The Assay of Thymidine Kinase 1 in Sera from Haematological and Solid Tumours with AroCell TK 210 ELISA: Comparison with TK-Liaison and its Clinical Applications

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ABSTRACT

- Thymidine kinase 1 (TK1) is a pyrimidine salvage pathway enzyme involved in DNA precursor synthesis and its expression is S phase dependent.
- During uncontrolled cell proliferation, high levels of TK1 leak into the blood and form stable aggregates which in turn indicate cell turnover (1).
- TK1 enzyme activity (e.g. DiaSorin LIAISON[®] TK) is an established biomarker for hematological malignancies. However, the activity based assays may under-estimate TK1 levels particularly in sera from patients with different solid tumours due to the presence of inactive TK1 complexes (2, 3).
- To overcome this issue, AroCell has developed an ELISA for TK1 protein measurements. The AroCell TK 210 ELISA kit uses monoclonal antibodies against specific exposed epitopes in the C-terminal region of the human TK1 protein (Fig1) and a unique sample pre-treatment buffer that reduces the high molecular weight complexes and exposes the TK1 epitope for antibody binding.
- A recent study demonstrated that TK 210 ELISA had significantly higher sensitivity for differentiating healthy from breast cancer patients compared to the TK1 activity assays (4).

OBJECTIVES

patients with hematological and solid tumor diseases.

MATERIALS AND METHODS

- Serafrom patients with hematologic malignancies (N=51; Leukemias and lymphomas (N=35), Myeloid dysplastic syndrome (N=8) multiple myelomas (N=7)) were collected from the Akademiska University Hospital, Uppsala, Sweden.
- Samples from solid tumors [breast cancer (n=60); benign hyperplasia and prostate carcinoma patients with a known PSA values (in the range 2 to 10 μg/L) (n=60)] along with healthy individuals (N=102) were collected from the University Medical Centre, Ljubljana, Slovenia.
- TK1 protein levels in serum samples were determined using the AroCell TK 210 ELISA kit as described (www. arocell.com; www.e-labeling.eu/ARO1001-15-5).
- TK1 activity in the same sera were determined by TK-Liaison assay following the assay procedure as described (www.DiaSorin.com).

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Fig 1: A model of human TK1 sub unit.

• The main objective of this study was to evaluate the performance of the AroCell TK 210 ELISA kit in comparison with the TK-Liaison assay in sera from



Fig 2: TK 210 ELISA calibration curve.

CONCLUSIONS

- This study showed that the AroCell TK 210 ELISA kit is a robust, precise and sensitive alternative to TK-Liaison assay when studying TK1 in hematological malignancies.
- The AroCell TK 210 ELISA had higher sensitivity for TK1 in patients with solid tumors compared to TK-Liaison potentially widening the clinical applicability of TK1 in cancer management.

RESULTS

- In healthy individuals, the TK1 protein levels were in the range of 0.1 to 0.35 µg/L with a median value of 0.2. The TK1 protein levels in men had higher median compared to women (0.21 vs 0.19) with no significant difference.
- Both the TK 210 ELISA and the TK-Liaison assays showed significantly higher TK1 levels in hematological and solid tumors compared to blood donors (P<0.0001).
- ROC curve analysis demonstrated that at 96% specificity, the TK 210 ELISA (cut-off value of 0.34 μ g/L) gave higher sensitivity of 0.43 to separate healthy subjects from those with all those malignancies compared to TK-Liaison assay (cut-off value of 10 U/L) of 0.36.
- The relative performance of assays differed depending on the type of malignancies, TK 210 ELISA and TK-Liaison had similar sensitivities (0.65) for hematological malignancies (Fig 3A).
- In case of solid tumors, TK 210 ELISA had higher sensitivity (0.35) compared to TK-Liaison (0.26) at the specificity of 0.96 (Fig 3B)
- A regression analysis of the TK 210 ELISA (y) and TK-Liaison (x) assays for all the three groups gave an equation of y = 0.15 + 0.150.017x (rs = 0.78, n = 265) after excluding high TK1 sera from Acute myeloid leukemia patients (N=8).
- The correlation value in hematological malignancies (rs=0.92, Fig 4A) was higher than for healthy individuals (rs=0.67, Fig 4B)
- The correlation between TK 210 ELISA and TK-Liaison was low in patients solid tumors (rs=0.50, Fig 4C) as ELISA measures both active and inactive forms of TK1 while the other not.





Fig 4: Correlation b/n TK-Liaison and TK 210 ELISA (A) hematological sera excluding Liaison values above 100 (N=8). (B) Blood donors (C) Solid tumors.

-----AUC = 0.92 vs 0.95AUC = 0.85 vs 0.83---- TK_Liaison ---- TK_210_ELISA ---- TK_Liaison ---- TK_210_ELISA

Fig 3: Comparison of ROC curves of TK 210 ELISA and TK-Liaison. (A) for hematological malignancies (B) for solid tumors.



100-Specificity