METASTATIC BREAST CANCER IS ASSOCIATED WITH INCREASED LEVELS OF SOLUBLE INHIBITORY IMMUNE CHECKPOINT PROTEINS AND DECREASED LEVELS OF SOLUBLE STIMULATORY IMMUNE CHECKPOINT PROTEINS

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Background Checkpoint proteins regulate the immune system. Breast cancer (BC) cells exploit the up-regulation or down-regulation of these proteins to evade anti-tumor immune responses. Soluble forms of immune checkpoint molecules (ICM) can be measured in human plasma; however, their biological and clinical significance remain essentially unknown. The present analysis aimed to measure plasma ICMs in metastatic BC patients (pts) and compare them to healthy controls.

Methods Soluble forms of ICM and RANTES, arginase and TGF-b1 were measured using Multiplex® bead array and ELISA technologies, respectively. Plasma samples from 20 metastatic breast cancer (MBC) pts and 45 healthy controls were analyzed for each protein. Data was prospectively obtained. Measured levels were compared between MBC pts and healthy controls using a non-parametric test (Mann-Whitney), p-values below 0.05 were considered statistically significant.

Results The median age of the cohort was 53 years (range 34–79 years). The main metastatic sites included liver (10 pts), bone (8 pts), and lung (6 pts), with brain-, nodal-, re-cur- tum- and skin metastasis presenting in one patient each. The performance status was as follows; PS=0 (11 patients), PS=1 (7 patients,) and PS=2 (2 patients). The median neutrophil-lymphocyte ratio (NLR) was 3.18 (range 0.35 – 10.97). The soluble co-stimulatory molecules, GITR (p<0.0011), GITRL (p< 0.0000), CD27 (p< 0.0049), CD86 (p< 0.0000) and ICOS (p< 0.0157), as well as the co-inhibitory molecules, PD-L1 (p< 0.0022), CTLA-4 (p< 0.0002) and BTLA (p<0.0145) levels were significantly lower in MBC pts compared to healthy controls. Inhibitory molecules TIM-3 (p< 0.0001) and LAG-3 (p<0.0000) were significantly higher than those of healthy controls. Other biomarkers with raised serum concentrations included TLR (p<0.0039). Serum CD80 (p<0.0992), PD-1 (p<0.2325), HVEM (p<0.062,6) and RANTES (p<0.4861) levels were not significantly different between the MBC pts and the healthy controls (table 1).

Conclusions We identified lower levels of CD27, CD28, CD40, ICOS, GITR, GITRL, CD86, PD-L1, CTLA-4, BTLA, arginase, and TGF-β1, and higher levels of TIM-3 and LAG-3 immune checkpoint molecules in MBC pts compared to healthy controls. These results indicate that a down-regulation of soluble ICM pathways and an up-regulation of some inhibitory ICM pathways are associated with MBC patients. To our knowledge, this is the first study to describe soluble immune checkpoint molecules in MBC pts.

Ethics Approval Ethics approval was granted by The Research Ethics Committee, Faculty of Health Sciences, University of Pretoria (Ethics Committee Approval Numbers 517/2017.