METHODS FOR AUTOMATED IN SITU ANALYSIS OF BIOMARKERS IN HUMAN PLACENTAL CHORIONIC TISSUE –

QUANTIFICATION OF THE RECEPTOR FOR ADVANCED GLYCATED END PRODUCTS (RAGE) IN HUMAN HEALTHY AND PRE-ECLAMPTIC PLACENTAS

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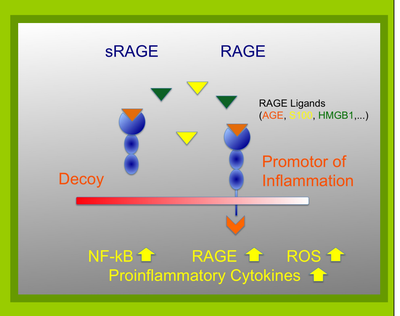
INTRODUCTION

1.Tissue-Cytometry combines microscopy with automated image segmentation and computer-based parameter quantification. By enabling protein localization and reproducible parameter measurements in large tissue areas, it is an essential tool to analyse protein expression in healthy and diseased tissues. However, algorithms for automated segmentation and analysis of multinucleated as well as a-nuclear (erythrocytes) cells are lacking.

2. Binding of ligands to **receptor for advanced glycation edproducts (RAGE**) leads to up-regulation of RAGE. Activation of RAGE contributes to chronic inflammatory states found in e.g. diabetes mellitus or Alzheimer disease. Soluble RAGE (sRAGE) lacks the ability to transduce signals, can act as endogenous RAGE-antagonist and may be used in therapy.



3. Pre-eclampsia (PE) is a leading cause of maternal and fetal mortality and morbidity. The complex biology of PE is still poorly understood and effective therapies are lacking. However, PE is characterized by systemic inflammation, suggesting promotion of inflammation via activation of the ligand-RAGE axis. Indeed, elevated serum-levels of RAGE-ligands in women with PE were demonstrated. Detailed quantitative analysis of RAGE expression and localization in PE compared to healthy placentas are lacking, but are required to understand RAGE-involvement in the development of PE.

AIMS

1. Development of algorithms for segmentation of placental tissue bearing multinuclear syncytiotrophoblast (STB) and auto-fluorescent erythrocytes.
1. Automated RAGE measurement in healthy and PE placentas .

SUMMARY

1.Algorithms to perform Tissue-Cytometry of placental tissue were developed.

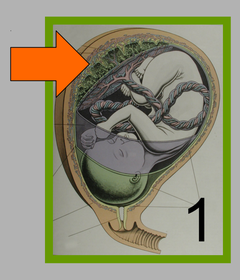
1.Elevated RAGE expression in the STB of PE-placentas correlating with PE-severity supports contribution of ligand-RAGE axis to inflammation.

MATERIAL AND METHODS

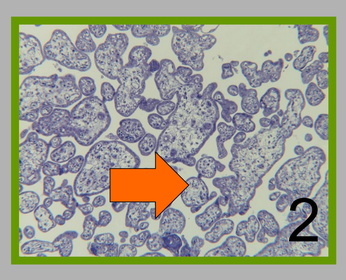
1. Formalin-fixed paraffin-sections of chorionic tissue from PE (n=14) and healthy (n=13) placentas were processed by immunofluorescence microscopy (IFM). Frozen healthy tissue was used for RT-(q)PCR and westernblotting.
2. Digital fluorescence- and corresponding transmitted light-images (9 x 9 images/sample) were acquired with TissueFAXS, an epi-fluorescence microscope with motorized stage (TissueGnostics GmbH, Vienna, Austria).
3. Algorithms were developed combining classical digital image-processing and pattern recognition approaches with machine-learning techniques. The F1-score was computed to compare human ground truth data with algorithm output.

BACKGROUND

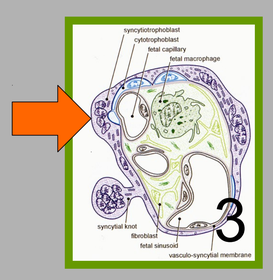
1. In the human placenta (1), chorionic villi are the major functional units (2). The multinucleated STB (3; Cytokeratin 7^+/$CK7^+$) covers the surface of the villi and faces maternal blood.



Placenta



Chorionic villi (semithin section, HE-staining)

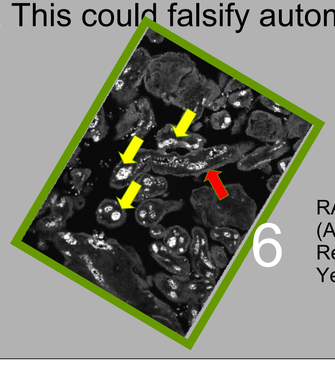


STB covering villus

2. Automated segmentation of the STB could be based on CK7-expression (4). As the STB covers all villi, this would also enable automated detection of total villus area (TVA) composed of STB and non-STB (stromal core) tissue (5).

CK7-expression in chorionic villi analysed by IFM.
Red arrow indicates extravillous space (maternal blood)
Blue arrow indicates stromal core of the villi (non-STB cells)
STB (orange) and stromal core (blue) sum up to total villus area

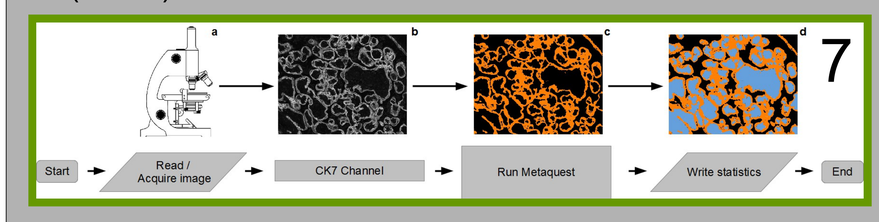
3. Placental sections (6) contain many fetal erythrocytes which exhibit bright autofluorescence. This could falsify automated quantification of proteins in IFM.



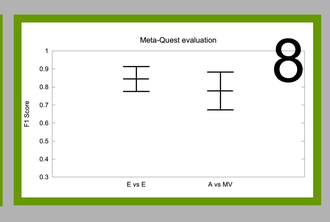
RAGE-expression in placental villi detected by IFM (Alexa-568-conjugated secondary antibodies).
Red arrow indicates RAGE in STB.
Yellow arrows indicate auto-fluorescent erythrocytes.

RESULTS

1. Algorithm (7) for detection of placental STB and TVA and validation data (8) obtained from comparison of the computer-masks to human expert mark-ups (n= 31).

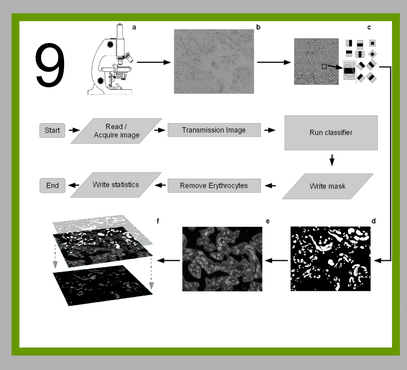


Workflow of the algorithm. Orange indicates the resulting STB-mask, blue the stromal core-mask. STB and stromal core add up to TVA

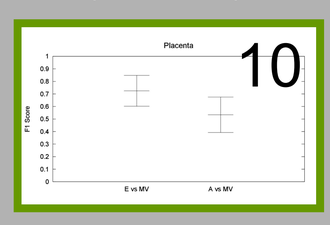


F1-Score for Expert vs Expert and Algorithm vs Majority Vote (n ≥ 2) pixelwise comparison of STB masks.

2. Algorithm (9) for detection of erythrocytes and validation data (10, n= 31).

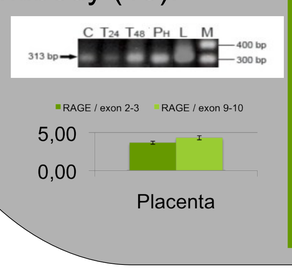


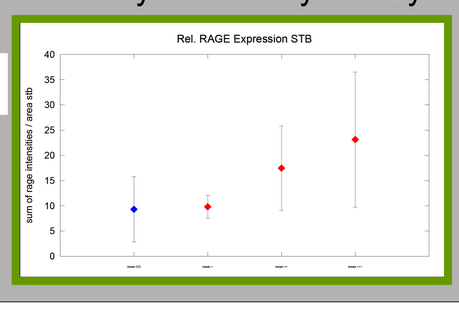
Workflow of erythrocyte detection. A transmission image serves as input for a classifier (cascade of boosted decision trees of haar-like features). The output mask can later be combined with different fluorescence channels to localize erythrocytes and eliminate their associated autofluorescence.

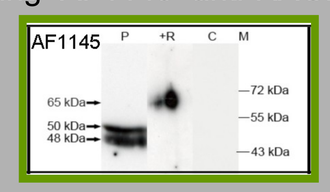


F1-Score for Expert vs Majority Vote (n ≥ 2) and Algorithm vs Majority Vote (n ≥ 2) pixelwise comparison of erythrocyte masks.

3. RAGE mRNA (11) was detected in placental tissue (P) and isolated (C) and cultured (T) trophoblasts. RAGE protein expression was compared between healthy and PE placentas by Tissue-Cytometry applying AF1145 anti-RAGE antibody (13).







RAGE per STB-area in healthy (blue) and PE-placentas (red). mild (n=3), medium (n=4), and severe (n=7) PE).

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