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Effect of Biomodulina-T® and VA-MENGOC-BC® on lymphocyte subpopulations in older adults

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ABSTRACT

Introduction: The elderly population suffers from the natural process called immunosenescence, which may be related to the high mortality rates it has against the SARS-CoV2 virus, which is why therapies that improve the immune status are required. The combined treatment of the VA-MENGOC-BC® (V-BC) vaccine and the Biomodulina T® (BT) drug could achieve this purpose. This treatment could immunomodulate both the innate and adaptive branches of the immune system simultaneously.
Objective: To determine the effect of BT and V-BC on the immunomodulation of lymphocyte subpopulations in older adults.
Methods: Our study was carried out in 30 apparently healthy Cuban adults over 65 years of age. The study included three groups of 10 subjects per treatment: a combination of both and the monotherapies. Before and 7 days after treatment, 2 mL of peripheral blood was drawn from each subject. Multiparametric flow cytometry was used to identify lymphocyte subpopulations. For the comparison between the groups, point estimates and the confidence intervals of the Odds Ratio were made.
Results: We found that subpopulations of B lymphocytes and natural cytotoxic T (NKT) cells increased only with the administration of BT. Additionally, combination treatments and V-BC did not generate statistically significant immunomodulatory changes in any of the studied lymphocyte subpopulations.
Conclusions: BT presented an immunoenhancing effect on the B and NKT lymphocyte subpopulations of older adults. The three-dose treatment scheme a novel and specific treatment strategy for this formulation. We also were verified that the combined application of V-BC and BT did not have the expected benefits. All these findings suggest that BT administration is a promising approach for immune restoration and to offering protection in elderly patients against COVID-19.

1. Introduction

The Cuban population has a life expectancy of 78 years and 20.1% of the country's people are older than 60 years. Advanced age is correlated with unfavorable changes within the immune system, which are called immunosenescence (Saavedra et al., 2019). Age-related immune deterioration is associated with increased morbidity and mortality in older adults (Larbi and Fulop, 2014). Immunosenescence shows changes in

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Table 1
Distribution of lymphocyte subpopulations percentages in older adults, according to type of treatment received.

Immunophenotypes [RRV (Tuttle and Gapin, 2018; Bae et al., 2019)]	Treatments	Median Before treatment ± SD/ Median After treatment ± SD	OR [95%CI]	p
	Combination	37.75 ± 12.99/ 31.68 ± 16.38	0.97 [0.90;1.03]	0.326
$CD3^+/CD4^+$	V-BC	24.50 ± 15.33/ 33.43 ± 12.69	1.05 [0.98;1.13]	0.154
[30.3–55.7]	BT	36.37 ± 16.29/ 34.25 ± 11.33	0.98 [0.92;1.05]	0.668
	Combination	13.86 ± 10.21/ 17.97 ± 10.15	1.01 [0.93;1.11]	0.650
$CD3^+/CD8^+$	V-BC	19.26 ± 10.35/ 22.68 ± 9.30	0.95 [0.86;1.05]	0.307
[13.2–42.9]	BT	16.03 ± 7.61/16.49 ± 8.29	1.06 [0.94;1.19]	0.385
	Combination	5.53 ± 10.41/6.63 ± 10.57	1.02 [0.93;1.11]	0.597
$CD19^+$	V-BC	11.72 ± 10.69/ 10.18 ± 10.12	0.96 [0.88;1.05]	0.450
[5.4–49.5]	BT	5.09 ± 2.11/10.15 ± 2.97	2.42 [1.14;5.13]*	<0.001*
	Combination	29.63 ± 21.96/ 10.74 ± 18.52	0.97 [0.92;1.02]	0.112
$CD3^-/CD56^+$	V-BC	24.36 ± 21.60/ 28.01 ± 20.11	1.02 [0.98;1.07]	0.367
[3.7–28.0]	BT	15.07 ± 23.08/ 15.67 ± 15.59	0.99 [0.94;1.03]	0.940
	Combination	9.28 ± 25.11/ 10.29 ± 9.11	0.97 [0.91;1.03]	0.520
$CD3^+/CD56^+$	V-BC	12.11 ± 22.72/6.88 ± 17.37	0.99 [0.94;1.04]	0.450
[0.9–20.1]	BT	9.34 ± 6.46/18.94 ± 15.5	1.26 [1.02;1.56]	0.005*

(RRV) Ranges of normal reference values in percentages.
(SD) standard deviation; (OR) odds ratio; (CI) confidence interval.
* Statistically significant.

the frequency, phenotype and function of innate and adaptive immune cells, and there are even limitations in the immune response after the application of vaccines (Pera et al., 2015; Crooke et al., 2019). Specialists agree that immunosenescence can be reversed by enhancing activation of the innate and adaptive immune system, through the manipulation of cytokines and the restoration of naïve lymphocytes production (Nikolich-Žugich, 2018).

The pandemic generated by SARS-CoV2 represents a danger for the elderly, since COVID-19 (coronavirus disease 2019) causes high mortality rates in this population (Khan et al., 2020; Tay et al., 2020; Mathew et al., 2020). Therefore, it is essential to find immunotherapies to prevent contagion or progression of older adults' patients to serious condition.

Biomodulina T® (BT) is a Cuban product registered in 1994 by the laboratory of Biomodulators ("Laboratorio de Biomoduladores") (Rodriguez Martin, 2020; Rodriguez Martin et al., 2020). This drug is a polypeptide fraction obtained from bovine thymus. Saavedra et al. reported the successful application of BT in the partial restoration of the immunosenescent $CD4^+$ and $CD8^+$ T cell compartments in older adults (Saavedra et al., 2019; Suárez-Formigo and Saavedra-Hernández, 2020). This product increases the capacity of T lymphocytes to produce cytokines, thereby enhancing the function of these cells (Rodriguez Martin, 2020).

The VA-MENGOC-BC® (V-BC) vaccine was registered in Cuba by the Finlay Vaccine Institute and has been incorporated into the Cuban National Vaccination Program for more than 30 years. The V-BC vaccine is applied to actively immunize against meningococcal disease caused by serogroups B and C. It is composed of a complex of vesicles of the outer membrane of meningococcus B forming a stabilized particle with the capsular polysaccharide of meningococcus C, this complex has demonstrated its immunoenhancing effects on the innate immune system (Sierra-González, 2019; Sotolongo Padrón et al., 2007).

This study is part of a clinical trial registered and approved by the Center for State Control of Medicines (Public Registry Code: RPCEC00000315; date: 5/22/2020) carried out on the elderly population. In this clinical trial, the synergistic immunomodulatory capacity of BT and V-BC against monotherapies was evaluated. New and universal prevention strategies are needed to contain COVID-19 beyond vaccines. This combined treatment could be able to attenuate the natural process of immunosenescence. Therefore, this research seeks to determine the effect of BT and V-BC on the immunomodulation of lymphocyte subpopulations in older adults.

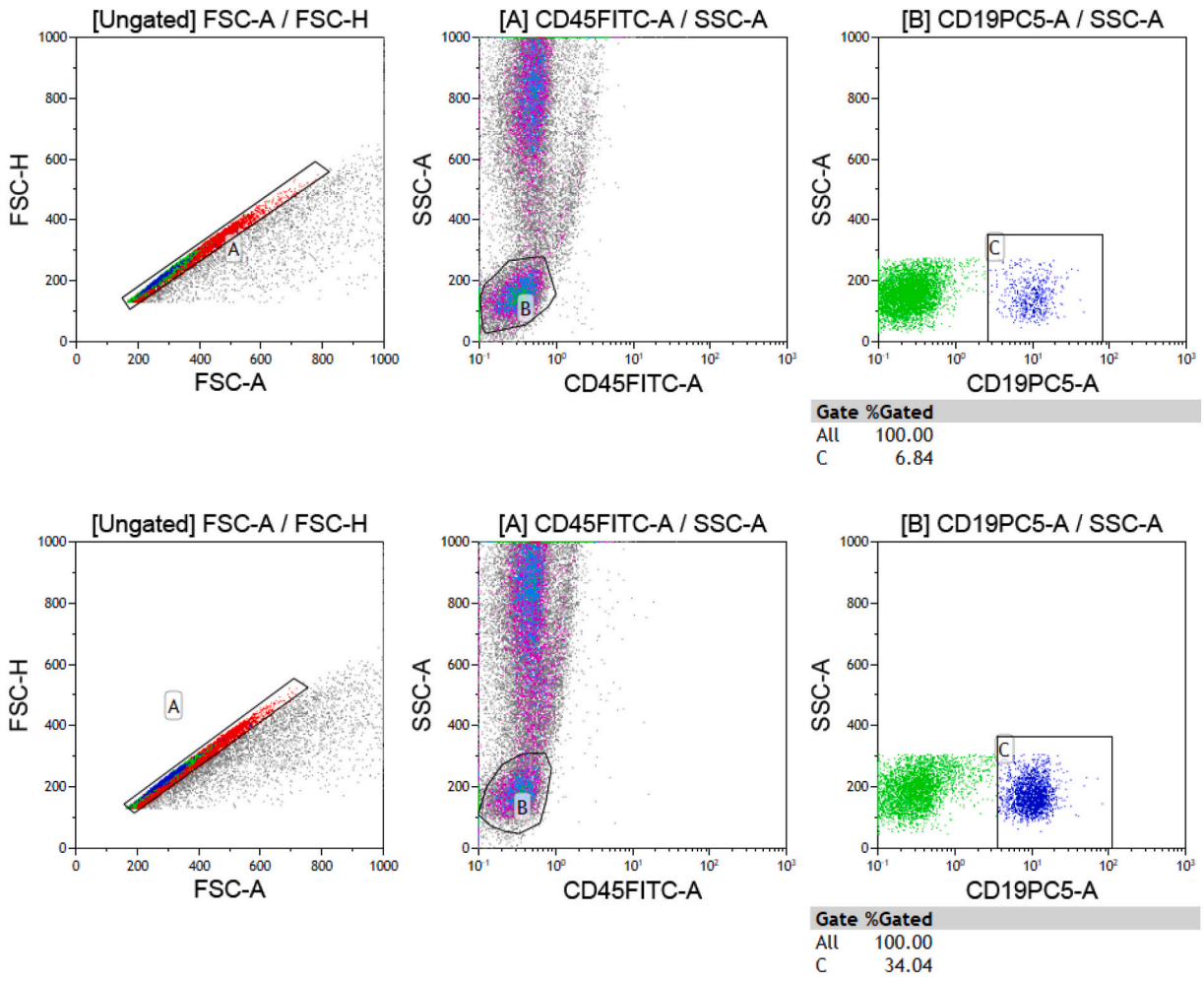
2. Materials and methods

2.1. Patients and treatments

The study lasted 30 days, for which 60 subjects were randomly selected from a Cuban clinic, with the following inclusion criteria: subject 60 years of age or older, without distinction of sex, apparently healthy with no severe cognitive problems and their consent in writing to participate in the study. The clinical trial excluded people with a history of acute infectious disease at the time or 7 days prior to their treatments administration; history of acute allergic processes or history of severe allergic reactions; history of chronic diseases in the decompensation phase; subjects with known hypersensitivity to any of the components of the research products; subjects with immunomodulatory treatment (immunostimulant or immunosuppressive); immunocompromised or immunosuppressed subjects (e.g. HIV, autoimmune disease), or who have received V-BC or BT three months before the start of the investigation. These criteria excluded the main influences on immunity and allowed reasonably healthy older adults to participate in this study. The 30 subjects were randomized into three treatment groups:

- Group I ($n = 10$), three doses of BT administered on alternate days, followed by the administration of a single dose of V-BC seven days after the first dose of BT.
- Group II ($n = 10$), single dose of V-BC.
- Group III (n = 10), three doses of BT, administered on alternate days.

The duration of treatment was 8 days in group I, 1 day in group II and 5 days in group III. Approximately 2 ml of peripheral blood (PB) per



Figs. 1 and 2. Analysis strategy of B lymphocytes, before and after treatment with Biomodulina T, respectively. A: Discrimination of singlets events from FSC. B: Separation of lymphocytes using dotplot SSC/CD45FITC. C: Discrimination by $CD19^{+}$ antigen from SSC.

subject were drawn in tubes (Vacutainer®) with ethylenediaminetetraacetic acid (EDTA), before and 7 days after treatment.

Each 3 mL bulb of BT contains 3 mg of thymus fraction. The 0.5 mL V-BC dose contains: 50 μg of meningococcal B outer membrane vesicles, 50 μg of meningococcal C capsular polysaccharide and 0.05 mg of thiomersal.

The routes of administration were intramuscular in the gluteal region for BT, and intramuscular in the deltoid region for V-BC.

Standard effective treatment with BT consists of administering 3 mg intramuscularly two or three times a week for six weeks. In total 12 to 18 doses of BT 3 mg each one.

In this study, BT was administered in a lower dose of 3 mg three times a week for one week in groups I and III. In total only three doses of BT were administered.

2.2. Flow cytometry techniques

The identification and evaluation of lymphocytes was developed by the multiparametric flow cytometry (FC) technique with monoclonal antibodies (MAb) conjugated to fluorochromes. All samples were processed and analyzed in the Immunology laboratory of the Institute of Hematology and Immunology. The processing of the samples began with the incubation of 50 μL of blood with MAb, in 15 mL tubes for 20 min at 4 °C and protected from light. The following panel of MAb conjugated with fluorochromes was used:

Monoclonal antibodies	Fluorochromes (brand)
Anti-CD3	FITC/PerCP (Miltenyi)/(Beckman Coulter)
Anti-CD4	FITC (Beckman Coulter)
Anti-CD8	FITC (Beckman Coulter)
Anti-CD19	PC5 (Miltenyi)
Anti-CD45	PC5/PerCP (Miltenyi)
Anti-CD56	PE (Miltenyi)

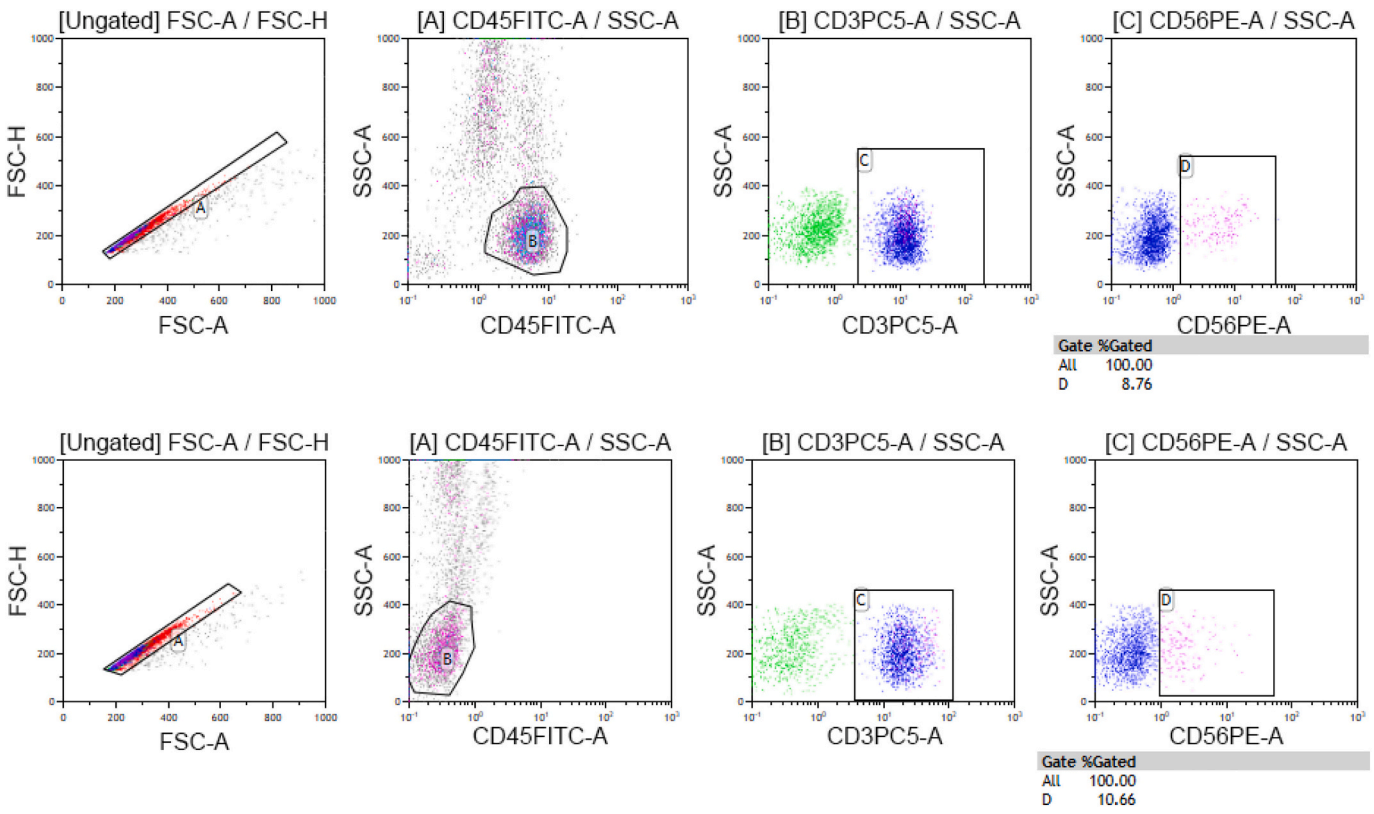
FITC: fluorescein isothiocyanate, PE: phycoerythrin, PC5: phycoerythrin cyanine 5, PerCP: peridinin chlorophyll protein.

Subsequently, the red blood cells were lysed with the Red Blood Cell Lysis Solution (10×) from Miltenyi Biotec®, according to their recommendations. The cells were then washed twice with 0.9% sodium chloride and centrifuged for 10 min at 365*g*.

A cytometer (Gallios® from Beckman Coulter, USA) was used to study the samples, with a minimum of 50,000 acquired events. The data obtained were analyzed with the Kalluza Analysis® software version 1.2 for Microsoft®.

Absolute counts were obtained by double platform; results obtained were combined in an automatic hematological counter (SYSMEX 1000i®) and by cytometry, using de following formula:

$$\text{Absolute count}(\text{cells}/\mu\text{L}) = \text{lymphocyte count}(\text{number of cells}/\mu\text{L in blood count}) \times \%\text{ of the cellular subpopulation of interest} \div 100$$



Figs. 3 and 4. Analysis strategy of natural cytotoxic T cells (NKT) before and after treatment with Biomodulina T, respectively. A: Discrimination of singlets events from FSC. B: Separation of lymphocytes using dotplot SSC/CD45FITC. C: Discrimination by $CD3^+$ antigen of T lymphocytes from SSC (Figs. 1 and 2). D: Selection by $CD56^+$ antigen from SSC (Figs. 3 and 4).

2.3. Statistical analysis

The statistical analyses were performed with compare Groups package (Subirana et al., n.d.) of R statistical system (R Computing, Vienna, Austria). Shapiro-Wilk test for normality were used to decide if each variable were normal or non-normal distributed. Statistical significance among the two studied time points was evaluated using non-parametric Mann Whitney *U* test or Student's *t*-test. We calculated odds ratios (OR) and their associated 95% confidence intervals (CI). In all analyses, $p < 0.05$ was considered statistically significant.

2.5.

Ethics

The Research Ethics Committees of the IHI approved this study. Declaration of Helsinki was applied in all processes.

3. Results

From the total number of patients in the different treatments, the $CD4^+$ T lymphocyte subpopulation showed a slight downward trend in the combination and BT treatments (60%, respectively), however there was a slight upward trend in the V-BC treatment (70%). The $CD8^+$ T lymphocyte subpopulation showed a very similar rise for the three treatments (BT: 70%; V-BC: 60%; combination: 70%).

The $CD19^+$ (B lymphocyte) subpopulation showed statistically significant growth in 100% of the patients who received BT treatment (Table 1). Only in this group the *p* value and the effect size measured by the OR, allow us to consider that the immunomodulation of B lymphocytes is directly related to the application of BT. Treated patients with V-BC reported a reduction of this parameter in 60% from the total number of patients; in turn, in the combination treatment a balance was observed, where 50% showed an increase compared to the pre-treatment state (an effect that can take place at the expense of BT), and 50% showed a decrease.

On the other hand, the subpopulation of natural killer (NK) cells decline after BT (80%) and the combination (70%) treatments, however the patients treated with V-BC increased this population in 60% from the total of patients.

Natural cytotoxic T lymphocytes (NKT) presented statistical significance, but only in patients treated with BT, where 90% from the total of patients treated increased their baseline values (Table 1). There was a reduction of the NKT values in V-BC treatment, while those treated with the combination showed a balance between increase (50%) and decrease (50%). Figs. 1 and 2, show the analysis strategy for B cells, Figs. 3 and 4 for NKT.

The absolute values also showed statistical significance in B and NKT lymphocytes in BT treatment (Table 2), although with an insignificant effect (OR) on all the measured subpopulations.

A more specific analysis of the effect was also carried out considering the ranges of normal reference values (RRV) for each subpopulation (data not shown). Regarding the effect size measured by the OR, this analysis generated statistically significant results only in B cells of the subjects treated with BT, both in percentage (OR 10.8, 95% CI [1.22; 343]; $p = 0.057$) and absolute values (OR 7.88, 95% CI [1.12; 86.7]; $p = 0.070$). In this same group, the percentage values before treatment showed that 60% of the patients treated only with BT were outside RRV. After being treated, in said group 90% of all patients were within RRV. The absolute values showed that 70% of all patients were outside RRV before treatment, and 80% of these were found within RRV after being treated with BT.

Table 2
Distribution of lymphocyte subpopulations absolute counts in older adults, according to type of treatment received.

Immunophenotypes [RRV (Arango Prado et al., 2020; Kokuina et al., 2019)]	Treatments	Median Before treatment ± SD/Median After treatment ± SD	OR [95% CI]	p
	Combination	864.7 ± 345.0/869.5 ± 453.0	1.00 [1.00;1.00]	0.678
$CD3^+/CD4^+$	V-BC	452.6 ± 394.4/579.7 ± 273.4	1.00 [1.00;1.00]	0.370
[479–1792]	BT	769.2 ± 388.9/644.2 ± 415.9	1.00 [1.00;1.00]	0.913
	Combination	313.2 ± 267.6/412.7 ± 325.7	1.00 [1.00;1.00]	0.496
$CD3^+/CD8^+$	V-BC	283.8 ± 285.8/324.8 ± 378.3	1.00 [1.00;1.00]	0.364
[248–1101]	BT	302.0 ± 229.2/362.7 ± 235.8	1.00 [1.00;1.01]	0.564
	Combination	92.89 ± 302.2/126.3 ± 319.0	1.00 [1.00;1.00]	0.650
$CD19^+$	V-BC	176.4 ± 227.9/157.9 ± 236.3	1.00 [1.00;1.00]	0.450
[114–1491]	BT	81.44 ± 44.86/214.6 ± 93.45	1.02 [1.00;1.04]	0.005*
	Combination	500.5 ± 515.0/192.7 ± 569.1	1.00 [1.00;1.00]	0.131
$CD3^-/CD56^+$	V-BC	472.2 ± 347.3/760.7 ± 332.5	1.00 [1.00;1.00]	0.244
[70–652]	BT	336.8 ± 582.2/363.7 ± 177.3	1.00 [1.00;1.00]	0.880
	Combination	237.3 ± 535.1/245.5 ± 305.7	1.00 [1.00;1.00]	0.762
$CD3^+/CD56^+$	V-BC	150.2 ± 793.2/115.3 ± 300.0	1.00 [1.00;1.00]	0.940
[21–402]	BT	181.1 ± 148.4/442.3 ± 224.4	1.01 [1.00;1.01]	0.007*

(RRV) Ranges of normal reference values in cells per μL.
(SD) standard deviation; (OR) odds ratio; (CI) confidence interval.
* Statistically significant.

4. Discussion

The pandemic generated by SARS-CoV2 has caused a very complex epidemiological context for the elderly, which is why it is necessary to maintain an optimal immune status in this risk group. The purpose of this research is to provide information about the immunomodulatory action of the combined treatment of V-BC and BT, as strategy to prevent and decrease the risk of more serious complications from COVID-19.

In this study, only BT treatment showed statistical significance in percentage values, specifically in B and NKT cells (Table 1). On the other hand, the absolute values also showed statistical significance in these same treatment groups and lymphocyte subpopulation, but only in *p* values (Table 2). This indicates that in absolute values there is no significant effect, although according to the p values in these cell subpopulations it is not caused by chance (Table 2).

By narrowing the data analysis to the RRV for each subpopulation, the effect size can provide more clinically valuable information. In this analysis, the effect size is significant in BT treatment, but only in B lymphocytes. Although these results were significant in percentage: OR 10.8, 95% CI [1.22; 343]; $p = 0.057$, and absolute values: 7.88, 95% CI [1.12; 86.7] $p = 0.070$. It is necessary to highlight that according to the *p* values in these results, the effect could be caused by chance.

The V-BC vaccine is applied to actively immunize against meningococcal disease caused by serogroups B and C. The immunization schedule for this vaccine comprises two doses applied with a 6 to 8 week interval. In this study, only a single dose was applied in groups I and II. This vaccine induces a Th1-type immune response according to studies by Pérez et al., assessed in terms of delayed-type hypersensitivity and lymphocyte proliferation (Pérez et al., 2001). In addition, other studies indicate that proteoliposomes or outer membrane vesicles from *Neisseria meningitidis* B (the active pharmaceutical ingredient of V-BC), may become promising adjuvants for new vaccines (Rodríguez et al., 2005; Pérez et al., 2007).

In this study, BT was administered in a lower dose of 3 mg three times a week for one week. The results obtained in this study correspond with the dose and the time of treatment used with BT. The dose used in this study is a lower dose than the standard effective dose reported in previous studies, where a conventional treatment was administered for six weeks (Saavedra et al., 2019; Rodriguez Martin, 2020). In previous studies the effective dose reported for BT was 3 mg two or three times a week for six weeks (Rodriguez Martin, 2020). In another study, the administered dose of BT was 3 mg once or twice a week for 6 weeks (Saavedra et al., 2019). In said report, the sample extraction to study the lymphocyte subpopulations was done four weeks after the end of the treatment. Probably for these reasons, a broader stimulation was obtained in lymphocyte subpopulations of T cells after the administration of BT. The administration of the standard active dose of BT, promotes a larger immunomodulatory effect in various lymphocytes subpopulations. Clinical trials in immunodeficiency, autoimmune diseases, and infectious diseases show that BT restores $CD3^+$, $CD4^+$, and $CD8^+$ T-cells and their function and enhances the immune response against infections (Rodriguez Martin et al., 2020). In older adults, BT increases the numbers of $CD4^+$ naïve T-cells, $CD4^+$ recent thymic emigrant (RTE) T-cells, $CD8^+$ stem cell-like memory (SCM) T-cells, and $CD4^+$ $CD31^+$ naïve T-cells. BT also improves the proliferative capacity of $CD4^+$ T-cells and the ability of $CD4^+$ T-cells to produce IFN-γ and helps restore the Th1 response (Saavedra et al., 2019; Rodriguez Martin, 2020; Suárez-Formigo and Saavedra-Hernández, 2020; Rodriguez Martin et al., 2020). This immune restoration in older adults and immunocompromised individual improve the immune response and helps contain COVID-19 (Rodriguez Martin et al., 2020).

Maturation, differentiation, and rearrangement of the T and NKT lymphocytes T-cell receptor alpha/beta (TCR αβ) occurs in the thymus. This organ begins its involution after reaching sexual maturity, and during aging the extrathymic function of the liver becomes prominent. For this reason, NKT cells migrate directly from the bone marrow to hepatic tissue to achieve their development (Wang et al., 2011; Waldhauer and Steinle, 2008). BT treatment promotes the recovery of the mass and normal size of the thymus in children with thymic hypoplasia (Rodriguez Martin, 2020). In our report, BT treatment may have restored the thymus capability to generate mature NKT cells, which would explain the result obtained. Thymosin-α-1 (Tα1) is a polypeptide hormone generated by thymic epithelial cells, capable of increase the number of T cells, promoting their differentiation and maturation (King and Tuthill, 2016). Sugahara et al. informed demonstrated that 24-week treatment with Tα1 increased the number of intrahepatic NKT cells in 7 patients (between 25 and 41 ages) with chronic hepatitis B, but this fact was not reported on PB (Abo et al., 2000). NKT's significant reports obtained in our study, represent novel evidence in the application of BT in elderly patients. NKT cells participate in various ways in the immune response such as: tumor rejection, early protection against microbial

pathogens and in the control of autoimmune diseases (Mocchegiani and Malavolta, 2004; Sugahara et al., 2002; Emoto and Kaufmann, 2003). Therefore, maintaining normal percentages of these lymphocytes in PB, would help support commonly weakened immunosurveillance in the elderly (Klibi et al., 2020; Tuttle and Gapin, 2018; Bae et al., 2019).

Aging individuals have a significant reduction in B-cell repertoire diversity and this correlates with their health status. The number of circulating B cells has been shown to decrease significantly with age and changes in the relative frequencies of different B-lymphocyte cell compartments have been reported. This age group has decreased numbers of B-cell lineages but increased prevaccination mutation load in their repertoire, resulting in a less efficient response, and the diversity of the lineages is thus greatly reduced as compared with that in young individuals (Pinti et al., 2016).

Our study also has some limitations. The absence of statistical significance in the combined treatments of both products and in V-BC monotherapy, makes us recommend future research with a larger sample size. We also recommend using other markers that allow to deeply describe other subpopulations of T lymphocytes and B and cells of the innate immune system. Furthermore, future studies should include the evaluation of higher doses of both drugs, as well as a longer treatment time and evaluation of the results, mainly for BT.

5. Conclusions

The evidence presented allows us to ensure the immunoenhancing effect of BT on the B and NKT lymphocyte subpopulations of older adults. The treatment scheme used and the results obtained from it, suggest new lines of research in which to deepen, and it also provides a novel and specific treatment strategy for this formulation. In addition, we were able to verify that the combined application of V-BC and BT did not have the expected benefits. The results obtained in this study, propose BT as a promising strategy to reverse immunosenescence and restore the immune response in older adults, decreasing the risk of more serious complications from COVID-19.

Declaration of competing interest

The author, participating institutions and researchers declare no conflict of interest. In this study, all the ethical principles typical of human research were applied. The identity of the study participants was kept anonymous to maintain their privacy.

References

Abo, T., Kawamura, T., Watanabe, H., 2000. Physiological responses of extrathymic T cells in the liver. Immunol. Rev. 174, 135–149. https://doi.org/10.1034/j.1600-0528.2002.017415.x.

Arango Prado, Md.C., Villegas Valverde, C.A., Soto Pardeiro, P., Torres López, G., Morales, A.M., Faxas García, M.E., 2020. Caracterización de los inmunofenotipos linfocitarios de sangre periférica en pacientes con cáncer. Rev. Cuba. Hematol. Inmunol. Hemoter. 36 (2), 16–21.

Bae, E.A., Seo, H., Kim, I.K., Jeon, I., Kang, C.Y., 2019. Roles of NKT cells in cancer immunotherapy. Arch. Pharm. Res. 42 (7), 543–548. https://doi.org/10.1007/s12272-019-01139-8.

Crooke, S.N., Ovsyannikova, I.G., Poland, G.A., Kennedy, R.B., 2019. Immunosenescence and human vaccine immune responses. Immun. Ageing 16, 25. https://doi.org/10.1186/s12979-019-0164-9.

Emoto, M., Kaufmann, S.H.E., 2003. Liver NKT cells: an account of heterogeneity. Trends Immunol. 24 (7), 364–369. https://doi.org/10.1016/s1471-4906(03)00162-5.

Khan, S., Ali, A., Shi, H., Siddique, R., Shabana, Nabi G., et al., 2020. COVID-19: clinical aspects and therapeutics responses. Saudi Pharm. J. 28 (8), 1004–1008. https://doi.org/10.1016/j.jsps.2020.06.022.

King, R., Tuthill, C., 2016. Chapter seven - immune modulation with thymosin alpha 1 treatment. In: Litwack, G. (Ed.), Vitamins and Hormones, 102. Academic Press, pp. 151–178.

Klibi, J., Amable, L., Benlagha, K., 2020. A focus on NKT cell subset characterization and developmental stages. Immunol. Cell Biol. 98 (7), 607. https://doi.org/10.1111/imcb.12378.

Kokuina, E., Breff-Fonseca, M.C., Villegas-Valverde, C.A., Mora-Díaz, I., 2019. Normal values of T, B and NK lymphocyte subpopulations in peripheral blood of healthy Cuban adults. MEDICC Rev. 21 (2–3), 16–21. https://doi.org/10.37757/MR2019.V21.N2-3.5.

Larbi, A., Fulop, T., 2014. From "truly naïve" to "exhausted senescent" T cells: when markers predict functionality. Cytometry A 85 (1), 25–35. https://doi.org/10.1002/cyto.a.22351.

Mathew, D., Giles, J.R., Baxter, A.E., Oldridge, D.A., Greenplate, A.R., Wu, J.E., et al., 2020. Deep immune profiling of COVID-19 patients reveals distinct immunotypes with therapeutic implications. Science (New York, N.Y.) 369 (6508), eabc8511. https://doi.org/10.1126/science.abc8511.

Mocchegiani, E., Malavolta, M., 2004. NK and NKT cell functions in immunosenescence. Aging Cell 3 (4), 177–184. https://doi.org/10.1111/j.1474-9728.2004.00107.x.

Nikolich-Žugich, J., 2018. The twilight of immunity: emerging concepts in aging of the immune system. Nat. Immunol. 19 (1), 10–19. https://doi.org/10.1038/s41590-017-0006-x.

Pera, A., Campos, C., López, N., Hassouneh, F., Alonso, C., Tarazona, R., et al., 2015. Immunosenescence: implications for response to infection and vaccination in older people. Maturitas 82 (1), 50–55. https://doi.org/10.1016/j.maturitas.2015.05.004.

Pérez, O., Lastre, M., Lapinet, J., Bracho, G., Díaz, M., Zayas, C., Taboada, C., et al., 2001. Immune response induction and new effector mechanisms possibly involved in protection conferred by the Cuban anti-meningococcal BC vaccine. Infect. Immun. 69 (7), 4502–4508. https://doi.org/10.1128/IAI.69.7.4502-4508.2001.

Pérez, O., Lastre, M., Cabrera, O., del Campo, J., Bracho, G., Cuello, M., Balboa, J., et al., 2007. New vaccines require potent adjuvants like AFPL1 and AFCo1. Scand. J. Immunol. 66, 271–277. https://doi.org/10.1111/j.1365-3083.2007.01981.x.

Pinti, M., Appay, V., Campisi, J., Frasca, D., Fülöp, T., Sauce, D., et al., 2016. Aging of the immune system: focus on inflammation and vaccination. Eur. J. Immunol. 46 (10), 2286–2301. https://doi.org/10.1002/eji.201546178.

Rodriguez Martin, R.R., 2020. History of the Discovery and Development of Biomodulina T (InmunyVital®), a Useful Immunomodulator With a Broad Range of Clinical Applications. https://doi.org/10.2139/ssrn.3678181.

Rodriguez Martin, R.R., Gonzalez Gonzalez, O., Rodriguez Gonzalez, C., Rodriguez Gonzalez, R.R., 2020. Biomodulina T (InmunyVital®) restores T cells and helps contain COVID-19. Front. Immunol. 11, 606447 https://doi.org/10.3389/fimmu.2020.606447.

Rodríguez, T., Pérez, O., Ménager, N., Ugrinovic, S., Bracho, G., Mastroeni, P., 2005. Interactions of proteoliposomes from serogroup B Neisseria meningitidis with bone marrow-derived dendritic cells and macrophages: adjuvant effects and antigen delivery. Vaccine 23, 1312–1321. https://doi.org/10.1016/j.vaccine.2004.07.049.

Saavedra, D., Fuertes, S.A., Suárez, G.M., González, A., Lorenzo-Luaces, P., García, B., et al., 2019. Biomodulina T partially restores immunosenescent CD4 and CD8 T cell compartments in the elderly. Exp. Gerontol. 124, 110633 https://doi.org/10.1016/j.exger.2019.110633.

Sierra-González, V.G., 2019. Cuban meningococcal vaccine VA-MENGOC-BC: 30 years of use and future potential. MEDICC Rev. 21 (4), 19–27. https://doi.org/10.37757/MR2019.V21.N4.4.

Sotolongo Padrón, F., Campa Huergo, C., et al., 2007. Cuban meningococcal BC vaccine: experiences & contributions from 20 years of application. MEDICC Rev. 9 (1), 16–22. https://doi.org/10.37757/MR2007V9.N1.6.

Suárez-Formigo, G.M., Saavedra-Hernández, D., 2020. Biomodulina T may restore immunity in older adults. MEDICC Rev. 22 (3), 54–56. https://doi.org/10.37757/mr2020.V22.N3.11.

Subirana, I., Sanz, H., Vila, J.. Comparegroups 4.0: Descriptives by Groups. Available online: https://cran.r-project.org/web/packages/compareGroups/vignettes/compareGroups_vignette.html. (Accessed 21 February 2021).

Sugahara, S., Ichida, T., Yamagiwa, S., Ishikawa, T., Uehara, K., Yoshida, Y., et al., 2002. Thymosin-a1 increases intrahepatic NKT cells and CTLs in patients with chronic hepatitis B. Hepatol. Res. 24 (4), 346–354. https://doi.org/10.1016/S1386-6346(02)00145-6.

Tay, M.Z., Poh, C.M., Rénia, L., MacAry, P.A., Ng, L.F.P., 2020. The trinity of COVID-19: immunity, inflammation and intervention. Nat. Rev. Immunol. 20 (6), 363–374. https://doi.org/10.1038/s41577-020-0311-8.

Tuttle, K.D., Gapin, L., 2018. Characterization of thymic development of natural killer T cell subsets by multiparameter flow cytometry. In: Reinhardt, R.L. (Ed.), Type 2 Immunity: Methods and Protocols. Springer New York, New York, NY, pp. 121–133.

Waldhauer, I., Steinle, A., 2008. NK cells and cancer immunosurveillance. Oncogene 27 (45), 5932–5943. https://doi.org/10.1038/onc.2008.267.

Wang, J., Geiger, H., Rudolph, K.L., 2011. Immunoaging induced by hematopoietic stem cell aging. Curr. Opin. Immunol. 23 (4), 532–536. https://doi.org/10.1016/j.coi.2011.05.004.