

## LETTER TO THE EDITOR

# Serum Level of Soluble CD226 Receptor in Healthy Individuals is Highly Variable and Associated with Age

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### To The Editor,

CD226 (DNAM-1) is a molecule that has been recently given a great deal of attention due to its immunomodulatory properties and possible usefulness in assessment of tumors and autoimmune disorders activity. This cellular transmembrane activating immunoreceptor is expressed predominantly on the surface of natural killer cells, most CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells, platelets, and monocytes (1). CD226, also known as DNAM-1 (DNAX Accessory Molecule-1), was originally described as an adhesion molecule that controls NK cell-mediated cytotoxicity (2). CD226 is also speculated to be an important suppressor of autoimmune responses (3). Single nucleotide polymorphism (rs763361) in CD226 gene, which leads to one amino acid substitution (Gly307Ser) in CD226, was reported to be associated with the development of several autoimmune diseases, including type 1 diabetes (T1D), multiple sclerosis, autoimmune thyroid disease (AITD) and rheumatoid arthritis (RA) (3). Interestingly, we and others reported that patients with distal 18q deletion syndrome - a natural model of CD226 haploinsufficiency, seem to be at higher risk of developing autoimmune conditions including T1D, AITD or vitiligo, coexisting with antibody and T regulatory cells deficiency (4). Membrane CD226 (mCD226) expressed on peripheral blood mononuclear cells might be shed from cell membranes by certain proteases and appear in the serum in its soluble (sCD226) form (5,6). There is a substantial body of evidence showing that sCD226 affects the interaction between CD226 and its ligands, resulting in modulation of CD226-mediated immune responses (7).

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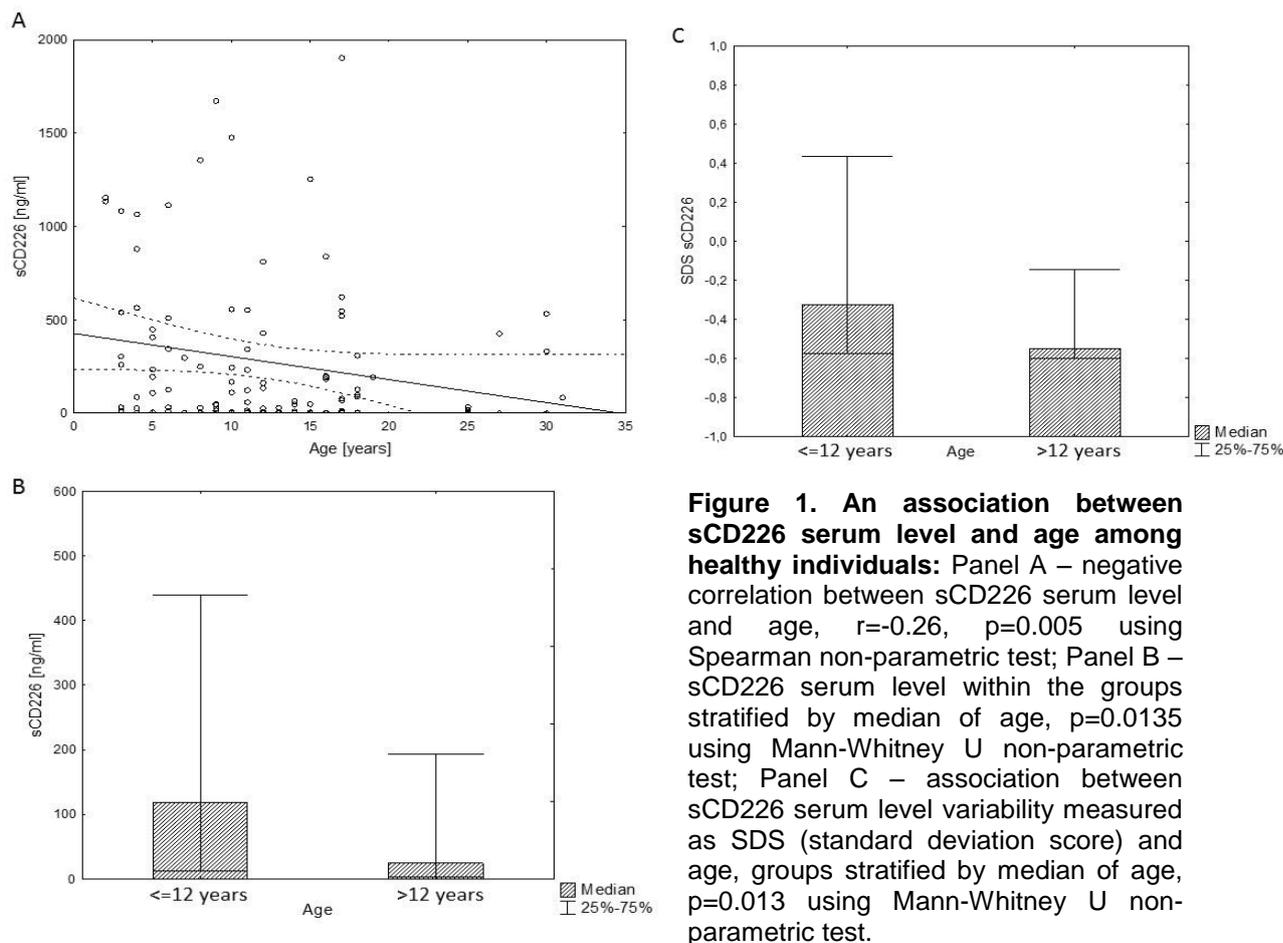
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sCD226 protein directly inhibits cancer cell proliferation giving hope that intervention in the CD226/nectin-2-dependent pathway may be used in the treatment of a variety of tumors (8). sCD226 is also potentially a predictive biomarker for the development of Graft versus Host Disease (GVHD) (5). Moreover, serum level of sCD226 could be considered as an independent risk factor for the prediction of RA within healthy individuals and also for RA disease activity (9).

However, a broad range of levels of sCD226 was observed even among healthy people (5). Since this variability could result from putative confounding factors which might affect further analysis we decided to evaluate the effect of age and sex on serum level of sCD226 among our group of children and young adults of Caucasian origin (n=118, aged 2-31 years, M/F 48/70). The study was approved by the local Ethics Committee and informed consent was obtained from each study subject and/or legal guardian. All serum samples were collected and stored at  $-20^{\circ}\text{C}$  until use. The sCD226 concentration was analyzed using commercial ELISA kit (RayBiotech, USA) with monoclonal antibodies (epitope aa19-247) following the manufacturer's instructions. Like others (5), we observed highly variable serum levels of sCD226 in a group of healthy individuals (median 55.56 ng/ml, quartiles 5.02-333.11 ng/ml and range 0.36-3506.79 ng/ml). Standard deviation score (SDS) for sCD226 level ranged from -0.6 to 7.7 standard deviations from average value for whole study group. A negative correlation between sCD226 and age was found ( $R=-0.26$ ,  $p=0.005$ ) (Figure 1A and 1B). Interestingly, detailed analysis revealed that SDS of sCD226 was also associated with age with the higher values in younger subjects; median -0.32 (-0.58-0.44) vs. -0.55(-0.60--0.015;  $p=0.013$ ) for the groups below and above median age, respectively (Figure. 1C). No significant influence of sex on the level of sCD226 was found; median 48.92 (4.17-342.6) and 78.54 (7.92-320.86) ng/ml for women and men respectively ( $p=0.54$ ). It is difficult to explain why the higher levels were found in the youngest age group. One could speculate that this reflects a process of development, organogenesis and maturation of the immune system in young children and higher activity of certain matrix metalloproteases (MMPs) involved in the proteolytic activation of all important biologically active proteins/peptides in this period (10). It is also unclear why healthy adult people showed a broad range of serum sDNAM-1 levels (5). Kanaya *et al.* speculate that this could be caused by individual variability in MMP activities among study subjects (5).

In conclusion, we found that both serum sCD226 level and its variability are independently associated with the age in general pediatric population. We believe that our comments will be helpful while performing future studies on sCD226, especially concerning pediatric subjects in which age-adjusted measurements of sCD226 should be recommended. Our results are also interesting considering novel treatment strategies with blocking antibodies against TIGIT/CD96/DNAM or CD155.



## ACKNOWLEDGEMENTS

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