INTERNATIONAL SOCIETY OF ONCOLOGY AND BIOMARKERS

43rd Annual ISOBM Congress 2016 Chicago

Evaluation of an improved version of the AroCell TK 210 ELISA for determining thymidine kinase 1 protein levels in serum samples

Jagarlamudi KK[1,2], Holmgren S[3], Venge P[4], Eriksson S[1,2]

1. Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, VHC, Po Box -7011, SE 75007, Uppsala, Sweden.
2. AroCell AB, Viding's Alle 32B, SE-754 50 Uppsala, Sweden.
3. Diagnostics development, Uppsala, Sweden.
4. Department of Medical Sciences, Uppsala University, Sweden.

INTRODUCTION

- Thymidine kinase 1 (TK1) is a pyrimidine salvage pathway enzyme involved in DNA precursor synthesis and its activity is cell cycle dependent.
- During uncontrolled cell proliferation TK1 leaks into the blood and forms stable aggregates which in turn indicates cell turnover.
- Serum TK1 activity is an established marker for blood malignancies and several available activity based assays include TK-REA and TK-Liasion [1, 2].
- TK1 forms aggregates in serum with different enzyme activities, and especially in subjects with solid tumours there is a large fraction of inactive TK1 protein, leading to decreased discrimination between healthy and pathological values [3].
- Antibody based assays provide an alternative way for TK1 measurements, with superior sensitivity in samples from solid tumour patients compared to the TK1 activity assays [4].
- The AroCell TK 210 ELISA have been demonstrated to provide increased discrimination between TK1 serum levels in healthy subjects and those with breast cancer [5].
- This study demonstrated how changing the composition of the zero calibrator standard increased the capacity to determine the TK1 protein levels in healthy individuals.

TK 210 ELISA PROCEDURE

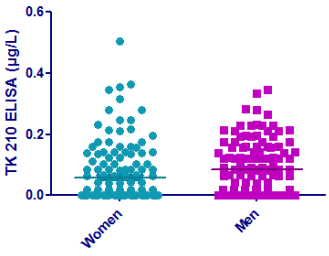
- Calibrators, controls and serum samples were diluted **1:1 in the sample dilution buffer** and incubated at room temperature (RT) for **60 min**.
- Plates with coated antibody were prewashed 4 X 3 min with wash buffer (WB).
- Incubate the plate with prepared calibrators, controls and samples **at RT for 2h**.
- Wash 4 X in WB .
- Add Biotinylated anti-TK1 antibody diluted in reagent buffer
- Incubate at RT 60 min.
- Wash 4 X in WB
- Add Strep– HRP and incubate for **30 min** at RT.
- Wash 4 X.
- Add Substrate TMB and Incubate for **15min**.
- Add Stop solution and measure the absorbance at **450 nM**.
- TK1 protein concentrations were determined by 4-PL analysis.

HYPOTHESIS

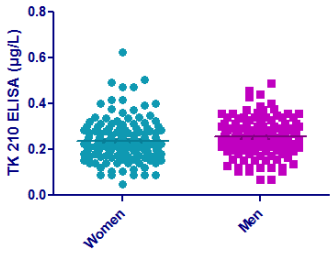
- A serum matrix is important that it stabilizes the TK1 protein in assay calibrators but it increases the zero calibrator value and reduces the ability to measure TK1 in sera from healthy persons.
- Our hypothesis was that an altered composition of the zero calibrator could improve the sensitivity of the AroCell TK 210 ELISA for sera with low TK1 protein levels.

RESULTS

- TK1 protein levels in 271 healthy persons [148 women and 123 men] were determined using the AroCell TK 210 ELISA. TK1 protein levels were estimated using both a serum matrix based and sample dilution buffer based zero calibrators.
- Using the sample dilution based zero calibrator significantly increased the mean TK1 protein levels found in healthy compared to serum matrix based zero calibrator [0.25 ng/mL compared to 0.08 ng/mL]. Figures 1A and 1B shows the TK1 protein distribution in women and men with the different zero calibrators.
- Fig 2 shows the age distribution of TK1 protein levels in this group of healthy persons, using the serum dillution buffer zero calibrator. Mean and median TK1 protein levels for each group are shown in Table 1.
- Over all the mean TK1 protein levels may be slightly increased between 30 to 60 and decresed after 60 years of age, but these differences were not statistically significant.



1A. Serum matrix zero calibrator



1B. Sample dilution buffer zero calibrator

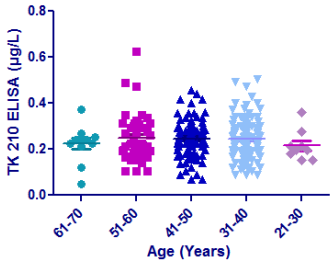


Figure 2. Serum matrix zero calibrator

	TK 210 ELISA (µg/L)			
Age	N	Mean	Median	SD
61-70	12	0.22	0.24	0.08
51-60	45	0.27	0.23	0.16
41-50	80	0.25	0.24	0.08
31-40	120	0.24	0.24	0.08
21-30	9	0.21	0.2	0.07

Table 1. Mean and median TK1 protein levels in different age groups

CONCLUSION

- ☑ Using the improved zero calibrator matrix increased the sensitivity of AroCell TK 210 ELISA so that TK1 protein could be detected in sera from more than 95 % of healthy persons compared to approximately 50% when using the earlier zero calibrator matrix.
- ☑ Increasing the accuracy of determining TK1 levels in healthy samples offers the potential for better discrimination between healthy and pathological resulting in increased clinical value of the assay.

Acknowledgements:

This work was funded by AroCell AB, Uppsala, Sweden

References

1. Kallander CF, et al. Serum deoxythymidine kinase gives prognostic information in chronic lymphocytic leukemia. Cancer 1984, 54:2450-2455.
2. Öhrvik A, et al. Sensitive nonradiometric method for determining Thymidine kinase 1 activity. Clin. Chem. 2004 50: p.1597-1606.
3. Jagarlamudi KK, et al. Breast and prostate cancer patients differ significantly in their serum Thymidine kinase 1 (TK1) specific activities compared with those hematological malignancies and blood donors: implications of using serum TK1 as a biomarker. BMC cancer, 2015, 15:66
4. He, Q, et al. Concentration of thymidine kinase 1 in serum (S-TK1) is a more sensitive proliferation marker in human solid tumors than its activity. Oncol. Reports, 2005, 14: p. 1013-1019.
5. Kiran Kumar J et al, A clinical evaluation of the TK 210 ELISA in sera from breast cancer patients demonstrates high sensitivity and specificity in all stages of disease. Tumor Biology, 2016 (Epub a head print).