# Macrophages of the antral mucosa in children with Helicobacter pylori infection and after eradication

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## Abstract

Our study included 59 children, aged 12.2 ± 4.6 years, with Helicobacter pylori infection and 29 children, aged 11.0 ± 4.2 years, with past H. pylori infection after spontaneous eradication with positive IgG antibodies against H. pylori and with functional disorders of the gastrointestinal tract, without H. pylori infection, with normal IgG concentration against H. pylori. All biopsy specimens from each of the study groups were stained by an immunohistochemical method for the evaluation of $CD68^+$ macrophages in the antral mucosa.

Histopathological changes in the antral mucosa of children with Helicobacter pylori are characterized by an increased infiltrate of macrophages, dependent on the severity grade of inflammation.

**Key words:** macrophages, antral mucosa, Helicobacter pylori, children.

## Introduction

Infiltrating cells synthesize and produce mediating molecules which influence the recruitment and activation of further inflammatory cells and potentiate the triggering activity of cytokines and chemokines, for example: neutrophils express IL-1, IL-8, TNF-α [3], and macrophages, which are the source of MIP1-α [1,2,4]. Integrins: CD11a/CD18, CD11c/CD18, α4b1 and α4 β7, play an essential role in the inflammatory process. These are adhesive molecules present in numerous cells and built up of two polypeptides (α and β), fixed in the cell wall. The macrophage-activating complex-CD11b/CD18 (Mac-1), located on monocytes and neutrophils, belongs to one of them. Being a receptor for iC3b, it takes part in a complement-dependent bacteria phagocytosis and lysis [5]. It is the expression of macrophages, which initiates a local inflammatory state in the course of Helicobacter pylori infection [2, 6].

The aim of the study was to assess the expression of macrophages in the inflammatory infiltrate of the antral mucosa in children with H. pylori infection and after eradication.

## Material and methods

A total of 106 patients, divided into three groups with regard to the presence of H. pylori infection, were included in the study. Group I - 59 children (29 girls and 30 boys; the age range: 2-19 years) with chronic gastric mucosa inflammation in the course of H. pylori infection with a positive titre of antibodies in IgG class against H. pylori. Group II - 29 children (14 girls and 15 boys; the age range 3-19) with past H. pylori infection, but with a maintaining positive titre of antibodies in IgG class against H. pylori. Group III - 18 children (12 girls and 6 boys; the age range 5-17) with functional disorders of the gastrointestinal tract, without H. pylori infection, with normal IgG concentration against H. pylori (the control group). Endoscopy and histopathological examinations of the stomach, basing on the Sydney System [7] were performed in all the children. The urease test was performed in the course of endoscopy in all the children. The specimens were fixed in 10% buffered formalin. They were processed, oriented on the edge, embedded in paraffin, cut in sequential 5-μm sections and stained by H+E for the evaluation of inflammation and by the Giemsa method, used to identify H. pylori bacteria. All the biopsy specimens from each study group were stained by an immunohistochemical method for the evaluation of $CD68^+$ macrophages in the antral mucosa. The ABC method was used, according to commercial protocol. The

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*Table 1.* CD68+ cells in the antral mucosa in children.

| Examined groups | CD68+ cells in the antral mucosa/mm² | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Number of patients (N) | Minimum (min) | Maximum (max) | Arithmetic mean (x) | Median (M) | Standard Deviation (SD) | Low Quartile | High Quartile |
| Group I | 23 | 19 | 95 | 54.1 | 60 | 19.4 | 76.0 | 146.0 |
| Group II | 17 | 0 | 54 | 11.3 | 8 | 15.3 | 3.0 | 10.0 |
| Group III | 7 | 0 | 45 | 9.4 | 4 | 15.9 | 1.5 | 7.0 |

*Figure 1.* Correlation between the expression of CD68+ cells and the severity grade of antral gastritis in children with Helicobacter pylori infection (Group I)

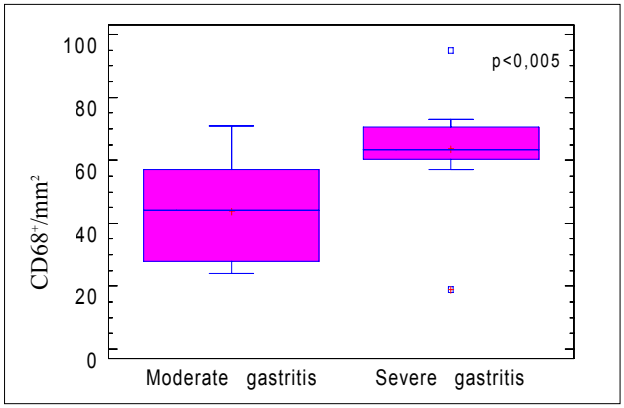
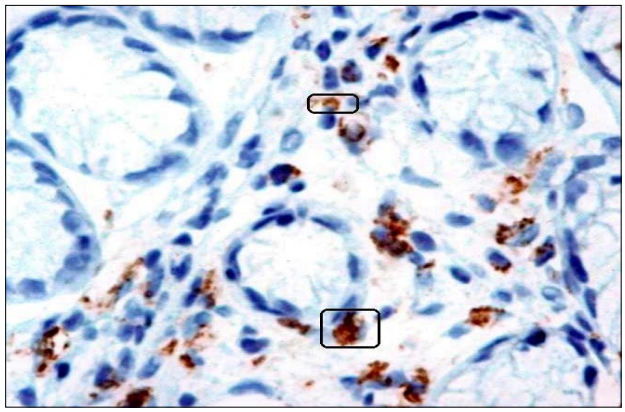



*Photo 1.* Antral mucosa. Immunohistochemical reaction with CD68+. Mag. x 400.



number of CD68+ macrophocytes was counted in discrete areas, measuring 0.785 mm² each, by using a light microscope. All the counts were performed, using a magnification of 200x. The numbers of positively stained cells were presented as the mean values per 1 mm² of analysed gastric section area. All the CD68+ macrophages counts were performed by a single observer (I. K.), who was unaware of either the H. pylori status or of the subject's clinical group.

An analysis of the preparations and their photographic documentation were performed with on Olympus Bx50 light microscope, with a video circuit and a Pentium 120 PC computer with the Lucia G (Nikon) software for microscope image analysis.

Descriptive statistics included the measures of central tendency: the arithmetic mean (x), median (Me) the measures of dispersion: standard deviation (SD), and the minimum (min) and maximum (max) result. The levels of the parameters were compared by means of the U Mann-Whitney test for independent or paired trials. The differences were regarded as statistically significant at $p<0.05$.

All the clinical and laboratory tests were performed in children with a prior consent of their parents and guardians, and of the Bioethical Board of the Medical University of Białystok.

## Results

According to Sydney's Classification, the severe grade activity was found in 69.5% of the infected children and the moderate grade activity in 30.5% of these children. Neither severe nor moderate grade activity was found in children with past infection and after eradication, whereas mild grade activity was revealed only in 20.7% of the group. The analysis of antral gastritis by means of Chi² proved statistic significance ($p<0.001$) in the examined groups. The quantitative analysis of CD68+ macrophages in the antral mucosa showed a significant increase in their expression in children with H. pylori infection (54.1 cells/mm²). In children with past H.pylori infection, the expression of macrophages (11.3 cells/mm²) could be compared to their expression in the controls (9.4 cells/mm²). Photo 1.

When evaluating CD68+ cells in the inflammatory infiltrate, statistically significant differences were revealed between Group I and Group II ($p<0.001$) and between Group I and Group III ($p<0.01$). Table 1.

The quantitative analysis of CD68+ cells showed statistically significant differences in relation to the severity of antral gastritis ($p<0.005$). The expression of macrophages (CD68+) increased in the inflammatory infiltrate, together with the severity of the inflammation. In the children, infected with H. pylori (Group I) (Fig. 1), the expression of CD68+ cells equalled on the average 43.82 cells/mm² in a moderate inflammation of the antral mucosa. In case of severe inflammation of the antral mucosa, the mean expression of CD68+ cells was 63.58 cells/mm² ($p<0,005$). The expression of CD68+ cells in children with H. pylori infection was 45.75 cells/mm² in the antral mucosa with a moderate activity inflammation. In case of severe activity inflammation, the mean expression of CD68+ cells amounted to 55.89 cells/mm².

## Discussion

When assessing $CD68^+$ cells in the antral mucosa, their predominating expression was revealed in children with H. pylori infection. Statistically significant differences were also reported between Group I and II ($p<0.001$) and between Group I and III ($p<0.01$). A positive correlation was found between the expression of $CD68^+$ cells and the severity grade of antral gastritis ($p<0.005$). The expression of macrophages increased directly proportionally to the severity grade of antral gastritis. In children, who spontaneously eradicated Helicobacter pylori, the expression of macrophages was lower in the inflammatory infiltrate, what may prove suppression of inflammatory process, due to an effective immune response in the examined patients. An increased expression of $CD68^+$ macrophages in the inflammatory infiltrate in the lamina propria, associated with Helicobacter pylori infection, was observed by Kusugami et al. [2]. They described a chemokine-macrophage inflammatory protein-1α, for which $CD68^+$ macrophages are target cells. The authors found an increased synthesis and secretion of MIP-1α in more than 50% of patients with Helicobacter pylori, located in the antrum [2].

Phagocytosis is ineffective in Helicobacter pylori infection, in spite of the increased expression of macrophages. Numerous mechanisms were developed by Helicobacter pylori, such as: mechanisms inhibiting oxygenic killing, interactions with lysosomal proteins, a protective activity of surface bacterial proteins: fetuin, heparin sulphate, hyaluronic acid, vitronectina in the presence of complement elements [8, 9].

## Conclusion

Histopathological changes in the antral mucosa of children with Helicobacter pylori are characterized by an increased infiltrate of macrophages, dependent on the severity grade of inflammation.

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