A New ELISA for Quantification of the blood biomarker TK1 in Canine malignancies

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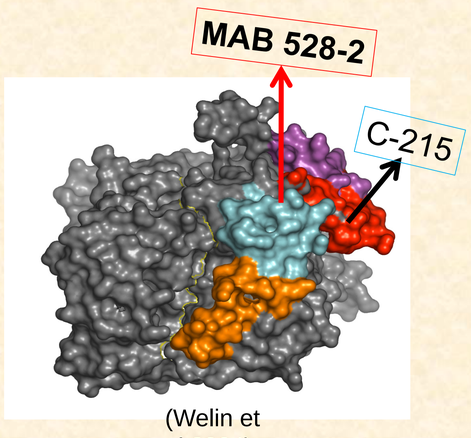
Introduction

- Thymidine kinase 1 (TK1) is a ATP dependent enzyme involved in DNA precursor synthesis and its activity is cell cycle dependent.
- Un controlled cell proliferation which is a main characteristic of cancer progression results in leakage of TK1 into the blood.
- Serum TK1 activity is an established marker for blood malignancies and several activity based assays are available, including TK REA, TK Liasion and [^{3}H]-dThd phosphorylation (1, 2, 3).
- The development of TK1 peptide antibodies has shown an alternative for TK1 measurements, especially in case of solid tumour diseases, where TK1 activity assays have limited sensitivity (4).
- Here we attempt to develop a sandwich ELISA for determining TK1 protein levels in different canine malignancies. Furthermore, we compared its performance with the results obtained with a TK1 activity assay.

Method

- The new robust TK210-ELISA was developed based on anti-TK1 antibodies that were produced against the C-terminal (C-215) region, which served as a detector and against the active site of TK1 as a catcher antibody (MAB 528-2, provided by AroCell AB, Uppsala).
- Pre-incubation of samples with a special serum dilution buffer (from AroCell AB) is a crucial step for consistent results. Recombinant canine TK1, diluted in the serum dilution buffer, was used to create a standard curve (Fig 2).



(Welin et al.2004)

Fig 1

Procedure for the anti dog TK1 ELISA

- Recombinant dog TK1 and serum samples were diluted **1:1 in the serum dilution buffer** and incubated at RT for 60 min.
- Plates with coated antibody were prewashed 4 X 3 min with wash buffer.
- The plate with prepared calibrators, controls and samples is incubated **at RT for 2h**.
- Washed 4 X in wash buffer (WB) .
- Biotinylated anti-TK1 antibody diluted in wash buffer
- Was added and Incubate at RT 60 min.
- Wash 4 X in WB
- Streptavidine– HRP was added and incubate 60 min at RT.
- Wash 4 X in WB.
- The coloremetric substrate TMB was added
- Incubate for**: 15 min.**
- The Stop solution was added and the absorbance at **450 nM recorded**

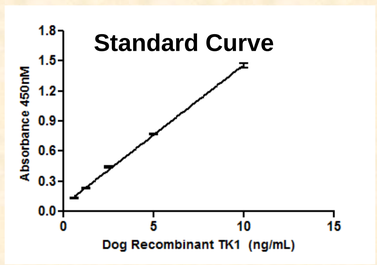


Fig 2

Results

- Standards with different concentrations of recombinant dog TK1 ranging from 0.6-10 ng/mL were used.
- The Lowest level of detection was 0.46 ng/mL and within-run CVs were less than 10%. Serum TK1 protein levels were determined by using the calibrations curve as shown in Fig 2.

TK1 protein and activity levels in Canine malignancies

- ✓ The TK1 ELISA performance was evaluated and compared with the activity assay results for Canine haematological and solid tumours (Fig 4 and 5).
- ✓ TK1 protein levels in sera from dogs with haematological (n=43) and solid tumours (n=55) were significantly higher compared to healthy dogs (n=42) (Fig 4a &5a).
- ✓ However, significantly higher TK1 activity levels were found only in haematological tumours but not in sera from the solid tumour group in comparison with healthy dogs (Fig 4b& 5b).

CONCLUSIONS

- ✓ **The new anti dog TK1 ELISA could detect increased TK1 protein levels in sera from dogs with solid tumours which was not seen with the TK activity assays.**
- ✓ **The new TK1 ELISA was sufficiently sensitive for monitoring chemotherapy of dogs with malignant lymphoma and thus serve as a valuable tool in veterinary medicine.**

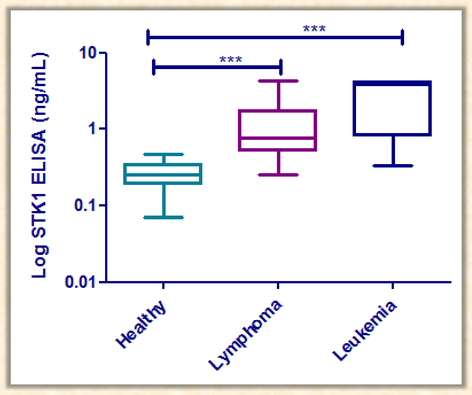


Fig 4a

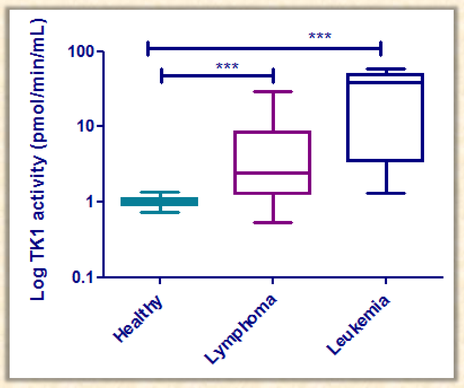


Fig 4b

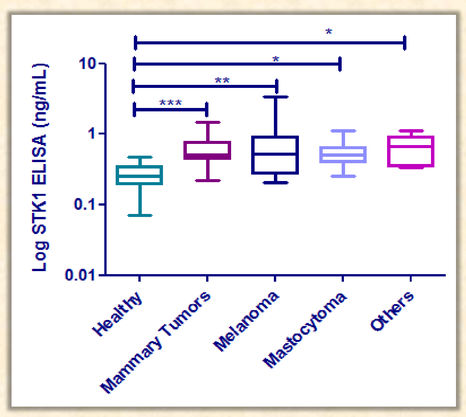


Fig 5a

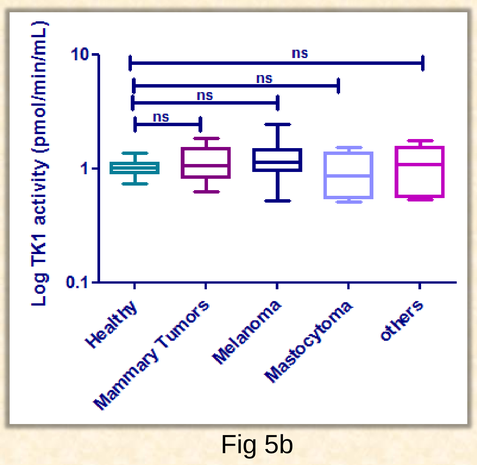


Fig 5b

ROC curve analysis of TK1-ELISA and TK1 activity assays

- ✓ The ROC curve analysis of the results showed that both assays are sensitive enough for differentiating sera from dogs with haematological tumours from healthy dogs.
- ✓ The new TK1 ELISA also had a higher sensitivity for dogs with solid tumours (60%) (Fig 6a) compared to the results with the TK1 activity assay (20%) (Fig 6b).

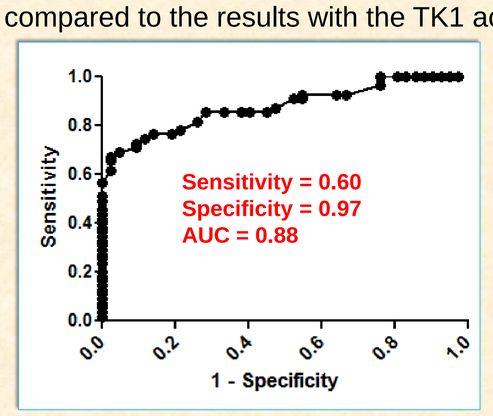


Fig 6 a

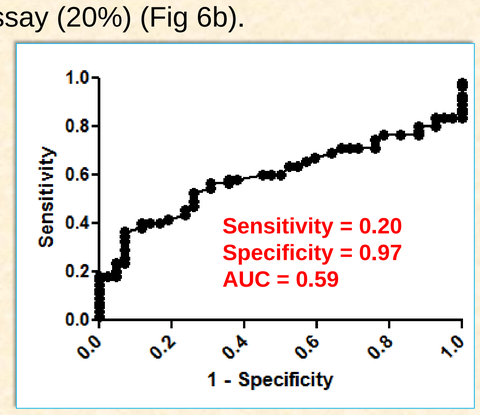


Fig 6 b

TK1 activity and protein levels in Canine Lymphoma during therapy

- ✓ 7 dogs with malignant lymphoma were follwed during therapy and serum samples were collected after each dose.
- ✓ The TK1 protein levels showed similar patterns as the TK activity levels (Fig 7a & 7b).
- ✓ In case of dogs in remission, both TK1 activity and TK1 protein levels reached normal values after one or two doses of therapy.
- ✓ 2 out of 7 dogs had increased TK1 activity and protein levels after therapy which correlated with tumor relapse in these dogs.

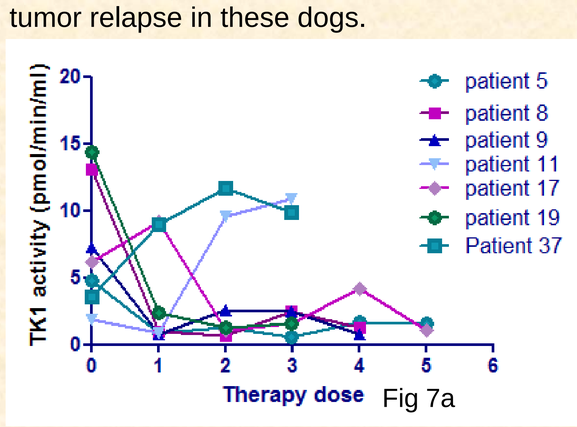


Fig 7a

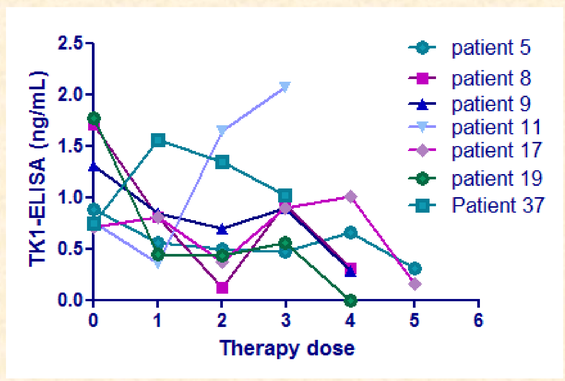


Fig 7b

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