School of Doctoral studies

-summary of PhD thesis-

Title: Role of microRNA in conjunction with inflammatory markers in pediatric and adult gastritis

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Introduction: Gastritis constitutes a major health problem, both in adults and children. *Helicobacter pylori (H. pylori),* its main etiological factor, is directly responsible for inflammation persistence and initiation of carcinogenetic processes. Due to its direct involvement in the development of gastric cancer and its systemic, extra-digestive impact, multiple molecular biology studies have tried to describe the physiopathological mechanisms triggered by this pathogen. Regulatory role of intra- and intercellular signaling molecules on mechanisms involved in gastric cancer genesis was intensely studied, with microRNAs being proven as key elements of intercellular communication, with essential implications in modulation of innate and adaptive immune response triggered by *H. pylori.* Literature data proved that microRNA molecules present expression alterations in the context of gastric inflammation and/or neoplasia. Therefore, they have been proposed by multiple authors as diagnostic and prognostic biomarkers.

Objectives:

The first study included 84 patients and its objective was to evaluate the diagnostic sensibility and specificity of IgA and IgG antibodies directed against *H. pylori* in children.

The second study analysed the impact of paediatric gastritis on haematological parameters and included 173 subjects. This study focused on the variation of platelet/lymphocyte and neutrophil/lymphocyte ratios (PLR and NLR), as non-invasive inflammatory biomarkers, as well as of erythrocyte, platelet indices and mean platelet volume in children with gastritis, by comparing these parameters with subjects without histhopatological changes of the gastric mucosa.

Study 3 included 194 patients and analysed a possible relationship between paediatric gastritis and expression of miR-155, by performing a comparison with cases without microscopic changes of the gastric mucosa.

Study 4 compared gene expression of miR-155 between two adult study groups, one with gastritis of various aetiologies and the other one with dysplastic changes of the

gastric mucosa, and was conducted on 27 patients. A *secondary objective* of this study was to establish possible differences regarding expression of miR-155 between adults and children with inflammatory modifications of the gastric mucosa.

General methodology: The four studies presented within this thesis were conducted prospectively and included patients of paediatric ages (1-18 years old) and adults with chronic dyspeptic symptoms or positive history of preneoplastic modifications of the gastric mucosa (chronic atrophic gastritis, intestinal metaplasia). Each patient underwent a paraclinical evaluation, as well as an upper digestive endoscopy with biopsy sampling from the corporeal and antral gastric mucosa. In order to determine gene expression of miRNA-155 (miR-155), additional biopsies were harvested. Division of patients within study groups was based upon microscopic examination findings.

Results: Study 1 identified a sensitivity of 50% and a specificity of 88.88% for IgA antibodies directed against *H. pylori,* whereas values of 81.25% and 81.08% were detected for the same parameters in the case of IgG antibodies. Thus, the study proves the diagnostic inferiority of serology in comparison with histopathological examination in children.

Study 2 found a lack of significant association between PLR, NLR and gastric inflammation in children, in spite of a slight increase in the value of these parameters in relation to *H. pylori* gastritis. Lymphocyte count could represent a predictive marker for non- *H. pylori* gastritis in children, as lymphocytes presented significantly higher values in these patients ($p<0.01$). Severity of gastritis was significantly associated with anaemia in paediatric patients, independently of *H. pylori* infection, severe gastritis in children being associated with compellingly lower values of haemoglobin and hematocrit ($p=0.04$ and $p=0.02$).

Study 3 did not identify significant variations of miR-155 expression in relation to paediatric gastritis ($p=0.16$). Still, *H. pylori* infection, chronic or severe inflammation of the gastric mucosa seem to cause an augmentation in miR-155 expression, but the obtained results were insignificant ($p=0.44$, $p=0.12$ and $p=0.45$).

Study 4, performed on an adult population, compared expression of miR-155 between a study group with gastritis of various aetiologies and a group with dysplastic changes of the gastric mucosa. A decrease in miR-155 expression was noted in patients with preneoplastic gastric lesions, as opposed to patients with chronic gastritis, but these results were statistically insignificant ($p=0.46$). Moreover, this microRNA type was similarly expressed in the context of chronic inflammation in children and adults ($p=0.87$).

General conclusions: *H. pylori* serology presents a diagnostic sensitivity and specificity inferior to histology in paediatric gastritis. The impact of paediatric gastritis on haematologic parameters is not significant for most of these, but the results obtained within the current research prove that higher counts of lymphocytes are associated with non-*H. pylori* gastritis in children, while severe gastritis is significantly correlated with

lower haemoglobin and haematocrit values. MiR-155 expression seems to be augmented in association with *H. pylori,* chronic or severe gastritis in children, but this variation from healthy gastric tissue is not significant. Moreover, miR-155 expression presented an insignificant decrease in adults with preneoplastic lesions in comparison with those with chronic gastritis. Lack of statistical validity for this result was most probably caused by the limited number of adult patients, but it is important to underline that miR-155 presented similar expression in paediatric and adult chronic gastritis. Comparative, serologic and tissular analysis of miR-155 expression, as well as extension of the research on a greater study group might elucidate the utility of this microRNA as non-invasive biomarker of inflammation and carcinogenesis.

Originality of the thesis

The present PhD thesis brought significant contributions to pre-existing literature data regarding paediatric gastritis, as well as regarding miR-155 role in child's and adult's gastritis. Study 1 is the first one from Romania that evaluated diagnostic reliability of IgA and IgG antibodies directed against *H. pylori* in children. Impact of paediatric gastritis on haematological parameters was studied in the past, but the large number of analysed parameters, as well as classification of gastric inflammation based on multiple criteria, offered a wider image on the subject as opposed to previous reported data. Moreover, variation of miR-155 expression from healthy gastric tissue was investigated for the first time in children with *H. pylori*/non-*H.* pylori gastritis, with study 3 including a significant number of patients. Study 4, conducted on an adult population, tried to establish the progression of miR-155 expression from chronic gastric inflammation to premalignant lesions (gastric atrophy/intestinal metaplasia). These two studies which focused on miR-155 establish the grounds for future studies on the matter, highlighting the need for extending the research on larger population samples, as well as the importance of studying microRNA expression throughout each stage of Correa's cascade.