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## Loss of cd45 protein in canine small clear cell/t-zone lymphoma is due to absence of gene transcription.

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### Abstract

Canine small clear cell/T-zone lymphoma (TZL) is a peculiar lymphoma subtype characterized by an indolent clinical course and aberrant CD45-negative phenotype, easily recognized by flow cytometry (FC). Recent studies have described clinical presentation and behavior, but to date the mechanisms for CD45-negativity have never been investigated.

Aim of this study is to confirm the lack of surface protein using a different technique from FC and to investigate if CD45-absence in TZL is linked to the lack of the corresponding transcript and gene.

40 TZL cases and 17 controls (7 T-high grade lymphoma, 10 reactive lymphnodes) were included in the present study. Immunohistochemistry was performed with a different antibody respect with FC to confirm CD45 surface protein absence. Total RNA and genomic DNA were extracted from lymph-nodes aspirates. CD45 transcript amount was investigated by quantitative real-time RT-PCR and the corresponding gene fragment was analyzed by quantitative real-time PCR.  $\Delta\Delta C_t$  method was used for the relative quantification of transcript amount and DNA load compared to housekeeping genes.

All TZL cases were negative for CD45 at immunohistochemistry. CD45 transcript amount was significantly lower in TZL compared to controls ( $p=0.000$ ). This difference was not significant ( $p=0.584$ ) for CD45 DNA load, that was similar between TZL and controls.

These results highlight that CD45 protein is lacking on cell surface and gene transcription is absent in TZL, whereas the corresponding gene is not deleted. The data here reported support further studies for clarifying possible genomic or epigenomic factors involving CD45 gene transcription and for better clarifying the possible role of CD45 in lymphomagenesis.

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