TITLE EFFECT OF EXERCISE TRAINING ON LYMPHOCYTE SUBPOPULATIONS IN CHEMICALLY AND HORMONALLY INDUCED PROSTATE CANCER: FLOW CYTOMETRY ANALYSIS **CODE** S.04

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ABSTRACT **Introduction:** Long-term and regular exercise training is suggested to have an immunomodulatory effect, protecting against several diseases. This work aimed to analyse the effect of exercise training on peripheral lymphocyte subpopulations in a model of prostate cancer (PCa) chemically and hormonally induced.

Methods: Fifty-five male Wistar Unilever rats of 4 weeks of age were randomly divided into four experimental groups as follow: control sedentary group (SED+CONT; n=10), control exercised group (EX+CONT; n=10), induced sedentary group (SED+PCa; n=15) and induced exercised group (EX+PCa; n=20). Prostate lesions were induced through the sequential administration of flutamide (50 mg/kg, TCI Chemicals, USA), testosterone propionate (100 mg/kg, TCI Chemicals, Portland, USA) and N-methyl-N-nitrosourea (30 mg/kg, Sigma Chemical, Spain), and subcutaneous implantation of tubes filled with crystalline testosterone (Sigma Chemical, Spain). At eight weeks of age, exercised animals started the training in a treadmill (Treadmill Control LE 8710, USA), 5 days/weeks, for 53 weeks. Animals were sacrificed at 61 weeks of age through an intraperitoneal injection of ketamine (75 mg/kg, Imalgene® 1000, Merial S.A.S., France) and xylazine (10 mg/kg, Rompun® 2%, Bayer Healthcare S.A., Germany), followed by exsanguination by cardiac puncture. Peripheral blood of all animals was collected by intracardiac puncture and transferred into tubes containing EDTA salt as an anticoagulant for flow cytometry analysis. The following conjugated monoclonal antibodies were used: cyCD3-BV421, CD3-FITC, CD25-APC, CD45-BV510, CD127-PE, CD161-FITC, CD4-PE/Cy7, CD45RA-APC/Cy7, OX-82-PE and CD8a-PerCP. The flow cytometry immunophenotyping was performed in a BD FACSCantoTM II cytometer (BD Biosciences, USA) and data were analysed with InfinicytTM, flow cytometry software 1.7 version. The prostate was collected and stained with H&E for histopathological analysis. Statistical analysis was performed using SPSS 25. The differences were considered statistically significant at p<0.05.

Results: A higher level of CD161+NK cells were observed in EX+PCa group when compared with SED+PCa group (p<0.05). These results are in accordance with the literature which suggests that exercise training increase NK cells number. Moreover, long-term exercise training increased gama delta Tcells/CD3 ratio and decreased Treg/NK ratio in PCa-induced groups (p0.05).

Conclusion: These results reinforce the beneficial role of exercise in anti-tumour immune response. Additional studies are warranted to better understand these results.

CONFLICT OF INTEREST No potencial conflict of interest to report.

TITLE UTILITY OF CD326 TO DETECT EPITHELIAL CELLS IN SEROUS FLUIDS **CODE** S.05

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ABSTRACT The suspension cells in the serous fluids are essentially leukocytes and mesothelial cells. In cases of malignancy, tumor cells infiltrate the serous space through direct or lymphatic dissemination. Cytopathology, aided by immunocytochemistry, is considered the “gold standard” in the detection of malignant cells in effusions. CD326 (epithelial-specific antigen, clone Ber-Ep4) is a cell membrane glycoprotein on human epithelia. It is expressed by a large range of epithelial tumors, including skin, gastrointestinal, breast, and tumors of male and female genitourinary tract. A presence of epithelial cells in the body fluid should raise the suspicion of metastatic epithelial malignancy.
With this work, we assessed the interest in the investigation of CD326 positive cells in serous fluids through flow cytometry in a routine way in our hospital. The clinical pathology and the surgery services of our institution studied 32 serous fluids with clinical suspicion of a neoplastic etiology. 26 ascitic fluids and 6 pleural fluids, 13 female and 19 males with age comprised between 47 and 87 years and mean age of 69 years. All samples were evaluated, by flow cytometry and cytopathology. To 500 µL of serous fluid sample was added the monoclonal antibodies: 5µL of CD326 FITC, 10µL of CD33 PE, and 5µL of CD45 APC. After an incubation period of 15 min in the dark was added 2 ml of FacsFlow, centrifuged, and resuspended in 500 µL of FacsFlow. The acquisition was performed in a FACSCalibur; in a first step acquired all the events, in a second step performed a gate in CD45 negative events and acquired until 500 thousand events. The results were analyzed by the InfinicytTM 1.8. We search the presence of CD45-CD33-CD326+ cells; that identification leaves us to presume that positive sample for malignant epithelial cells and their absent negative. The results of the flow cytometry were compared with the cytopathology reports demonstrated a sensitivity of 92% and specificity of 89%. Of the 32 samples analyzed, 30 samples (94%) had concordant results and 2 samples (6%) discordant results. The discordant samples were ascitic fluid, one false negative and one false positive.
In the future is needed to establish the minimum value of frequency of CD45-CD33-CD326+ cells to assume a positive sample for malignant epithelial cells. Some samples have a low number of cells, to overcome this limitation must acquire the largest possible number of cells. The detection of CD45-CD33-CD326+ cells by flow cytometry is strongly indicative of the presence of malignant epithelial cells, presenting, in our study, a sensitivity of 92% and specificity of 89%.
Our work clearly shows that study of the CD326 expression by flow cytometry in effusions can be a useful method to identify non-hematological cells of epithelial origin in a routine laboratory.

CONFLICT OF INTEREST No potencial conflict of interest to report.