrates diminished percentage of GNLY cells in NK subpopulation (CD3-CD56+GNLY+) (Figure 3A) in CRC patients in comparison with controls ($p<0,05$) and the significant decrease in NKT cells (CD3+CD56+GNLY+) in patients with CRC (Figure 3B). A non-parametric test for independent samples (Mann-Whitney U Test) was used and statistically significant difference between the groups was $p<0,05$.

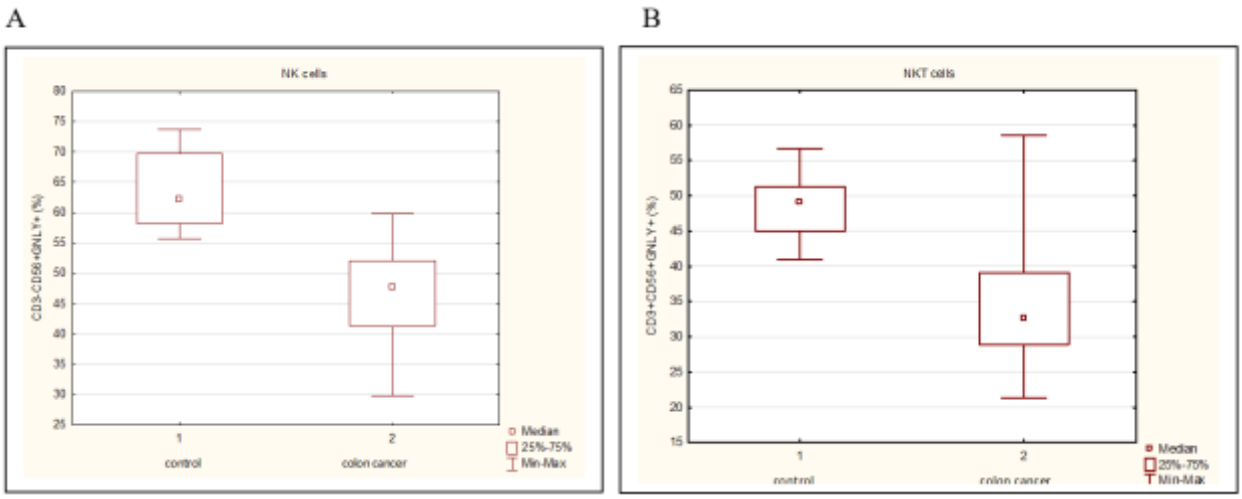

Figure 3. Proportion of GNLY+ cells in the peripheral blood subpopulations. 3A. Frequency of GNLY+ cells in NK cells (CD3-CD56+) ($p<0,05$). 3B. Frequency of GNLY+ cells in NKT cells (CD3+CD56+) ($p<0,05$). Mann-Whitney U Test as a non-parametric test for independent samples was used and a statistically significant difference in the value of P+ cells were found between patients with colorectal cancer and the control group ($p<0,05$). Abbreviations: GNLY - granulysin.

3.4. Distribution of P Expression in the Dukes Classification

Figure 4 A, B, C and D show a total P expression in PBL in patients with Dukes A, B, C and D compared with control. Mann-Whitney U Test showed a statistically significant difference in the value of P+ cells between patients with Dukes A, B, C and D CRC in comparison with the control group ($p<0,05$). Figure 4 E illustrates the summary of the significance of changes in perforin expression in different stages of CRC classified by Duke. ANOVA (one-way) test was used, and it showed a statistically significant difference in the values of total perforin between Dukes A and Dukes C, Dukes A and Dukes D, Dukes B and Dukes D with $p<0,05$. There is no statistically significant difference between Dukes A and Dukes B, between Dukes B and Dukes C and Dukes D.

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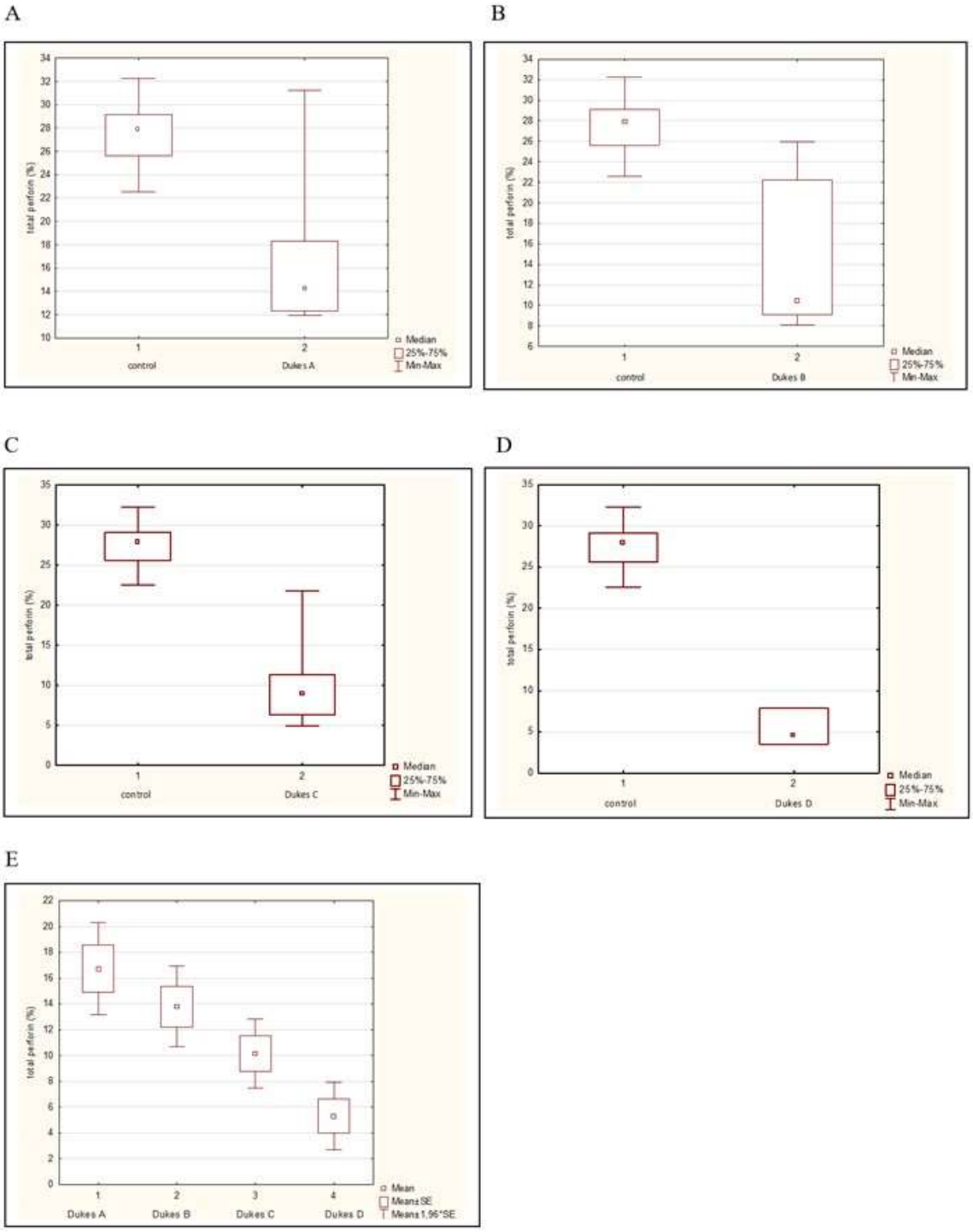

Figure 4. Distribution of perforin expression in the Dukes classification. 4A, 4B, 4C and 4D. Total perforin (P) expression in (PBL) of patients with Dukes A, B, C and D compared with control. Mann-Whitney U Test shown a statistically significant differences in the value of P+ cells between colorectal cancer patients with Dukes stage A, B, C and D in comparison with the control group ($p<0,05$). 4E. ANOVA (one-way) test was used and shown a statistically significant difference in the values of total perforin between Dukes A and Dukes C, Dukes A and Dukes D, Dukes B and Dukes D with $p<0,05$. There is no statistically significant difference between Dukes A and B, between Dukes B and C, and Dukes C and D.

3.5. Distribution of GNLY Expression in the Dukes Classification

Figure 5 illustrates the changes in GNLY expression in different stages of tumors according to Dukes classification. Total GNLY expression in PBL in patients with Dukes A, B, C and D in

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comparison with control was statistically diminished. Mann-Whitney U Test showed a statistically significant difference in the value of GNLY+ cells between patients with Dukes A, B, C and D CRC in comparison with the control group ($p<0,05$) (Figure 5 A, B; C and D). ANOVA test (one-way) was used and shown a statistically significant differences in the values of total GNLY between all 4 groups of Dukes (Figure 5E).

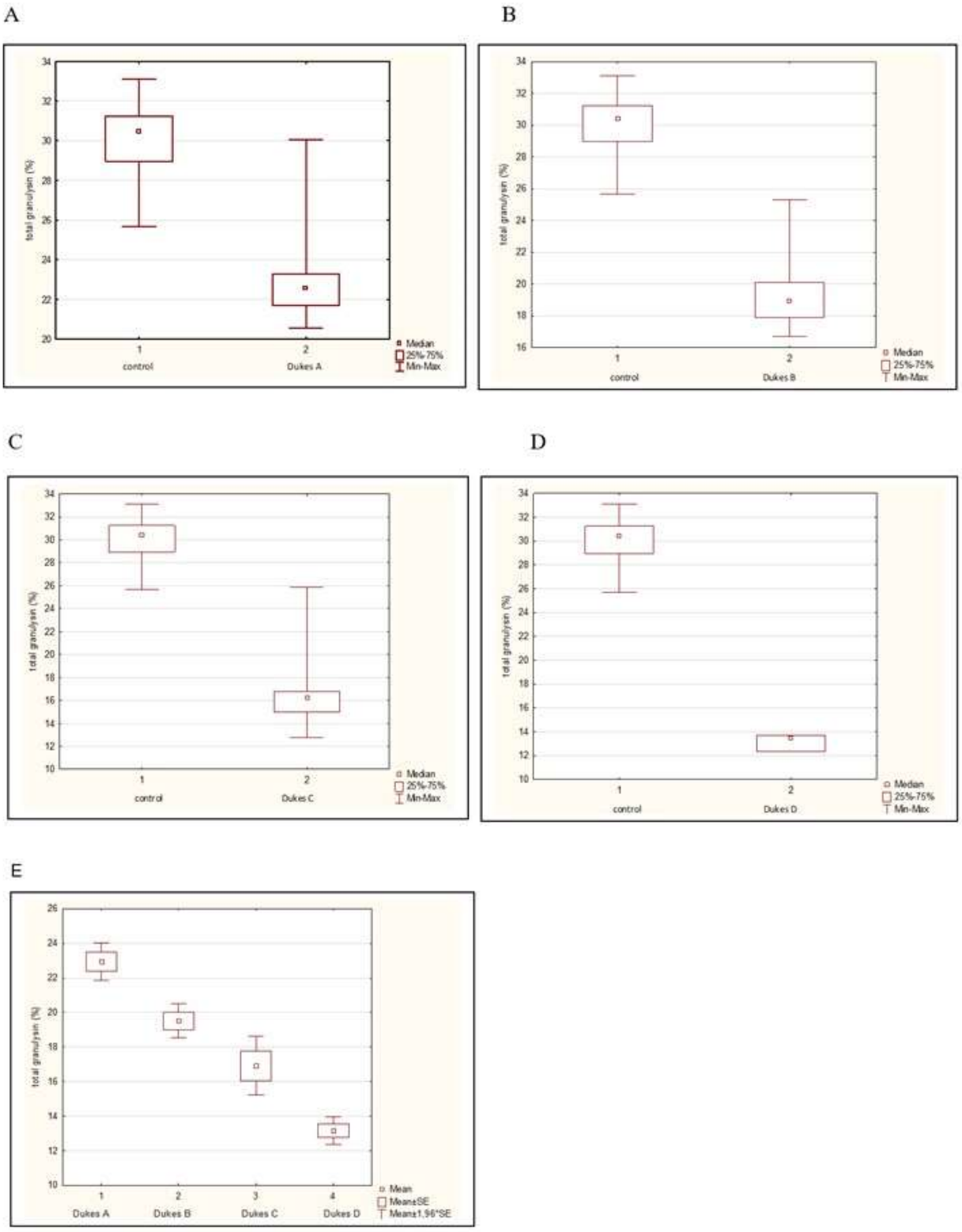

Figure 5. Distribution of GNLY expression in the Dukes classification. 5A, 5B, 5D and 5D. Total GNLY expression in PBL in patients with Dukes A, B, C and D compared with control. Mann-Whitney U Test shown a statistically

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significant difference in the value of GNLY+ cells between patients with Dukes A, B, C and D CRC in comparison with the control group (p<0,05). 5E. ANOVA test (one-way) was used and shown a statistically significant differences in the values of total GNLY between all 4 groups of Dukes.

3.6. Correlation Between Perforin and GNLY Expression in Colorectal Cancer Patients

The correlation coefficient, i.e. the degree of association between the variables total GLNY and total perforin in patients with CRC, is statistically significant (p<0.05) and is r (X,Y)=0.49, indicating a good association. This is a positive and incomplete correlation, i.e. a linear increase in one variable corresponds to a linear increase in the other variable (Figure 6). The Spearman's rank correlation coefficient is used.

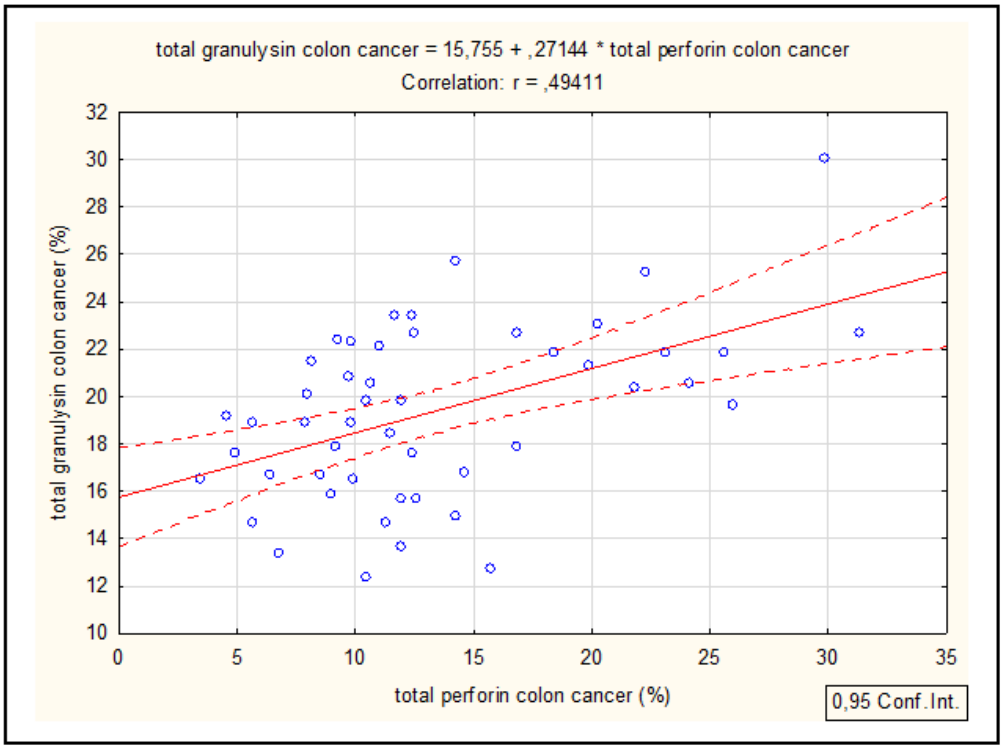

Figure 6. Correlation between the percentage of total perforin and total GNLY in colorectal cancer patients. Correlation between total GNLY and total perforin in patients with colorectal cancer is statistically significant (p<0.05) and is r (X,Y)=0.49, positive and linear increase determined by Spearman's rank correlation coefficient.

4. Discussion

This investigation includes the examination of the effects of released cytotoxic molecules perforin and GNLY from the PBL of CRC patients. Changes in their functions may contribute to the immunologic escape in antitumor activities. Perforin is a cytotoxic molecule released from activated lymphocytes and plays very important role in the elimination of tumor cells and virus-infected cells, usually during the effector phase of the immune response. Our obtained results show reduced values of total perforin, as well as double positive cells in peripheral blood (perforin expression is significantly reduced within NK and NKT cells (Figure 1A and Figure 2). These results point to a reduced cytolytic potential of patients with colorectal tumors, and a consequent reduction in the ability to eliminate tumor cells as well as infected cells, which can lead to more frequent infections and general weakness of the organism, which is a characteristic of patients with malignancies. The low potential of cytotoxic activity was particularly observed in the low number of CD3-CD56+P+ cells. NK cells are the ones that have the highest value of perforin in healthy individuals. In patients with CRC, perforin was released from the granules, and the cells have a weakened cytolytic ability.

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In our previous works, we proved elevated values of regulatory T cells (Tregs) in the carcinomatous area, and we can conclude that it is a matter of dynamic changes and migration of these cells [40,41]. Tregs use important perforin/granzyme pathway to control the immune response. Contradictory data have shown that perforin and Granzyme B expressed by Tregs can inhibit tumor clearance, which contradicts the roles of these cytotoxic molecules expressed by cytotoxic T-cells and NK-cells and participating in antitumor immune responses [42]. Killer cells can fuse with the appropriate target cell, discharge cytolytic granules, including perforin, into the intercellular space. This process is dependent on the presence of Ca2+ ions [43]. Perforin monomers bind and polymerize in the target cell membrane, primarily around the central opening or pore. Thus, the membrane permeability of the specific target cell is disrupted, homeostasis within the cell is disrupted, endocytosis is stimulated, and osmotic lysis of these target cells is developed. Furthermore, target cell's damages created by the perforin pores (perforin is necessary for cytotoxicity mediated by granules), the communication of the target cell with mediators of cytotoxic lymphocytes is enabled, and thus perforin affects the lysis mediated by cytotoxic lymphocytes. Figure 4 illustrates a statistically significant difference in distribution of perforin expression in different stages of tumors classified by Dukes. Our results indicated that the percentage of total perforin significantly diminished in accordance with tumor progression. Nakanishi H et al. [44] were the first who described the observation that decreasing of P+ cells in CD8+ subpopulations in tumor tissues depends on tumor progression and represents the suppression of local immunity in hosts. This immune suppression represents a key problem in modern immunotherapy, and many investigators try to find possible mechanisms that may act and change this negative immune scene. Nowadays, dietary supplementation is very popular, and Hanson et al. [45] represent the effects of high doses of vitamin E substitution therapy in CRC patients with increased NK activity. But this augmentation was not a result of increased perforin secretion, nor the number of NK cells. The reason for this effect was diminished oxidative stress due to the minor increase of the induction of NKG2D expression. Vitamin E as an antioxidant may stimulate the function of NK cells in CRC patients. Moreover, perforin may regulate the mucosal inflammatory reaction in colitis-associated cancer [46] and our results with significantly diminished the percentage of P in NK and NKT subpopulations (Figure 2) may point this perforin function in incidence of CRC, as well as our positive correlation between the percentage of total perforin and total GNLY (Figure 6) in which, for the first time, has shown GNLY-perforin cytotoxicity in CRC. New data try to establish GNLY as cytotoxic agent who has a possibility to retard in vivo development of tumor. It has shown that GNLY-treated tumor tissues developed the augmentation of NK cell infiltration and apoptosis [47]. It is well known that GNLY is a cytotoxic molecule present in NK cells, as well as in cytotoxic T cells and is responsible for cell-mediated immune response against tumor growth and infection. Our data have shown (Figure 1B) that total GNLY expression is significantly lower in patients with CRC which indicates the fact that the cytotoxic ability of PBL is significantly reduced in these patients, and may be associated with a worse outcome of the disease and a weaker response to therapy. Cytolytic molecules may be involved in endoplasmic reticulum membrane permeability, and may act on the augmentation of the intracellular Ca2+ levels, together with the production of ROS and stimulation the changes of mitochondrial membrane potential [48,49]. This intracellular cascade of activation contributes to development the caspase-dependent apoptosis, via cytochrome c release and some other pro-apoptotic factors. Figure 5 indicates that GNLY expression is reduced in all tumor types classified by Dukes, and the reduction is proportional to the stages of the disease. Weakened immune response represents one of the biggest challenges in the therapy of patients with CRC, and any knowledge that helps in understanding complex immune events can contribute to faster diagnosis and more effective treatment. Investigation of Kishi A et al. [50] have shown that the patients with malignancies had significantly fewer GNLY-positive NK cells, but they do not examine the correlation between these two types of cytotoxic molecules and their interactions with the tumor stages.

5. Conclusions

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Patients with malignancies develop immune suppression which represents a major problem for effective immunotherapy. GNLY and perforin cytotoxic potential is severely impaired in CRC patients and indicates the importance of restoring cytolytic potential as one of the possible therapeutic targets. Preserved cytolytic potential may diminisher the intraoperative risk, contribute to better postoperative recovery, immediately after the operation and later during postoperative oncology therapy. Diminished expression of perforin and GNLY in patients with CRC, along with reduced expression of these cytotoxic molecules in NK and NKT cells correlate with cancer progression, and the determination of cytotoxic potential may prove to be a good assessment of the patient's immune status, and they may represent a new targeted therapy.

Author Contributions: Conceptualization, L.L., I.Š.L. and I.M.Š.; methodology, L.L., Z.B., I.Š.U., Đ.B. and I.Š.L; validation, A.Đ. and I.K.; formal analysis, A.Đ.; investigation, L.L., I.Š.L. and I.M.Š.; writing—original draft preparation, L.L., I.Š.L., I.M.Š. and J.R.; writing—review and editing, I.M.Š. and J.R.; visualization, A.Đ. and I.L. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of University Clinical Hospital Centre Mostar,Bosnia and Herzegovina (No 46/26; 21 June 2017 and 27 February 2026).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent was also obtained from the subjects to publish this paper.

Data Availability Statement: The data presented in this study are available on request from the corresponding authors. The data is not publicly available due to privacy and ethical restrictions.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

CRC	Colorectal cancer
FACS	Fluorescent-activated cell sorting
GNLY	Granulysin
IBD	Inflammatory bowel disease
NSAID	Non-steroid anti-inflammatory drugs
PBL	Peripheral blood lymphocytes
PBMC	Peripheral blood mononuclear cells

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