# Proceedings

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## Computer-assisted quantification of caix membrane immunoreaction destined for the clear cells in renal carcinoma. A pilot study.

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### Introduction/ Background

Carbonic Anhydrase IX [CAIX] has been considered as a candidate prognostic factor in clear-cell renal carcinoma [CCRC], however the supporting evidence is conflicting. CAIX is strongly induced by hypoxia via HIV-1α, and in CCRC via mutations to the VHL gene. CAIX expression could be identify as an immunohistochemical predictor of CCRC patients outcome but the published studies related to the patients prognosis have based on the diverse quantification protocols of CAIX expression (TMAs vs. whole tissue section; semiquantitative vs. computerised image analysis; with/without intensity scoring; with various software). The available commercial image analysis tools are mainly for general purpose e.g. software for breast carcinoma HER2 membrane immunoreaction has been used in various tumour tissue studies. However the cytological images of CCRC and breast carcinoma show essential differences related to the nuclei (size, outlines, intracellular location) and nuclear/cytoplasmic proportion which could influence the measurement credibility in maladjusted algorithm.

### Aims

The aim of our study was to evaluate an algorithm for quantification of the membranous CAIX expression specifically dedicated to CCRC ("snake variant") in comparative analysis to applied HER2 breast cancer algorithm for CCRC.

### Methods

In the quantitative analysis of the specimen, the image processing follows: recognition of the cell nuclei; segmentation of the immunoreactive cell membranes; the assignment of the membrane segments to an individual cell. The last step is challenging for analysis due to frequent discontinuities in membranous immunoreaction, great variability of cellular counters and intracellular nuclei location. Because the classical watershed method for the individual cell separation is insufficient, the snake active contour method was applied, starting from each nucleus outline. The built gradient image allowed to select the most adequate parameters in the snake adaptation process. The recognized snake represents the membrane associated with the particular cell. The material includes records of 39 patients with the histopathologically verified diagnosis of CCRC who had nephrectomy (between 2009-2011) and were treated with tyrosine kinases agents (the Clinic of Oncology registry). 74% (29 out 39) of patients presented stage I - T1 N0; 20,5% - stage III and 5,4% stage TII. The formalin-fixed tissue sections of the resected CCRCs (the Pathology Department registry) were immunostained for CAIX protein using CAIX antibody (clone NB100-417) (Antibodies-online GmbH) with EnVisionTM (DAKO) according to the manufacture recommendations. The representative digital images were selected from each Whole Slide Image (scanned with Aperio, under 20x) and were assessed automatically by 3 independent observers using two algorithms: "snake variant" and "breast HER2". The extend of staining (percentage) was scored in the 10% intervals of CAIX positive carcinomatous cells and the intensity of immunoreaction was evaluated in 3 grade scale (1-3).

*Results*

The obtained results have been under investigation for the intra- and inter-observer accuracy as well as for the comparative data analysis of both types of algorithm. The statistical analysis has been incorporated. This approach explores a new possibility of the computerised quantitative estimation of the membrane CAIX immunoreaction destined for the specific clear cells in renal carcinoma.