Dysregulation of soluble immune checkpoint proteins in newly diagnosed early breast cancer patients.

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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 remains essentially unknown. The aim of the present analysis was to measure the pre-treatment ICM in newly- diagnosed BC patients (pts) and compare them to healthy controls.

Methods: Soluble forms of ICM, as well as cytokines and chemokines, were measured using Multiplex® bead array and ELISA technologies. Plasma samples from 98 BC pts and 45 healthy controls were analyzed for each protein. Data was prospectively obtained. Measured levels were compared between BC pts and healthy controls using a non-parametric test (Mann-Whitney). P-values below 0.05 were considered statistically significant.

Results: Soluble stimulatory molecules GITR ($p<0,000002$), GITRL ($p< 0,007$), CD27 ($p< 0,002$), CD28 ($p<0,003$), CD40 ($p< 0,003$), CD80 ($p< 0,009$), ICOS ($p< 0,0006$), as well as inhibitory molecules PD-L1 ($p< 0.0000001$), CTLA-4 ($p< 0,005$), TIM-3 ($p< 0,00006$), HVEM ($p< 0,00002$) TLR-2 ($p< 0,05$), levels were significantly lower in early BC pts compared to healthy controls. When analyzed according to BC characteristics (TNBC vs. non-TNBC, tumor size, stage, nodal status and age) no significant difference was detected between the soluble levels of these ICM between the different subsets. Additionally, serum CXCL5 ($p< 0,000001$), CCL23 ($p< 0,04$), IL-16 ($p< 0,00005$), interferon-α ($p< 0,03$) and IL1-RA ($p< 0,03$) were significantly lower compared to healthy controls. Serum CX3CL1 or fractalkine ($p< 0,024465$) was significantly higher compared with healthy controls. Serum interferon-γ ($p<0,2$), IL-6 ($p<0.6$) and IL-2 ($p<0.6$) levels were not significantly different between the BC pts and the healthy controls.

Conclusion: We identified low levels of both the stimulatory and inhibitory immune checkpoint molecules, in newly diagnosed, non-metastatic BC pts compared to healthy controls. These results indicate that early BC is associated with a down-regulation of both soluble stimulatory and inhibitory immune-checkpoint pathways. Newly diagnosed early BC pts have a generalized immune-suppression independent of subtype (TNBC vs non-TNBC) and stage, which, to our knowledge, is the first study to describe soluble immune checkpoints in early BC pts.