

Perforin and granzyme B gene expression is associated with a short survival time in patients with multiple myeloma

Associação da expressão gênica de perforina e granzima B a um menor tempo de sobrevida em pacientes com mieloma múltiplo

Asociación de la expresión génica de perforina y granzima B con un tiempo de supervivencia más corto en pacientes con mieloma múltiple

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Bruna Maria Bereta de Souza

ORCID: <https://orcid.org/0000-0002-0278-4369>
Universidade Federal do Triângulo Mineiro, Brazil
E-mail: brunabereta@hotmail.com

Fernanda Bernadelli De Vito

ORCID: <https://orcid.org/0000-0002-7407-4972>
Universidade Federal do Triângulo Mineiro, Brazil
E-mail: fernanda.vito@uftm.edu.br

Marianna Licati Calado

ORCID: <https://orcid.org/0000-0003-1899-7585>
Universidade Federal do Triângulo Mineiro, Brazil
E-mail: mariannacalado@gmail.com

Leonardo Rodrigues Oliveira

ORCID: <https://orcid.org/0000-0003-1882-1694>
Universidade Federal do Triângulo Mineiro, Brazil
E-mail: leonardorodoli@hotmail.com

Marcos Vinícius da Silva

ORCID: <https://orcid.org/0000-0002-2966-7621>
Universidade Federal do Triângulo Mineiro, Brazil
E-mail: marcosuftm@gmail.com

Virmondes Rodrigues Júnior

ORCID: <https://orcid.org/0000-0001-8706-4223>
Universidade Federal do Triângulo Mineiro, Brazil
E-mail: virmondes.rodrigues@uftm.edu.br

Vânia Tietsche de Moraes-Hungria

ORCID: <https://orcid.org/0000-0002-4327-1957>
Clínica São Germano, Brazil
E-mail: hungria@dialdata.com.br

Helio Moraes-Souza

ORCID: <https://orcid.org/0000-0001-8049-348X>
Universidade Federal do Triângulo Mineiro, Brazil
E-mail: helio.moraes@uftm.edu.br

Abstract

Objectives: Perforin and granzyme B are essential proteins for protective immune responses mediated by Cytotoxic T Lymphocytes (CTLs) and Natural Killer cells (NK) against cancers, especially those of hematological origin. Our study investigated polymorphisms in the perforin gene (PRF1) and quantified the levels of the perforin and granzyme B proteins in patients with multiple myeloma (MM). *Methods:* The PRF1 coding region was evaluated in 58 patients with MM and 78 healthy individuals using direct sequencing. Quantitative real-time PCR was performed to quantify gene expression, and flow cytometry was used to determine the intracellular protein levels. *Results:* We did not observe differences in the allele frequencies of polymorphisms in the PRF1 gene as well as in perforin and granzyme B protein expression between patients with MM and healthy individuals. However, reduced expression of perforin or granzyme B genes was associated with a shorter survival time. In addition, patients with MM had significantly more CTLs expressing perforin and granzyme B, and had an increased number of NK cells. *Conclusion:* Our study suggests that the gene expression profile of perforin and granzyme B is a potential prognostic marker for MM.

Keywords: Perforin; Granzyme B; Multiple myeloma; Natural killer cell; Cytotoxic T Lymphocytes.

Resumo

Objetivos: A perforina e a granzima B são proteínas essenciais para as respostas imunes protetoras mediadas por Linfócitos T Citotóxicos (CTLs) e células Natural Killer (NK) contra cânceres, principalmente os de origem hematológica. Nosso estudo investigou polimorfismos no gene da perforina (PRF1) e quantificou os níveis das proteínas perforina e granzima B em pacientes com mieloma múltiplo (MM). **Métodos:** A região codificadora de PRF1 foi avaliada em 58 pacientes com MM e 78 indivíduos saudáveis por meio de sequenciamento direto. A PCR quantitativa em tempo real foi realizada para quantificar a expressão gênica e a citometria de fluxo foi usada para determinar os níveis de proteína intracelular. **Resultados:** Não observamos diferenças nas frequências alélicas dos polimorfismos no gene PRF1, bem como na expressão da proteína perforina e granzima B entre pacientes com MM e indivíduos saudáveis. No entanto, a expressão reduzida de genes de perforina ou granzima B foi associada a um menor tempo de sobrevivência. Além disso, os pacientes com MM tinham significativamente mais CTLs expressando perforina e granzima B e tinham um número aumentado de células NK. **Conclusão:** Nosso estudo sugere que o perfil de expressão gênica de perforina e granzima B é um potencial marcador prognóstico para MM.

Palavras-chave: Perforina; Granzima B; Mieloma múltiplo; Células natural killer; Linfócitos T citotóxicos.

Resumen

Objetivos: La perforina y la granzima B son proteínas esenciales para las respuestas inmunitarias protectoras mediadas por linfocitos T citotóxicos (CTL) y células Natural Killer (NK) frente a los cánceres, especialmente los de origen hematológico. Nuestro estudio investigó los polimorfismos en el gen de la perforina (PRF1) y cuantificó los niveles de las proteínas perforina y granzima B en pacientes con mieloma múltiple (MM). **Métodos:** La región codificante de PRF1 se evaluó en 58 pacientes con MM y 78 individuos sanos mediante secuenciación directa. Se realizó PCR cuantitativa en tiempo real para cuantificar la expresión génica y se utilizó citometría de flujo para determinar los niveles de proteína intracelular. **Resultados:** No observamos diferencias en las frecuencias alélicas de polimorfismos en el gen PRF1 así como en la expresión de la proteína perforina y granzima B entre pacientes con MM e individuos sanos. Sin embargo, la expresión reducida de los genes de perforina o granzima B se asoció con un tiempo de supervivencia más corto. Además, los pacientes con MM tenían significativamente más CTL que expresaban perforina y granzima B, y tenían un mayor número de células NK. **Conclusión:** Nuestro estudio sugiere que el perfil de expresión génica de perforina y granzima B es un marcador pronóstico potencial para MM.

Palabras clave: Perforina; Granzima B; Mieloma múltiple; Células asesinas naturales; Linfocitos T citotóxicos.

1. Introduction

Cytotoxic T Lymphocytes (CTLs) and Natural Killer (NK) cells are responsible for protection against tumor cells and virus infected cells. Upon activation, CTLs and NK cells release cytotoxic granules that are principally composed of perforin and granzyme B. The two proteins concurrently induce death of the target cell (Smyth, et al., 2000; Sankar, et al., 2022; Tibbs & Cao, 2022). Perforin polymerizes on the plasma membrane of the target cell and forms pores that lead to osmotic imbalance (Ivanova, et al., 2022). Together with granzyme B, perforin activates the caspase cascade, triggers the apoptotic pathway, and leads to the death of a neoplastic cell (Voskoboinik, et al., 2015).

Polymorphisms in the PRF1 gene and/or defects in its expression can cause a reduction in the cytotoxic function of CTLs and NK cells, and thereby adversely affect the elimination of tumor cells (Naneh, et al., 2014). Changes in the PRF1 gene have been associated with susceptibility to hematological neoplasms, and a wide spectrum of diseases such as familial hemophagocytic lymphohistiocytosis (LHF) and chronic inflammatory diseases (Stepp, et al., 1999; Santoro, et al., 2005; Clementi, et al., 2005; Cannella, et al., 2007; Voskoboinik, et al., 2013; Mehta, et al., 2021). In contrast to PRF1, there have been no reports thus far linking neoplasia cases to the presence of polymorphisms in the granzyme B gene (GZMB). However, the importance of the GZMB gene in the clearance of tumors has been demonstrated in granzyme B-deficient mice that have severe defects in the rapid induction of target cell apoptosis and have a reduced ability to eliminate transplanted halogenic tumors (Shrestha, et al., 1999; Revell, et al., 2005).

Among the investigations that analyze perforin-mediated immune responses against multiple myeloma (MM), we identified a study in an animal model, which showed that the immune response against a transplanted myeloma cell line was primarily mediated by NK cells and CD8+ T lymphocytes through the perforin and interferon gamma (IFN- γ) pathways

(Guillerey, et al., 2015). A previous study evaluated the membrane damage caused by purified perforin in tumor cells and observed that the plasma membranes of MM cells are highly susceptible to perforin-mediated lysis (Azuma, et al., 2004). Although several studies have associated polymorphisms in the PRF1 gene with hematological neoplasms, there are no reports investigating cases of patients with MM.

This study aimed to address the gap in our knowledge about the importance of the effector mechanism of CTLs and NK cells in patients with MM, and the impact of such mechanisms on the survival time of these patients. We evaluated the granule-mediated pathway of cell death by investigating the presence of polymorphisms in the coding region of the PRF1 gene, quantifying the gene expression for PRF1 and GZMB, and measuring the levels of perforin and granzyme B in cytotoxic granules. These study findings are expected to improve our understanding of the underlying mechanisms of immune response to tumor cells, and as certain the influence of genetic factors involved in the pathobiology of MM.

2. Methodology

This is a quantitative, case-control study, according to Rego (2010), approved by the Ethics Committee of the Universidade Federal do Triângulo Mineiro (protocol n ° 2677). All participants gave written consent.

Research Subjects

This study included 58 patients with MM, of which 32 were male and 26 were female, with a median age of 63 years (34–86 years) clinically monitored at the Clinical Hospital of Universidade Federal do Triângulo Mineiro and the Faculdade de Ciências Médicas da Santa Casa de São Paulo (FCMSCSP). The control group consisted of 78 individuals with no history of neoplasia, chronic or autoimmune infectious disease, of which 55 were male and 23 were female, with a median age of 46 years (23–76 years).

Polymorphism search in the coding region of the PRF1 gene

A search for polymorphisms in the PRF1 coding region was performed by direct sequencing. DNA was extracted from leukocytes obtained from total peripheral blood using the FlexiGene Mini DNA isolation kit (Quiagen, Hilden, Germany). Amplification of the sequences to be investigated was performed by polymerase chain reaction (PCR) using specific primers (Invitrogen Life Technologies, São Paulo, Brazil), for exon 2: forward (F2) 5'-CCTTCCATGTGCCCTGATAATC-3' e reverse (R2) 5'-GAAGCAGCCTCCAAGTTTGATTG-3'; and for exon 3: forward (F3) 5'-CAGTCCTAGTTCTGCCACTTA-3' e reverse (R3) 5'-CTAATGGGAATACGAAGACAGCC-3', following the technique previously described (Martínez-Pomar, et al. 2013). Sequencing was performed using the PCR products, the same amplification primers, and the ABI Prism Big Dye™ Terminator Cycle Sequencing Ready kit (Applied Biosystems, Foster City, USA), on an ABI Prism 3100x1 DNA sequencer (Applied Biosystems) following the manufacturer's instructions.

Gene Expression Analysis

To avoid possible interferences in the immune response of patients with myeloma, only patients who had not started chemotherapy treatment and healthy individuals matched by gender and age were underwent quantification of gene expression and intracellular expression of *PRF1* and *GZMB*. Among the 58 patients with MM who were included in the study, 15 had not yet started chemotherapy (Table 1).

Table 1 - Patient`s characteristics.

Patient	Gender	Age	Classification	Clinical Features	Durie Salmon	International Staging System	Therapeutic outcome	Survival time (months)
P1	M	43	IgG-Lambda	Low back pain and asthenia	IIIA	III	Very good partial response	54
P2	M	69	IgG-kappa	Left hip pain	IIA	I	Very good partial response	90
P3	F	64	IgG-Kappa	low back pain	IA	I	No treatment (asymptomatic multiple myeloma)	56
P4	M	74	IgA-Lambda	Dyspnea and edema of lower limbs	IIIB	IIIA	Death - no treatment	Not informed
P5	F	68	IgA-Kappa	Acute renal failure, anemia and thrombocytopenia	IIIB	III	Death - no treatment	1
P6	M	76	Unrealized	Not informed	Not informed	Not informed	Death - no treatment	1
P7	F	55	IgA-lambda	Right arm pain lasting 4 months	IA	II	Complete therapeutic response	30
P8	F	76	IgA-Lambda	Lower limb pain, asthenia, and weight loss	IA	III	Very good partial response	27
P9	F	71	IgG-Lambda	Adynamia, weakness, lower limb pain and dyspnea on exertion	IIIB	III	Very good partial response	21
P10	F	59	IgA-Kappa	Pathologic fracture of the left upper limb	IIIA	III	Very good partial response	21
P11	F	83	IgA-Kappa	Asymptomatic patient with detection of moderate anemia in preoperative cholecystectomy	IIIA	III	Very good partial response	17
P12	M	55	Kappa	Low back pain and weight loss	IIIA	II	Partial response	17
P13	F	73	IgG/Kappa	Inability to walk and tumor in left clavicle	IIIA	II	Very good partial response	16
P14	F	68	IgG/Kappa	Asthenia and adynamia associated with episodes of forgetfulness and lower limb pain	IIIA	III	Partial response	17
P15	M	38	Lambda	Presentation with diffuse tumor masses with initial evaluation motivated by headache and strabismus caused by skull base tumor	IIIA	Not informed	Death due to febrile neutropenia	3

Source: Authors.

Quantitative real-time PCR (qPCR) was used for the quantification of PRF1 and GZMB gene expression. Total RNA was extracted from peripheral blood using the QIAamp RNA Blood Mini (Qiagen, Hilden, Germany) kit and cDNA was generated by reverse transcription using GoScript™ Reverse Transcription System (Promega, Madison, USA). For amplification by qPCR, we used the inventoried TaqMan® assays: perforin (Hs00169473_m1), granzyme B (Hs01554355_m1) and β -actin (Hs99999903_m1) and the GoTaq® qPCR Master Mix kit (Promega). Gene expression values were normalized by endogenous β -actin gene (ACTB), and the expression levels of PRF1 and GZMB were calculated by the Δ cycle threshold (Δ CT) method using the $2^{-\Delta CT}$ formula.

In order to assess the impact of perforin and granzyme B deficiency on the survival of patients with MM, a threshold value was established from the median of the group. Thus, we divided the patients into two groups, the first with values of gene expression of perforin or granzyme B above the median, and the second with values of gene expression of perforin or granzyme B below the median (characterized as deficiency).

Immunophenotyping of CD8+ T and NK cells

Quantification of intracellular of perforin and granzyme B was performed by flow cytometry of 2×10^5 peripheral blood mononuclear cells from each individual, collected in a tube containing heparin, and isolated by density gradient separation using Ficoll-Paque PLUS (GE Healthcare, Uppsala, Sweden). Identification of CTLs and NK cells was performed using anti-CD3-APC, anti-CD8-Pe-Cy5, and anti-CD56-Pe-Cy7 antibodies (BD Pharmingen™, New Jersey, USA). The T cell activation was measured by expression of CD69 (PE) marker (BD Pharmingen™). For the quantification of intracellular protein levels of perforin and granzyme B, anti-perforin (PE) (Clone dG9), and anti-Granzyme B (FITC) (Clone GB11) (BD Pharmingen™) were used. Fifty thousand events were acquired on a FACSCanto II cytometer (Becton Dickinson, New Jersey, USA) and the results were analyzed using FlowJo 10 software (TreeStar, Ashland, USA).

Statistical analysis

Data analysis was performed using GraphPad Prism version 6.01 software (San Diego, USA). Comparison of the allele frequencies of the polymorphisms observed in patients and controls was performed using the Chi-squared test.

In relation to the results of protein quantification and gene expression by qPCR, after verifying the sample distribution through the Kolmogorov-Smirnov test, statistical analysis was performed using Student's t-test (for parametric distributions) or by the Mann-Whitney test (for non-parametric distributions). Survival analyzes were performed using the log-rank test to evaluate the impact of perforin and granzyme B deficiency in patients. Differences were considered significant when the p value was < 0.05 .

3. Results

Frequency of PRF1 gene polymorphisms

Five synonymous single nucleotide polymorphisms have been identified, three SNPs on exon 2 (G96A, G435A, G462A) and three SNPs on exon 3 (C822T, and C900T) of the PRF1 gene and all showed similar frequencies between patients with MM and healthy individuals. Regarding non-synonymous SNPs, all were identified on exon 2 of the PRF1 gene; R4H (G11A), A91V (C272T), R104C (C310T), and E261K (G781K) and the allele frequency of the SNPs also did not differ statistically patients with MM and healthy individuals (Table 2).

Table 2 - Non-synonymous polymorphisms found in the coding region of the PRF1 gene in patients with multiple myeloma and in healthy individuals.

		Allelic Frequency		<i>n- value</i>
		Wild allele	Mutated allele	
R4H (rs35418374)	P	115/116 (0.99)	1/116 (0.01)	0.47
	C	153/156 (0.98)	3/156 (0.02)	
A91V (rs35947132)	P	108/116 (0.93)	8/116 (0.07)	0.16
	C	151/156 (0.97)	5/156 (0.03)	
R104C (rs547723649)	P	115/116 (0.99)	1/116 (0.01)	0.24
	C	156/156 (1.0)	0/156 (0.0)	
E261K (rs758110629)	P	114/116 (0.98)	2/116 (0.02)	0.76
	C	154/156 (0.99)	2/156 (0.01)	

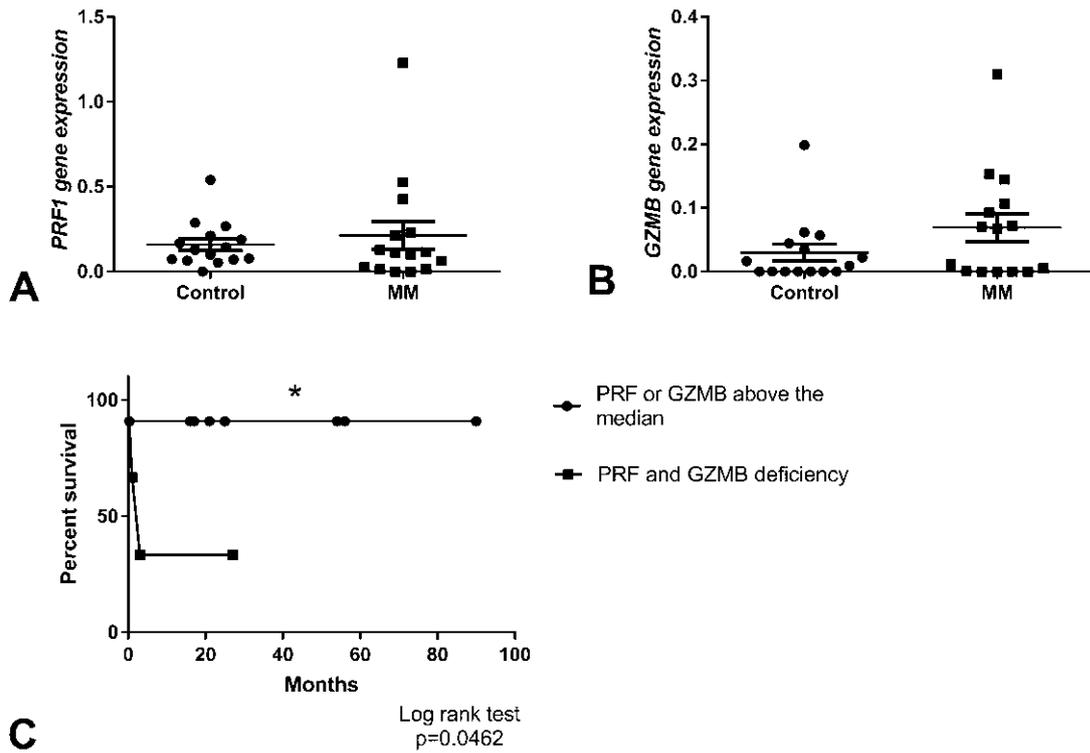
P: patients; C: controls (healthy individuals), Chi-square test: there was no significant difference in allele frequencies between patients and controls. Source: Authors.

Of the SNPs that were identified, A91V presented with a higher frequency compared to the others. The mutated 272T allele was found with an allelic frequency of 0.07 among patients with MM; six patients were heterozygous and one was homozygous. Despite this frequency was more than double that found in the group of healthy individuals, this difference was not considered statistically significant ($p=0.16$; OR=0.45 IC95% 0.14 – 1.4). The other non-synonymous polymorphisms (R4H, R104C and E261K) were found only in heterozygosity.

Lower expression of perforin and granzyme B correlated with lower survival of patients with MM

The values obtained by qPCR analysis demonstrated that there was no statistically significant difference in PRF1 and GZMB expression between patients with MM and healthy individuals ($p = 0.6778$ and $p = 0.2808$, respectively) (Figure 1A-B). However, we observed that patients with expression of PRF1 or GZMB above the median had significantly longer survival time compared to patients who had deficiency expression of both proteins ($p = 0.0462$) (Figure 1C).

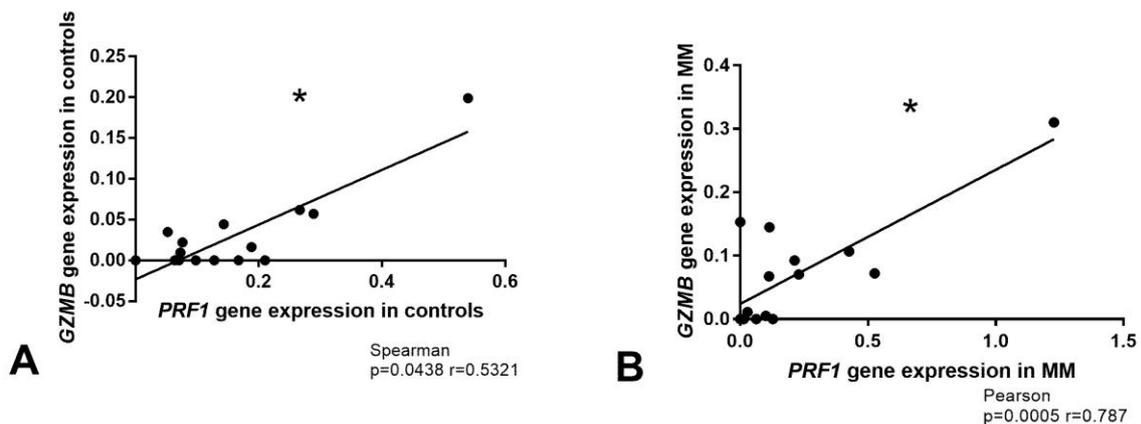
Figure 1 - Quantification of perforin and granzyme B expression and correlation with survival of multiple myeloma patients and controls. **A.** *PRF1* gene expression; **B.** *GZMB* gene expression; **C.** Comparison between survival curves of patients with *PRF1* or *GZMB* expression above the median and patients with deficiency expression of both proteins (gene expression below the group median).



Source: Authors.

We also observed a significant correlation between *PRF1* and *GZMB* gene expression in both MM ($p = 0.0005$, $r = 0.7870$) and healthy subjects ($p = 0.0412$, $r = 0.5321$) (Figure 2).

Figure 2 - Correlation between perforin gene expression and granzyme B. **A.** controls; **B.** healthy individuals.

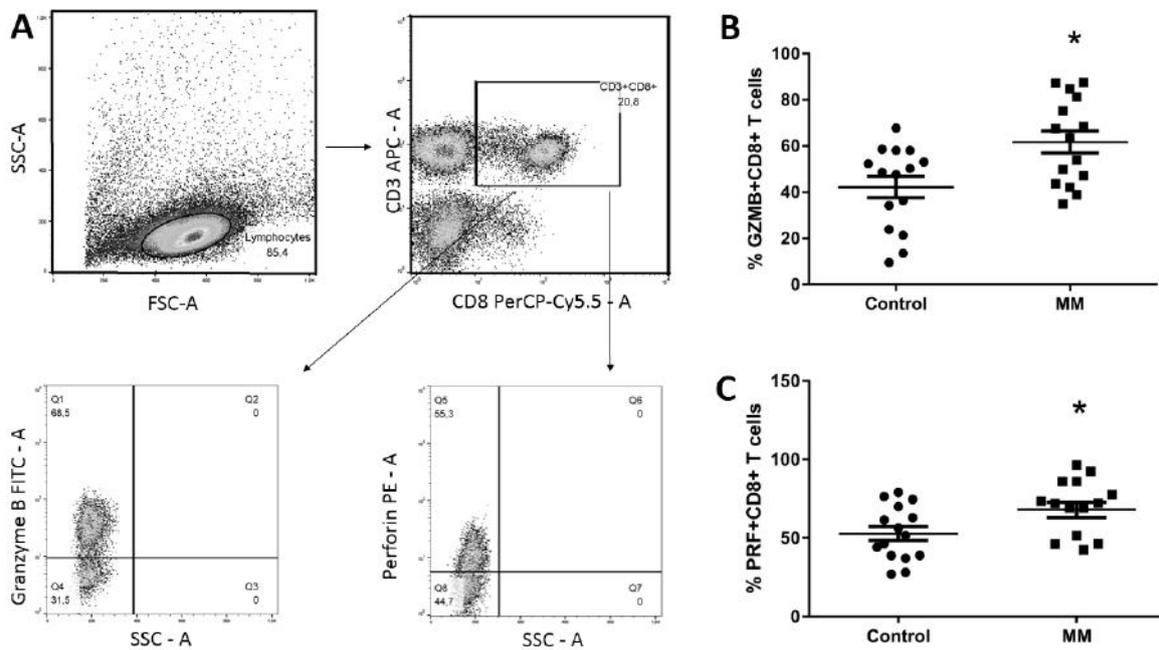


Source: Authors.

Increased number of lymphocytes expressing perforin and granzyme B

Regarding the expression of perforin and granzyme B, a significant increase was observed in the percentage of CTLs expressing granzyme B (Figure 3B $p = 0.007$) and perforin (Figure 3C, $p = 0.031$) in patients with MM.

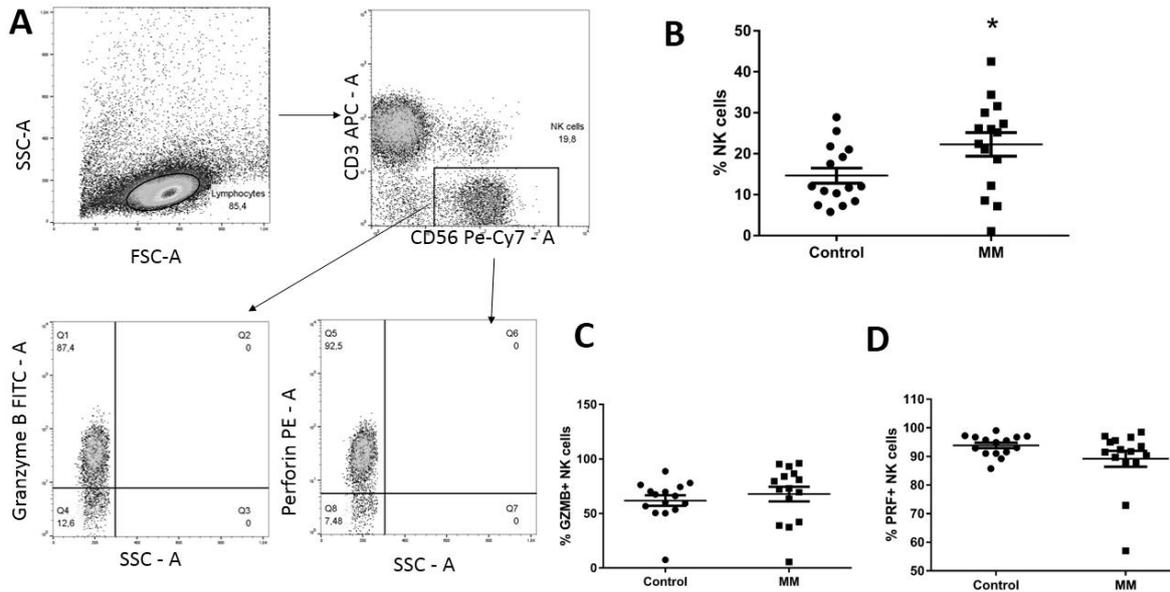
Figure 3 - Strategy for gating and quantification of subpopulations of CD8+ T lymphocytes expressing perforin and granzyme B. **A.** Gating strategy for separation of the lymphocyte population, separation of CD8+ T lymphocytes; and quantification of the percentage of cells expressing perforin and granzyme B. **B.** Quantification of CD8+ T lymphocytes expressing granzyme B. **C.** Quantification of CD8+ T lymphocytes expressing perforin.



Source: Authors.

Patients with MM also had a significant increase in the percentage of NK cells (Figure 4B, $p = 0.034$), but the proportion of these cells expressing granzyme B and perforin was not statistically different compared to healthy individuals (Figure 4C–D). Flow cytometric analysis showed that the percentage of CTLs (CD3+ CD8+) in patients with MM and controls did not differ significantly ($p = 0.236$).

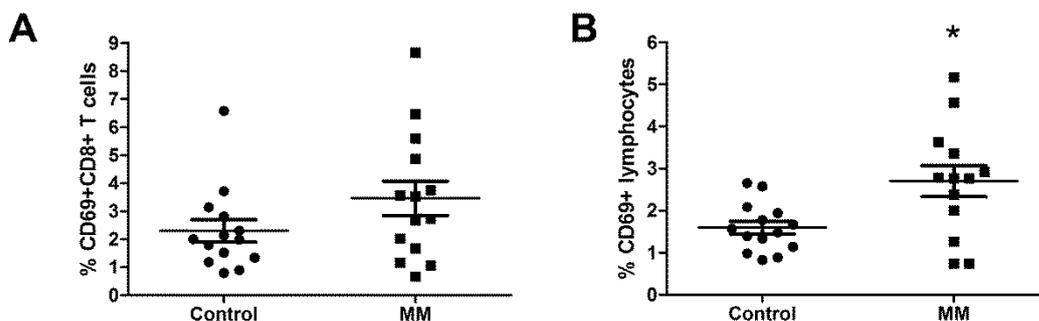
Figure 4 - Gating strategy and population quantification of NK cells expressing perforin and granzyme B. **A.** Gates strategy for separation of the lymphocyte population; separation of the NK cell population and quantification of the percentage of cells expressing perforin and/or granzyme B. **B.** Quantification of NK cells. **C.** Quantification of NK cells expressing granzyme B. **D.** Quantification of NK cells expressing perforin.



Source: Authors.

In addition, the activation of CTLs was evaluated through CD69 marker expression and patients with MM had a similar percentage of activated CTLs in relation to the control group ($p = 0.19$) (Figure 5A). However, when we evaluated the number of total lymphocytes expressing the CD69 marker, we noticed that patients with MM had greater activation compared to the control group ($p = 0.009$) (Figure 5B).

Figure 5 - Quantification of activated lymphocytes in patients with multiple myeloma. **A.** Quantification of CD69 + cytotoxic T lymphocytes. **B.** Quantification of CD69 + lymphocytes.



Source: Authors.

4. Discussion

In this study, we monitored the perforin-granzyme B pathway of cell death by evaluating gene expression and quantifying proteins in the intracytoplasmic granules of CTLs and NK cells. While gene sequencing was performed in patients regardless of the treatment phase, the other analyses for gene expression and protein quantification were limited either to patients who had not yet started treatment with chemotherapy drugs and to healthy individuals matched for sex and age. This ensured that changes in the immune response due to the patient's underlying disease were not taken into account, and that any bias attributable to the use of chemotherapeutic drugs or aging was eliminated.

We observed that the gene expression profiles of perforin and granzyme B did not differ between healthy individuals and patients with MM. Similarly, the allelic frequency of all identified polymorphisms did not have statistically significant differences between the two groups. However, when patients with MM were divided according to the level of gene expression, i.e., above or below the group median, a deficiency in the expression of perforin and granzyme B genes could be associated significantly with a shorter survival time in patients with MM. Thus, even if the inherited polymorphisms of the PRF1 gene have not been associated with MM, impaired cytotoxic responses may be associated with a poorer prognosis for these patients, demonstrating that the immune system plays a critical role in controlling the progression of, and response to, MM treatment.

Studies have shown that gene or protein expression profiles of perforin and granzyme B can be important prognostic markers for several types of cancers. For example, in case of colorectal cancer, amplified numbers of CTL and granzyme B expressing lymphocytes in the tumor infiltrate were associated with increased patient survival (Prizment, et al., 2017). Similarly, in patients with advanced stage lung cancer, a greater number of CTLs expressing perforin was associated with increased survival (Xu, et al., 2015). Moreover, a previous study by our group also showed that expression of these genes correlated positively with survival time in patients with non-Hodgkin's lymphoma (Souza, et al., 2018).

Although we were unable to associate an increase in the allelic frequency of the PRF1 gene polymorphisms with MM, we identified two SNPs with conflicting interpretations of pathogenicity (NCBI a; NCBI b). One of the SNPs that we identified and which was initially considered pathogenic according to the literature was G11A (rs35418374). It exchanges the amino acid arginine for histidine in codon four of the perforin protein. In our study, this change could only be identified in a heterozygous state, with no significant difference in allelic frequency between the two groups that were studied. There are conflicting interpretations regarding the pathogenicity of this polymorphism, since only a single study in the literature has reported an association between this alteration and aplastic anemia in a patient (Solomou, et al., 2007). However, recent clinical studies that have performed a more refined analyses confirm the clinical significance of this variation according to the Sherlock methods and the American College of Medical Genetics guidelines, and indicate that this polymorphism is benign (ACMG, 2015; Invitae Clinical Genomics Group, 2017).

The second SNP that we identified was C272T (rs35947132), which leads to substitution of the amino acid alanine for a valine in the codon 91 (A91V) of the perforin protein. This is a hypomorphic variant most commonly found in the PRF1 gene and is predominantly identified in populations of European or multiracial American origin, with frequencies of the mutated allele ranging from 0.02 to 0.04 (Exome Aggregation Consortium, 2016). In our study, although the frequency of the altered allele was twice as high among patients with MM (0.07), this difference was not statistically significant. However, this change causes protein misfolding and leads to decreased cleavage of the active form, rendering the protein more unstable and increasing its degradation, and consequently causing a decrease in the intracytoplasmic protein levels without interfering with gene expression (Trambas, et al., 2005; Voskoboinik, et al., 2005; Voskoboinik, et al., 2006; Brennan, et al., 2010).

Finally, protein quantification through flow cytometry allowed us to finalize the analysis of the granule-mediated pathway of death and helped us understand its key role in different lymphocyte populations. In our study, we observed a

significant increase in the number of both, CTLs expressing perforin and granzyme B, as well as NK cells. However, although the percentage of NK cells was higher in patients with MM than in healthy individuals, the proportion of NK cells expressing perforin and granzyme B was similar in both groups.

The expansion of T cells in patients with MM has been demonstrated previously in several studies (Moss, et al., 1996; Sze, et al., 2001; Raitakari, et al., 2003; Brown, et al., 2009). Raitakari et al (2000) observed that a majority of the expanded T-cells had the phenotype of cytotoxic T cells (CD8+CD57+ and perforin+). Reinforcing the key roles of CTLs and NK cells, which are the chief cells that express perforin and granzyme B, a study in an animal model demonstrated that the tumor burden in MM mice correlated inversely with the number of CD8+ T cells and NK T cells present in the bone marrow (Guillerey, et al., 2015).

We investigated cellular activation through the expression of the CD69 marker, and observed that there was no difference in the number of activated CTLs among patients with MM and healthy individuals. However, the percentage of activated total lymphocytes had increased. Previous studies have shown that T cell clones in MM patients can constitute up to 50% of the total blood lymphocytes, and despite the expression of cytolytic proteins, these cells are considered hyporesponsive *in vitro* (Suen, et al., 2016). However, the presence of such hyporesponsive cells could still be correlated with improved survival (Sze, et al., 2001; Raitakari, et al., 2003; Brown, et al., 2009). We believe that the hypo responsiveness of CTLs in patients with MM occurs due to chronic antigenic stimulation, and in this sense, CTLs may be more susceptible to reduced function in comparison to other types of lymphocytes (Wood, et al., 2009). Future studies examining activation and degranulation, and seeking to restore the effector function of these cells hold promise for immunotherapies directed toward the treatment of MM.

5. Final Considerations

Data from the literature and that which is presented here, shows that the expression of perforin and granzyme B genes as well as the expansion of CTLs may be correlated with longer survival in patients with MM. However, as opposed to flow cytometry, qPCR is a faster and more economical tool for gene expression analysis that can be used at a larger scale. In addition, the use of increasingly individualized therapeutic strategies brings benefits to the patient's quality of life and prognosis. Therefore, understanding the immune response of cancer patients to the underlying disease is of fundamental importance for identifying possible biomarkers that can predict the poor responders to conventional therapies at the time of diagnosis.

In view of this, it is essential to carry out studies on the role of these proteins in patients with multiple myeloma and also in other neoplasms, such as, for example, defining whether there is any moment in the course of the disease in which the quantification of these biomarkers is more relevant.

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