Anna Junga

THE COMPLEX MORPHOPATHOGENETIC ASPECTS OF INTRAABDOMINAL ADHESIONS DEVELOPMENT AND COURSE IN INFANTS

Summary of the Doctoral Thesis
for obtaining the degree of a Doctor of Medicine

Speciality – Morphology

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The Doctoral Thesis is available in the library of RSU and RSU homepage: www.rsu.lv

Secretary of the Doctoral Council of Medicine:
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LIST OF ABBREVIATION

CgA – Chromogranin A

FGF-2 – Basic fibroblast growth factor

FGFR1 – Fibroblast growth factor receptor 1

HBD-2 – Human beta defensin-2

HGF – Hepatocyte growth factor

IFN-γ – Interferon-γ

IL-1 – Interleukin-1

IL-4 – Interleukin-4

IL-6 – Interleukin-6

IL-7 – Interleukin-7

IL-8 – Interleukin-8

IL-10 – Interleukin-10

MMPs – Matrix metalloproteinases

MMP-2 – Matrix metalloproteinase-2

PGP 9.5 – Protein gene product 9.5

TGFβ – Transforming growth factor beta

TIMPs – Tissue inhibitors of metalloproteinases

TIMP-2 – Tissue inhibitor of metalloproteinase-2

TNFα – Tumour necrosis factor alpha

VEGF – Vascular endothelial growth factor

INTRODUCTION

Intraabdominal adhesions are fibrous connections between abdominal organs and/or abdominal organs and surfaces in the abdominal cavity. Intraabdominal adhesions may be classified as congenital or acquired. Acquired adhesions mostly are post-inflammatory or post-operative. The origin of adhesions most often is related to peritoneal injury or scarring (Schanaider et al., 2016; Zhang H. et al., 2016). The morphopathogenesis of intraabdominal adhesions is a complex process, characterised by the accumulation of extracellular matrix, tissue hypoxia and inflammation (Christodoulidis et al., 2013; Coccolini et al., 2013).

Transforming growth factor beta (TGFβ) is the most studied growth factor in case of adhesions so far, it regulates fibrotic processes by suppressing fibrinolysis (Chegini 2008). Other growth factors have also been investigated – hepatocyte growth factor (HGF) suppresses development of postoperative adhesions (Kosaka et al., 2008), basic fibroblast growth factor (FGF-2) decreases expression of inflammatory cytokines interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) (Kashpur et al., 2013), while increased level of fibroblast growth factor receptor 1 (FGFR1) causes defects of wound healing (Meyer et al., 2012).

Intensive vascular endothelial growth factor (VEGF) expression is characteristic in adhesions (Bi et al., 2017), which regulates angiogenesis and oxygen delivery to damaged tissue. Nerve fibers are identified in peritoneal adhesions, but general neuroendocrine system markers have not been identified yet.

There are still discussions about inflammatory response involvement in pathogenesis of adhesions. The reduction of IL-1 and tumour necrosis factor alpha (TNFα) suppresses inflammation and formation of adhesions (Liakakos et al., 2001; Bayhan et al., 2016; Wei et al., 2016; Zhang H. et al., 2016).

Interleukin-4 (IL-4) activates fibroblast proliferation, differentiation of myofibroblasts and collagen production, thereby promoting development of fibrosis (Luzina et al., 2012). Interleukin-8 (IL-8) poses appropriate conditions for fibrosis (Zimmermann et al., 2011) and IL-6 also corelates with high formation and accumulation of collagen; in addition, adhesions fibroblasts produce collagen even more (Vallée et al., 2017). Interleukin-7 (IL-7) promotes synthesis of IL-4 and interleukin-10 (IL-10) (Shmarov et al., 2016). IL-10 is an important adhesion limiting factor, its application has suppressed fibrosis in animal studies (Onishi et al., 2015). However, the interaction between inflammatory cytokines in case of intraabdominal adhesions is unclear, because of a lack of complex data. The appearance of human beta defensin-2 (HBD-2), main defensin of peritoneal membrane, in adhesions has also not yet been analysed.

Studies about the significance of matrix metalloproteinases and its tissue inhibitors are of high interest, because the balance between these enzymes provide formation and degradation of extracellular matrix (Ucar et al., 2010). Matrix metalloproteinasis-2 (MMP-2) is evaluated as a potential marker and target in the treatment of fibrosis (Horejs et al., 2017).

It has to be stressed that there mainly are studies about postoperative adhesions in adults or experimental animals, but there are few studies about adhesions in children. There are no guidelines to detect formation and development of adhesions, which affects diagnosis and therapeutic strategy selection. Nowadays, we can study morphopathogenetic processes at cellular level in the cases of adhesions.

The aim of this study is to evaluate and describe the morphopathogenetic factors which are related to the formation and development of adhesions, as well as describe their interactions.

To conduct the research, the following **objectives** were created:

1. to determine the relative amount and distribution of these factors in the tissues of intraabdominal adhesions:

a) tissue growth factors and their receptors (TGFβ, HGF, FGF-2, FGFR1);

b) angiogenesis affecting factors (VEGF);

c) general markers of diffuse neuroendocrine system (PGP 9.5, CgA);

d) markers regulating inflammation (IL-1α, IL-4, IL-6, IL-7, IL-8, IL-10, TNFα);

e) antimicrobial defense protein (HBD-2);

f) tissue degrading enzyme (MMP-2) and their inhibitor (TIMP-2);

2. to determine the relative amount and distribution of all factors mentioned above in the tissues of the control group;

3. to evaluate the possible correlation of the acquired morphological data.

Hypothesis of the study

Tissue growth, degeneration, fibrosis modulating factors, innervation and angiogenesis, as well as inflammation and antimicrobial defence regulating factors have predisposing and diagnostic significance in the formation and development process of intraabdominal adhesions.

Novelty of the study

For the first time, the intraabdominal adhesions of 49 patients under one year of age were investigated by immunohistochemical methods. The relative amount of immunoreactive structures of TGFβ, HGF, FGF-2, FGFR1, VEGF, PGP 9.5, CgA, IL-1α, IL-4, IL-6, IL-7, IL-8, IL-10, TNFα, HBD-2, MMP-2 and TIMP-2 were determined and documented in microphotographs. So far, only separate factors were investigated in case of adhesions, but, in the literature,

complex data about adhesions affecting/regulating factors cannot be found. It must be indicated that the material of this study is unique, because in the studies performed so far tissue from animals or adults was used.

Individual contribution

The author of this work has taken part in all stages of the research, performed the immunohistochemical visualisation and evaluation, acquiring scientific data and doing statistical analysis. The author has written all this work and she is the author of all microphotographs included in the scientific work.

Ethical aspects

The research work was performed in accordance with Helsinki declaration and the permission of Rīga Stradiņš University Ethics Committee on May 10, 2007.

Structure and volume of the Doctoral Thesis

The Doctoral Thesis were written in Latvian. It consists of five chapters: review of literature, material and methods, results, discussion and conclusions. The list of references consists of 266 sources. The volume of the Doctoral Thesis covers 139 pages, including 31 table and 56 figures (microphotographs).

1. MATERIALS AND METHODS

1.1. Morphological Investigation of the Material

Material for morphological studies was collected at Children's Clinical University Hospital within the period from March 2011 till September 2012. The study tissue material was obtained from patients who underwent abdominal surgery due to complete or partial bowel obstruction. Tissue specimen were obtained from 49 patients up to one year of age; six out of those underwent repeated surgery during which additional material was obtained, which is why together 57 tissue specimens were collected.

For histological and immunohistochemical analysis, the study material was processed at Rīga Stradiņš University Institute of Anatomy and Anthropology Laboratory of Morphology. After initial microscopic evaluation, 50 specimens were rated appropriate for morphological analysis and included in the study. 21 out of 50 specimens were evaluated as **congenital adhesions** (embryonic peritoneal adhesions, Ladd band), but 29 as **acquired adhesions** related to gastrointestinal perforation, diffuse peritonitis and repeated surgeries.

Most frequently adhesions were localised between the jejunal small intestinal loops and the proximal parts of ileum (21 cases) or at the duodenal region (ten cases). In four cases specimens were obtained from the distal parts of the ileum. In thirteen cases adhesions were forming a Ladd band, but in another two cases the anterior abdominal wall was involved.

The control group tissue material was obtained from eight patients with surgical repair of inguinal hernia.

All patients from the study group and control group were under one year of age.

1.2. Morphological Methods

1.2.1. Fixation of Studied Tissue Material

Tissue material fixation for 24 hours in Stefanini solution (Stefanini, et al., 1967) was performed at Children's Clinical University Hospital Department of Children Surgery immediately after the tissue material was obtained. After fixation, the study material was taken to Laboratory of Morphology of the Institute of Anatomy and Anthropology of Rīga Stradiņš University for further processing.

The fixed tissue material was dehydrated in alcohol solution of increasing concentration and degreased in xylol solution. Subsequently, samples were immersed in paraffin I for one hour and in paraffin II for two hours. Paraffin was poured in special cassettes with the help of a paraffin dispenser. Three to four micrometers thin tissue cuts were prepared by means of semi-automatic rotation microtome (Leica RM2245, Leica Biosystems Richmond Inc., USA) from the tissue material block and put on slides (HistoBond®+, Paul Marienfeld GmbH & Co. KG, Germany). The slides were placed into a thermostat for drying for 20–60 minutes at temperature 56 ºC. Further processing was done according to the routine histological staining method or immunohistochemical method.

1.2.2. Routine Histological Staining Method

To create a general overview of the morphological picture, the slides were processed for routine histological staining method. After drying into the thermostat, the cuts were deparaffinised in xylol, dehydrated in different concentrations of alcohols and stained with hematoxyline (code 05-M06002, Mayer's Hematoxylin, Bio Optica Milano S.p.A., Italy) un eosine (code 05-

B10003, Eosin Y alcoholic solution, Bio Optica Milano S.p.A., Italy). After staining, the preparations were rinsed with running water, dehydrated in different concentrations of alcohols and clarified with carboxylic acid and xylol. Then histological glue (code 6900002, Paul Marienfeld GmbH & Co. KG, Germany) for coverslip (Carl Roth GmbH + Co, Germany) attachment was used.

As a result, overview sections, in which the basophilic structures of the cell stained blue-violet, but the acidophilic – pink, were obtained. The stained preparations were examined using a light microscope (Leica DM500RB, Leica Biosystems Richmond Inc., USA), afterwards the preparations were processed in Image Pro Plus video analysis system and was fixated using a digital camera (Leica DC 300F, Leica Microsystem AG, Germany).

1.2.3. Immunohistochemical Method and Reagents

Using the biotin-streptavidin immunohistochemical method (Hsu et al., 1981) the following were identified in the tissue samples:

- **transforming growth factor β** (TGFβ, code orb7087, obtained from rabbit, working dilution 1:100, Biorbyt Ltd., United Kingdom);
- **hepatocyte growth factor** (HGF, code AF-294-NA, obtained from goat, working dilution 1:300, R&D Systems, Germany);
- **basic fibroblast growth factor** (FGF-2, code ab16828, obtained from rabbit, working dilution 1:200, Abcam, United Kingdom);
- **fibroblast growth factor receptor-1** (FGFR1, code ab10646, obtained from rabbit, working dilution 1:100, Abcam, United Kingdom);
- **vascular endothelial growth factor** (VEGF, code orb191500, obtained from rabbit, working dilution 1:100, Biorbyt Ltd., United Kingdom);

- **protein gene product 9.5** (PGP 9.5, code 439273A, obtained from rabbit, working dilution 1:100, ZYMED Laboratories, Invitrogen Corporation, USA);
- **chromogranin A** (CgA, code 910216A, obtained from rabbit, working dilution 1:100, Invitrogen Corporation, USA);
- **interleukin-1 alpha** (IL-1α, code sc-9983, obtained from mouse, working dilution 1:50, Santa Cruz Biotechnology, Inc., USA);
- **interleukin-4** (IL-4, code orb10908, obtained from rabbit, working dilution 1:100, Biorbyt Ltd., United Kingdom);
- **interleukin-6** (IL-6, code LS-B1582, obtained from mouse, working dilution 1:50, LifeSpan BioSciences, Inc., USA);
- **interleukin-7** (IL-7, code orb48420, obtained from rabbit, working dilution 1:100, Biorbyt Ltd., United Kingdom);
- **interleukin-8** (IL-8, code orb39299, obtained from rabbit, working dilution 1:100, Biorbyt Ltd., United Kingdom);
- **interleukin-10** (IL-10, code ab34843, obtained from rabbit, working dilution 1:400, Abcam, United Kingdom);
- **tumour necrosis factor alpha** (TNF-α, code sc-52250, obtained from mouse, working dilution 1:100, Santa Cruz Biotechnology, Inc., USA);
- **human beta defensin-2** (HBD-2, code sc-20798, obtained from rabbit, working dilution 1:100, Santa Cruz Biotechnology, Inc., USA);
- **matrix metalloproteinase-2** (MMP-2, code orb11061, obtained from rabbit, working dilution 1:100, Biorbyt Ltd., United Kingdom);
- **tissue inhibitor of matelloproteinase-2** (TIMP-2, code sc-21735, obtained from mouse, working dilution 1:50, Santa Cruz Biotechnology, Inc., USA).

Tissue sample fixation and preparation, embedment into paraffin blocks, microtomy and placement on slides was performed according to the scheme

described in section 1.2.1. Subsequently specimens were deparaffinised in xylol and dehydrated in alcohol solutions of different concentration. The deparaffinised tissues were washed twice for five minutes in TRIS buffer solution (code 2017X12508, Diapath S.p.A., Italy) and put in EDTA (code 2017X02239, Diapath S.p.A., Italy) boiling buffer up to 20 minutes in the microwave. When the samples had cooled down, they were washed twice for five minutes in TRIS buffer solution. Further, blocking for ten minutes in 3 % peroxidase solution was performed, then washed twice for five minutes in TRIS buffer solution. To decrease background staining, normal blocking serum for 20 minutes was used. All tissue samples were incubated with primary antibodies for one hour. All antibodies used in research were diluted with Antibody Diluent (code 938B-05, Cell Marque™, USA).

HiDef Detection™ HRP (code 954D-30, Cell Marque™, USA) polymer system was used for the **mice** or **rabbit** origin antibodies. After the primary antibody incubation and rinsing with TRIS wash buffer solution three times, HiDef Detection™ (code 954D-31, Cell Marque™, USA) reaction amplificator was applied for ten minutes at room temperature. Then preparations were rinsed with TRIS buffer solution three times for five minutes and incubated with HiDef Detection™ HRP (code 954D-32, Cell Marque™, USA) at room temperature for ten minutes. Again, rinsing with TRIS buffer solution was performed three times for five minutes. After this processing, tissue coating with liquid DAB Substrate Kit chromogenic system (code 957D-60, Cell Marque™, USA) up to ten minutes at room temperature was performed, resulting in a positive structures staining brown.

ImmunoCruzTM ABC (code sc-2023, Santa Cruz Biotechnology, Inc., USA) staining system was used for antigens of **goat** origin. In case ABC staining system was used, micropreparations were incubated by 1.5 % blocking serum TRIS buffer solution up to one hour at room temperature. Incubation of

preparations with primary antibodies for one hour at room temperature was performed. Rinsing with TRIS washing buffer three times for five minutes was done. After rinsing, the cuts were incubated with biotin-containing secondary antibody (biotinylated goat immunoglobulin) for 30 minutes. Thereafter samples were rinsed with TRIS buffer solution three times for five minutes. After rinsing avidin and biotin-horseradish peroxidase complex was added and incubation for 30 minutes at room temperature was performed. Subsequently samples repeatedly were rinsed with TRIS buffer solution three times for five minutes. After this processing, tissue coating with liquid DAB Substrate Kit chromogenic system up to ten minutes at room temperature was performed, resulting in a positive structure staining brown.

Regardless of the staining system, after the incubation with chromogenic substrate, rinsing with running water and counterstaining with hematoxylin (code 05-M06002, Mayer's hematoxylin, Bio Optica Milano S.p.A., Italy) for two minutes was performed. All samples were finally dehydrated with alcohol solutions of increasing concentrations (70º–96º) and clarified with carboxylic acid and xylol. Then slide adhesive for coverslip attachment was used. The stained preparations were examined using a light microscope (Leica DM500RB, Leica Biosystems Richmond Inc., USA), afterwards the preparations were processed in the Image Pro Plus video analysis system and was fixed using a digital camera (Leica DC 300F, Leica Microsystem AG, Germany).

For each preparation series, positive controls of the tissues indicated by the manufacturer, which always have positive reaction, were prepared. As negative control, the parallel cuts of the preparation, where primary antibody was substituted by antibody diluent Antibody Diluent, were used.

1.3. Data Processing Methods

The overview specimens were evaluated according to following criteria:

1) epithelial cell shape (flat or round);

2) characteristics of cells and fibers in the loose connective tissues in the subepithelium;

3) presence or non-presence of inflammatory cells;

4) vascularisation compared to the control group.

In order to access the extent of inflammatory cell infiltration, the leukocyte density was evaluated according to the following scheme:

- minimal infiltration (1): < 10 % inflammatory cells in the visual field;
- no marked infiltration (2): 10–25 % inflammatory cells in the visual field;
- moderate infiltration (3): 26–50 % inflammatory cells in the visual field;
- marked infiltration (4): > 51% inflammatory cells in the visual field (Erben et al., 2014).

1.3.1 Semiquantitative Counting Method

The semiquantitative counting method (Pilmane et al., 1995) was used for the registration of the relative amount of immunopositive structures:

0 – no positive structures in the visual field;

0/+ – occasionally positive structures in the visual field;

+ – few positive structures in the visual field;

+/++ – few to moderate positive structures in the visual field;

++ – moderate positive structures in the visual field;

++/+++ – moderate to numerous positive structures in the visual field;

+++/++++ − numerous to abundant positive structures in the visual field;

++++ − abundant positive structures in the visual field.

The amount of structures was analysed in five fields of view of randomly selected one section. The average amount of structures was chosen for further analysis.

1.3.2. Statistical Methods

To characterise the research group, descriptive statistic methods were used. For the description of each marker, median and interquartile ranges were used. For the comparison of groups, Mann-Whitney U-test was used (Riffenburgh, 2012a). To evaluate the cross-compliance of two variables Spearman's rank correlation coefficient (rs) was calculated (Riffenburgh, 2012b). The acquired results were interpreted: $rs \leq 0.35$ – weak correlation, $0.35 < rs < 0.7$ – moderate correlation, $rs \geq 0.7$ – strong correlation. Two-tailed p values of < 0.05 were considered as statistically significant. Statistical analysis was conducted using Statistical Package for the Social Sciences (SPSS) programme version 23.0 (IBM Corporations, USA).

2. RESULTS

2.1. Morphological Findings

The control group patient tissues were obtained during surgical repair of inguinal hernia and could be regarded as relatively normal histological findings.

Abnormal tissue changes were observed in all adhesion tissue specimen that were included in the study. Modifications in mesotheliocytes and fibroblasts were characteristics of adhesion tissue. Chaotically placed dense connective tissue fiber bundles and sometimes also large collagen fiber accumulations without fibroblasts were observed.

Inflammation with neutrophil and macrophage infiltration was observed in almost all overview specimen. A moderate to marked inflammation was observed in more than 50 % of the cases. Partly the inflammatory cells were located diffusely, partly perivascularly. Often the inflammatory cells were located around sclerotic arterioles. In the case of a diffuse marked inflammation, epithelioid cells were also observed.

In the overview specimen, hyperaemic blood vessels and neoangiogenesis were frequently observed.

2.2. Immunohistochemical Profile

2.2.1. Growth Factors and Their Receptors

In the control group tissues few to moderate (+/++) TGFβ positive cells (fibroblasts, mesotheliocytes and endotheliocytes) were observed in the visual field.

In adhesions group overall moderate (++) number of structures contained TGFβ. Most often TGFβ positive fibroblasts (see Fig. 2.1.) were observed, as

well as positive endotheliocytes, macrophages and epithelioid cells in some of the specimen. Compared to the control group, **TGFβ** positive structures were in **significantly higher counts seen in the adhesion group** ($U = 50.50$, $p < 0.001$).

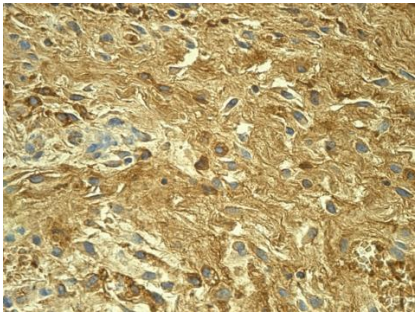

Fig. 2.1. **Numerous TGFβ positive fibroblasts, macrophages and connective tissue fibers in adhesions of a 39-day-old patient. TGFβ IMH, X 400**

HGF positive fibroblasts and macrophages in the control group were occasionally observed (0/+), while few to moderate (+/++) number of mesotheliocytes were seen.

In the adhesions, HGF positive fibroblasts, macrophages and mesotheliocytes most often were observed occasionally (0/+), in addition HGF positive **mesotheliocytes** were seen **significantly less** ($U = 60.50$, $p = 0.001$) than in control tissues. In some tissue samples fibroblasts were located in the submesothelium (see Fig. 2.2.).

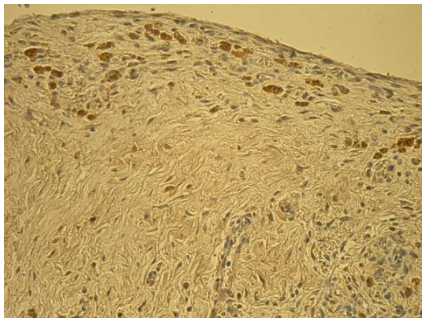

Fig. 2.2. **Moderate HGF positive fibroblasts of submesothelium in adhesions of a 71-day-old patient. HGF IMH, X 250**

In the control group tissues, moderate to numerous (++/+++) FGF-2 positive structures were found.

FGF-2 findings in the adhesion group were variable – from occasional (0/+) to abundant (++++) FGF-2 positive structures. Commonly, positive fibroblasts and macrophages were observed (see Fig. 2.3.), in some of the tissue samples endotheliocytes or mesotheliocytes were also found. A **statistically significant lower** ($U = 83.00$, $p = 0.007$) amount of **FGF-2** positive structures in adhesions was proven.

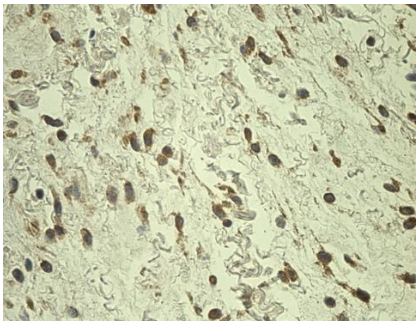

Fig. 2.3. **Moderate FGF-2 positive fibroblasts and macrophages in adhesions of a four-day-old patient. FGF-2 IMH, X 400**

In the control group tissues most often few (+) FGFR1 positive fibroblasts, endotheliocytes and mesotheliocytes were seen.

Moderate (++) number of FGFR1 positive fibroblasts and macrophages were detected in adhesions, sometimes also endotheliocytes were positive. Statistical tests confirmed that **significantly more FGFR1** positive structures (U = 81.00, p = 0.006) were seen **in the adhesions group** (see Fig. 2.4.).

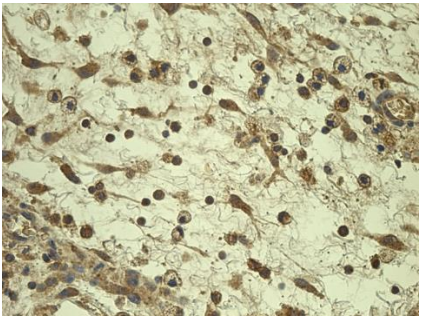

Fig. 2.4. **Numerous FGFR1 positive fibroblasts and macrophages in adhesions of a two-day-old patient. FGFR1 IMH, X 400**

2.2.2. Vascular Endothelial Growth Factor (VEGF)

The control group tissues showed few to moderate (+/++) or moderate (++) appearance of VEGF positive cells. The positive reaction for VEGF was found in endotheliocytes, mesotheliocytes, fibroblasts and macrophages.

In the adhesions group, a moderate (++) number of VEGF positive structures was detected. A positive reaction was found in endotheliocytes and mesotheliocytes, but there was **no statistically significant difference** between the groups. At the same time **VEGF** positive **macrophages** were seen in **significantly higher number** (U = 61.00, p = 0.001) than in the control group.

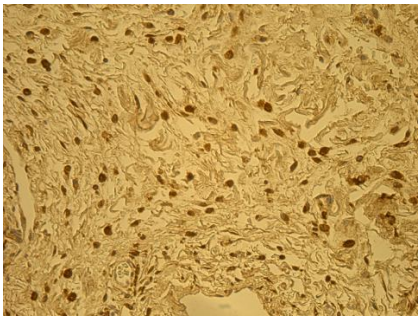

Fig. 2.5. **Moderate VEGF positive macrophages and fibroblasts in adhesions of a 48-day-old patient. VEGF IMH, x250**

2.2.3. General Markers of Diffuse Neuroendocrine System

PGP 9.5 positive nerve fibers, shape modified fibroblasts and mesotheliocytes in the control group tissues were observed in moderate (++) or moderate to numerous (++/+++) appearance.

In the adhesion group, PGP 9.5 positive structures (shape modified fibroblasts, nerve fibers) were mostly seen in few to moderate (+/++) or moderate (++) appearance. Most PGP 9.5 positive nerve fibers appeared around blood vessels (see Fig. 2.6.), some were found in the wall of the blood vessels. **PGP 9.5** findings in the study group were **significantly lower** than in the control group tissues ($U = 58.50$, $p = 0.001$).

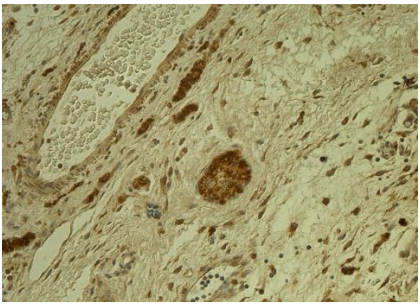

Fig. 2.6. **Moderate PGP 9.5 positive nerve fibers and shape modified fibroblasts in adhesions of a two-day-old patient. PGP 9.5 IMH, X 250**

In the control group, few (+) to moderate (++) CgA positive fibroblasts (also shape modified fibroblasts) were found in clusters and separate (0/+) positive endotheliocytes and mesotheliocytes were seen.

In the adhesion group tissues mostly separate (0/+) or few (+) positive endotheliocytes as well as sometimes macrophages were observed. In one case, CgA positive epithelioid cells were also seen. Few (+) to numerous (+++) fibroblasts contained CgA (see Fig. 2.7.). The number of positive structures in the adhesions and control group did not differ (**no statistical difference was found** ($U = 170.50$, $p = 0.491$)).

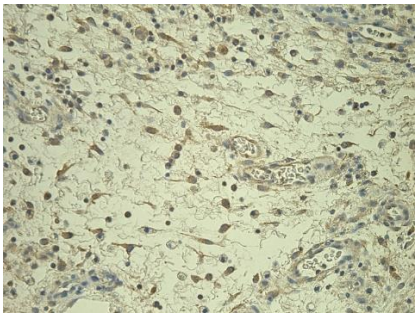

Fig. 2.7. **Moderate CgA positive fibroblasts and macrophages in adhesions of a 14-day-old patient. CgA IMH, X 250**

2.2.4. Inflammatory Cytokines

Moderate (++) numbers of fibroblasts and mesotheliocytes contained IL-1α in the control group.

In the adhesions, generally few to moderate (+/++) number of IL-1α positive macrophages (see Fig. 2.8.) and fibroblasts (also structurally changed fibroblasts) were detected. The amount of **IL-1α** found in adhesions was **significantly lower** (U = 98.50, p = 0.015) in comparison to the control group.

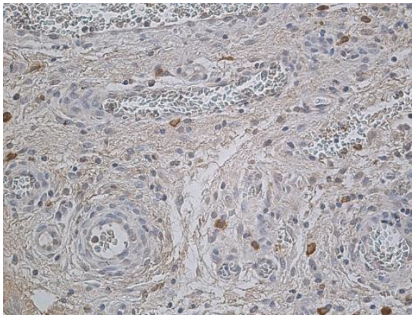

Fig. 2.8. **Few IL-1α positive macrophages in adhesions of a 14-day-old patient. IL-1α IMH, X 250**

In the control group tissues moderate (++) or moderate to numerous (++/+++) number of IL-4 positive fibroblasts and mesotheliocytes were observed.

Few (+) or few to moderate (+/++) number of IL-4 positive fibroblasts and macrophages in the adhesion group were seen (see Fig. 2.9.). The **difference between the number of structures containing IL-4** in the adhesion and control group were **statistically significant** (U = 60.50, p = 0.002)**.**

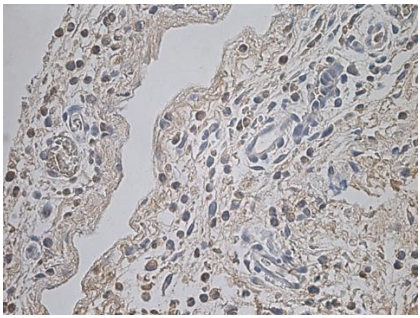

Fig. 2.9. **Few to moderate number of weakly IL-4 positive fibroblasts and macrophages in adhesions of a 30-day-old patient. IL-4 IMH, X 400**

Mostly moderate to numerous (++/+++) number of IL-6 positive fibroblasts and mesotheliocytes were detected in the control group.

In the adhesion group, IL-6 was observed in inflammatory cells (neutrophils, macrophages) and fibroblasts, as well as structurally changed fibroblasts. In some specimens IL-6 positive epithelioid cells (see Fig. 2.10.) were found. Generally, **IL-6** positive structures were seen in moderate (++) amount, which was **not statistically different** from the control group ($U = 146.50$, $p = 0.243$).

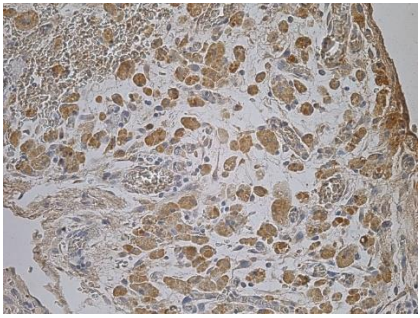

Fig. 2.10. **Numerous IL-6 positive macrophages and epithelioid cells in adhesions of a two-day-old patient. IL-6 IMH, X 250**

IL-7 positive fibroblasts and macrophages in the control group tissues were seen in moderate to numerous (++/+++) or numerous (+++) numbers. A positive reaction was also observed in mesothelium and endothelium.

IL-7 was visible in fibroblasts, macrophages, endotheliocytes (see Fig. 2.11.) and epithelioid cells of adhesions. Mostly numerous (+++), moderate to numerous (++/+++) or moderate (++) number of IL-7 positive structures were detected. The amount of the observed structures was **not significantly different** in comparison to the control group tissues ($U = 144.50$, $p = 0.190$).

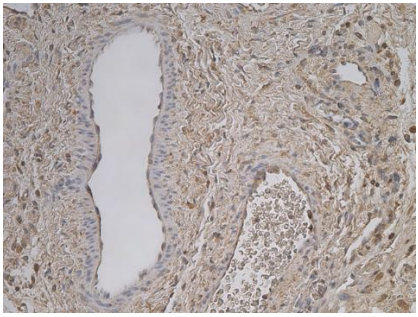

Fig. 2.11. **Moderate IL-7 positive endotheliocytes, weakly positive fibroblasts and macrophages in adhesions of a 19-day-old patient. IL-7 IMH, X 250**

In the control group, moderate to numerous (++/+++) number of mesotheliocytes and fibroblasts contained IL-8.

The IL-8 finding in the adhesion group tissues was variable. Mostly few (+) fibroblasts and macrophages contained IL-8, but some specimens showed few to moderate (+/++), moderate (++) (see Fig. 2.12.) or moderate to numerous (++/+++) positive structures. In general, **significantly less IL-8** positive structures were seen in the adhesion group compared to the control group ($U = 40.00$, $p < 0.001$).

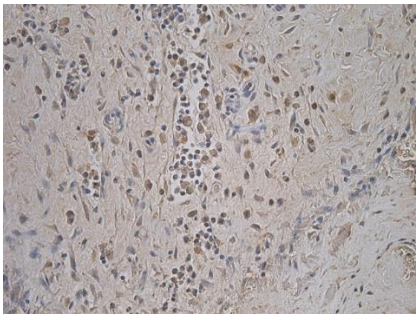

Fig. 2.12. **Moderate number of weakly IL-8 positive fibroblasts and macrophages in adhesions of a 56-day-old patient. IL-8 IMH, X 250**

In the control group, IL-10 positive structures were found in moderate (++) or moderate to numerous (++/+++) amount. Most frequently seen positive cells were mesotheliocytes, as well as macrophages and fibroblasts.

In the adhesion group, IL-10 positive macrophages, epithelioid cells, neutrophils and fibroblasts (see Fig. 2.13.) were observed. In most patients, moderate to numerous (++/+++) number of IL-10 positive structures were found. **No statistically significant difference** was detected between the groups ($U = 184.00$, $p = 0.769$).

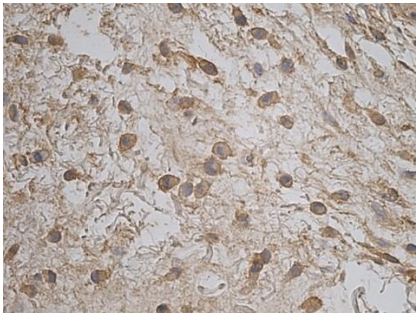

Fig. 2.13. **Moderate IL-10 positive fibroblasts and macrophages in adhesions of a patient less than a day old. IL-10 IMH, X 400**

Presence of TNFα was observed in all specimens of the control group. Positive macrophages and fibroblasts were mostly seen in a moderate (++) number.

TNFα containing cell finding in the adhesions group was incontestable – mostly or in 27 cases positive cells were seen in moderate (++) number. A positive reaction for TNFα was found in fibroblasts and macrophages (see Fig. 2.14.). In eight specimens, positive epithelioid cells were detected. There was **no statistically significant difference** between the groups (U = 124.00, p = 0.082).

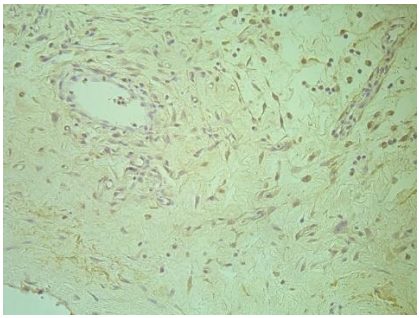

Fig. 2.14. **Moderate TNFα positive fibroblasts and macrophages in adhesions of a 56-day-old patient. TNFα IMH, X 250**

2.2.5. Human Beta Defensin-2 (HBD-2)

In the control group, moderate (++) or moderate to numerous (++/+++) number of HBD-2 positive fibroblasts and macrophages were found, but mesotheliocytes were seen in only few to moderate (+/++) number.

A positive reaction for HBD-2 usually was observed in fibroblasts and macrophages (see Fig. 2.15.) and there was **no statistically significant difference** between the groups. Only few (+) HBD-2 positive mesotheliocytes were detected and 17 specimens did not contain any HBD-2 positive

mesotheliocyte. In the study group tissues, positive **mesotheliocytes** were seen in **significantly lower** counts for HBD-2 ($U = 50.00$, $p < 0.001$).

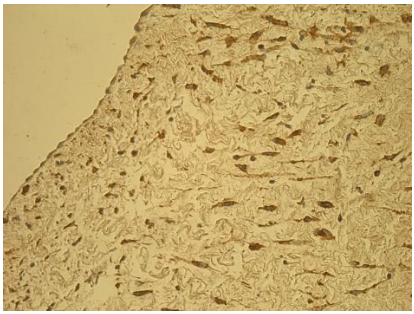

Fig. 2.15. **Moderate HBD-2 positive fibroblasts, macrophages and mesotheliocytes in adhesions of a 151-day-old patient. HBD-2 IMH, X 250**

2.2.6. Tissue Degrading Enzymes and Their Inhibitors

MMP-2 finding in the control group tissues was variable – from few (+) to moderate (++) numbers of positive structures. MMP-2 positive fibroblasts, macrophages, endotheliocytes and mesotheliocytes were found in all control specimens.

In the adhesions group tissues, few to moderate (+/++) number of MMP-2 positive inflammatory cells, mainly macrophages and neutrophils, epithelioid cells (see Fig. 2.16.), fibroblasts and endotheliocytes were detected. There was **no statistically significant difference** between the groups ($U = 174.00$, $p = 0.654$).

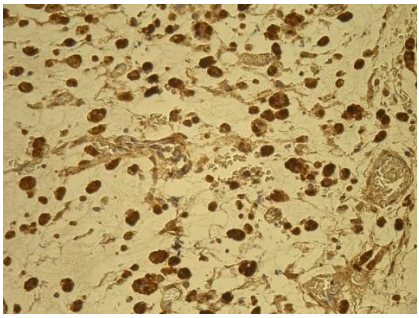

Fig. 2.16. **Numerous MMP-2 positive macrophages and epithelioid cells in adhesions of a two-day-old patient. MMP-2 IMH, X 250**

In the control tissues, TIMP-2 was observed in fibroblasts, macrophages and more prominent in mesotheliocytes and endotheliocytes. Overall, we detected moderate (++) number of weakly TIMP-2 positive cells.

The TIMP-2 findings in the adhesion tissues were variable – from few (+) to moderate to numerous (++/+++) positive structures. A positive reaction was seen in fibroblasts and inflammatory cells, mainly macrophages (see Fig. 2.17.). In the adhesion specimens, positive structures were found in **significantly lower counts for TIMP-2**. The most distinct difference was detected between TIMP-2 positive fibroblasts ($U = 108.00$, $p = 0.022$) and endotheliocytes ($U = 34.0$, $p < 0.001$).

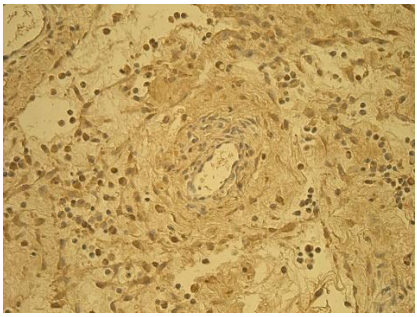

Fig. 2.17. **Moderate TIMP-2 positive fibroblasts, macrophages and endotheliocytes in adhesions of a 56-day-old patient. TIMP-2 IMH, x250**

2.3. Data about the Correlation between the Immunohistochemical Markers

A statistically significant tight, positive correlation was found between HBD-2 and MMP-2 finding ($r_s = 0.728$, $p < 0.001$).

Statistically significant moderately tight, positive correlations were observed between patients' age and CgA ($r_s = 0.549$, $p < 0.001$), patients' age and FGF-2 ($r_s = 0.354$, $p = 0.011$), as well as between immunohistochemically detected findings in different combinations (see Table 2.1.).

Table 2.1.

Statistically significant <u>moderately tight, positive correlations</u> in adhesions

Factor 1	Factor 2	r_s	p-value
HBD-2	*VEGF*	0.625	< 0.001
HBD-2	*IL-6*	0.614	< 0.001
HBD-2	*TIMP-2*	0.601	< 0.001
HBD-2	*TNFα*	0.596	< 0.001
TIMP-2	*VEGF*	0.593	< 0.001
TNFα	*TIMP-2*	0.588	< 0.001
IL-6	*TIMP-2*	0.583	< 0.001
IL-10	*IL-8*	0.550	< 0.001
IL-10	*VEGF*	0.548	< 0.001
HGF	*IL-6*	0.542	< 0.001

Table 2.1. continued

Factor 1	Factor 2	r_s	p value
HBD-2	*IL-10*	0.532	< 0.001
IL-6	*VEGF*	0.528	< 0.001
PGP 9.5	*TIMP-2*	0.524	< 0.001
PGP 9.5	*MMP-2*	0.515	< 0.001
VEGF	*MMP-2*	0.511	< 0.001
IL-7	*IL-4*	0.491	< 0.001
IL-4	*MMP-2*	0.490	< 0.001
FGF-2	*FGFR1*	0.490	< 0.001
MMP-2	*TIMP-2*	0.489	< 0.001
IL-4	*FGF-2*	0.481	< 0.001
HGF	*TIMP-2*	0.474	= 0.001
IL-1	*IL-7*	0.471	= 0.001
IL-10	*TIMP-2*	0.463	= 0.001
HGF	*TNFα*	0.460	= 0.001
HBD-2	*PGP 9.5*	0.453	= 0.001
HBD-2	*HGF*	0.443	= 0.002
IL-7	*IL-8*	0.440	= 0.001
IL-1	*IL-10*	0.438	= 0.002
TNFα	*IL-6*	0.437	= 0.003
IL-6	*PGP 9.5*	0.436	= 0.002
IL-10	*IL-7*	0.433	= 0.002
HGF	*PGP 9.5*	0.431	= 0.002
IL-7	*MMP-2*	0.431	= 0.002
TNFα	*PGP 9.5*	0.429	= 0.003
HGF	*IL-8*	0.423	= 0.003
IL-8	*VEGF*	0.415	= 0.003
HGF	*IL-10*	0.414	= 0.004
IL-1	*TIMP-2*	0.412	= 0.004
IL-6	*MMP-2*	0.401	= 0.005
IL-10	*IL-4*	0.398	= 0.005
FGFR1	*IL-7*	0.396	= 0.004
TNFα	*VEGF*	0.396	= 0.006
IL-7	*FGF-2*	0.394	= 0.005
IL-6	*IL-8*	0.381	= 0.007
TNFα	*IL-8*	0.376	= 0.010
IL-10	*MMP-2*	0.375	= 0.009
IL-8	*TIMP-2*	0.374	= 0.009
FGFR1	*IL-1*	0.369	= 0.008
IL-1	*IL-4*	0.365	= 0.010
VEGF	*PGP 9.5*	0.365	= 0.011
IL-10	*IL-6*	0.362	= 0.011
IL-8	*PGP 9.5*	0.361	= 0.012

Table 2.1. continued

Factor 1	Factor 2	r_s	p value
HGF	*MMP-2*	0.351	= 0.014
IL-8	*MMP-2*	0.351	= 0.014

Abbreviation: r_s – Spearman correlation coefficient

A statistically significant moderately tight, negative correlation was observed between VEGF and TGFβ findings (r_s = -0.401, p = 0.005) and PGP 9.5 and TGFβ (r_s = -0.386, p = 0.007).

3. DISCUSSION

Postoperative intraabdominal adhesions have been investigated in adults and there were experiments performed with animals in laboratory circumstances. However, there are few studies about the development of adhesions and their mechanisms in children, especially in newborns. There are also very few studies about the immunohistochemistry and morphology of adhesions, in the specimens obtained during surgery in infants and children.

The pathogenesis of adhesions is not fully understood, but it is known that in their formation mesothelial damage with a following fibrin matrix formation and disbalance of fibrinolysis, as well as the activation of pro- and anti-inflammatory factors, play a role. In the current study, the relative amount of immunoreactive structures for TGFβ, HGF, FGF-2, FGFR1, VEGF, PGP 9.5, CgA, IL-1α, IL-4, IL-6, IL-7, IL-8, IL-10, TNFα, HBD-2, MMP-2 un TIMP-2 in intraabdominal adhesion specimens in children up to one year of age was evaluated and compared to controls of relatively healthy peritoneum specimens. It must be highlighted that the material of this study is unique and has never been performed in such a group before, therefore the results can be compared only to literature data, obtained from animal studies of postoperative adhesions.

When analysing the specimens, round mesotheliocytes were seen in the adhesions. The change of the cell shape from flat to round might be proof of a mesothelial reaction, resulting in a disbalance of fibrin formation and fibrinolysis, therefore contributing to the formation of adhesions. Very likely, the change of these cell's shapes is not specific to adhesions, but indicates an increased cellular metabolic activity, which has been proved in other author's, who analysed the ultrastructure of mesotheliocytes, studies. Round, cube or cobblestone shaped mesotheliocytes have an increased cell volume, markedly increased mitochondria counts, a broadened granular endoplasmic reticulum and

a well-defined Golgi complex, indicating an increased metabolic activity (Nagai et al., 2013).

In the current study, modified fibroblasts in the adhesion specimens were also detected, most likely indicating a change in the functional activity of these cells. Indirectly, this is proved by the increased amount of collagen bundles in the adhesion specimens. Possibly, a change in fibroblast phenotype has occurred (possibly, into myofibroblasts), because it is characteristic for such cells to intensively produce fibers of the extracellular matrix and therefore to promote adhesions. This is reinforced by the increased finding of TGFβ positive cells in adhesions – one of the most crucial factors for myofibroblast activation (Bianchi et al., 2016). The Italian investigator Angelo Corti has described a CgA modified fibroblast activity with an increased synthesis of extracellular matrix proteins and stroma formation (Corti, 2004). Later, in the studies of other authors, in case of tissue damage, a differentiation from fibroblasts to myofibroblasts, with an increased synthesis of extracellular matrix proteins and extracellular matrix reorganising factors (MMPs, TIMPs), was described (Ravikanth et al., 2011; Darby et al., 2014). Myofibroblasts have been observed in several diseases linked to fibrosis (Bochaton-Piallat et al., 2016).

In the study, dense connective tissue bundles were observed, placed in different directions and collagen bundles without fibroblasts, in the adhesion specimens. Taking into account the mechanisms involved in the pathogenesis of adhesions, such a finding is typical for the formation of adhesions and can indicate fibrosis. It is thought that at the beginning of adhesion formation, there is an increased amount of connective tissue cells that gradually decreas, but the amount of fibers increases, forming large accumulations with very few cells. This is approved by the classification systems designed by other authors, in that the 1st degree (immature) adhesions are characterised by a small number of fibers, thin reticular, and many connective tissue cells, but the 4th degree (mature)

adhesions are characterised by dense connective tissue fibers and very few cells (Montalvo-Javé et al., 2016). Other authors mention that at the beginning of adhesion formation, few inflammatory cells are observed that gradually increase together with the proliferation of blood vessels, but in mature adhesions degenerative, fibrous tissues can be observed (Zhang S. et al., 2009).

In almost all of our adhesion specimens, an infiltration with leukocytes and macrophages was observed, proving the involvement of inflammation in the morphopathogenesis of adhesions. These findings are consistent with the studies of other authors, who investigated postoperative adhesions. The inflammation, provoked by peritoneal damage, promotes the formation of a thick fibrin matrix, which is the basis for the formation of adhesions (Wei et al., 2016). The inflammatory cells are capable of producing pro- and anti-inflammatory cytokines and growth factors that promote the formation of connective tissue and vascularisation (Binnebösel et al., 2008). In the current study, hyperemic blood vessels and neoangiogenesis were frequently observed that promote tissue remodelation. This is indirectly shown by the increase in VEGF and the positive correlation between this growth factor, inflammatory cytokines, and MMP-2.

In the study, in one part of the cases, inflammatory cells were located perivascularly, in the other part of the cases – diffusely. An innovative finding is the occurrence of epithelioid cells in adhesion tissues that was seen in the case of marked diffuse inflammation. It is thought that epithelioid cells indicate the formation of a granuloma and proves the chronicity of the inflammatory process. Other authors mention that these cells indicate an intense inflammatory response and promote fibrosis by the synthesis of fibroblast activating factors (Turk & Narayanan, 1982; Turner et al., 2014).

TGFβ is so far the most investigated growth factor in the pathogenesis of adhesions. In the study, moderate numbers of TGFβ positive structures in most adhesion specimens were observed; additionally, statistically significantly more

TGFβ positive structures were found in the study group. This proves an ongoing stimulation of adhesion forming structures, which is supported by the current finding of large accumulations of collagen bundles and adhesions. It may be believed that the increased amount of TGFβ positive structures is part of the adhesion pathogenesis and such data are also indirectly being supported by other author's findings. It is known that this factor regulates the formation of extracellular matrix and its remodelation and also promotes fibrosis (Verrecchia & Mauviel, 2007). The function of TGFβ is characterised by an increased synthesis of extracellular matrix components, as well as the proliferation, migration, and accumulation of cells with a mesenchymal origin (Pohlers et al., 2009). Increased expression of TGFβ expression is linked to fibrosis in various tissues and organs, thereof in peritoneal adhesions (Ghellai et al., 2000). Excess of TGFβ correlates with an increased upbuilt of connective tissue (Verrecchia & Mauviel, 2007).

A statistically significant moderately strong negative correlation between TGFβ and VEGF was observed, indicating the suppression of VEGF under the impact of TGFβ in intraabdominal adhesion tissues, which might be a compensatory mechanism in the morphopathogenesis of intraabdominal tissues. The findings of the current study is consistent with other author's data on TGFβ un VEGF interaction and involvement in peritoneal damage and fibrosis development (Kariya et al., 2018). TGFβ is responsible for VEGF structure protein stability and can also inhibit its expression, therefore regulating angiogenesis (Geng et al., 2013).

A negative correlation between TGFβ and MMP-2 findings was observed, which is consistent with the literature. TGFβ inhibits the activity of matrix metalloproteinases, therefore, not only it stimulates the accumulation of extracellular matrix components, but also delays its degeneration (Leask & Abraham, 2004).

A weak correlation between TGFβ and HBD-2 findings was observed, indicating the presence of inflammation and/or infection in the development of adhesions. TGFβ and inflammation mediators, for example, IL-1 and TNFα, all promote HBD-2 synthesis, therefore ensuring antimicrobial protection and promoting wound healing (Tang et al., 2017).

Only a few HGF positive structures were observed. It is noteworthy that statistically significant differences to the control group were not detected; however, in the control tissues, significantly more HGF positive mesotheliocytes were found. Possibly, the lack of this factor in adhesion tissues might promote the formation and development of adhesions, because HGF inhibits the TGFβ production in myofibroblasts, not only reducing the synthesis, but also the function of this factor, thus preventing peritoneal fibrosis (Gallo et al., 2015). HGF not only inhibits TGFβ, but also other fibrosis promoting factors, for example, platelet derived growth factor, connective tissue growth factor, interferon-γ (IFN-γ) (Kosaka et al., 2008; Nakamura & Mizuno, 2010; Ohashi et al., 2014).

In the current study, no link between HGF and TGFβ was detected; however, a statistically significant correlation was observed between HGF and TIMP-2, as well as between HGF and MMP-2. Possibly, HGF could be responsible for the regulation of extracellular matrix remodelation; however, this does not define MMP-2 and TIMP-2 levels. It is depicted in the literature that HGF considerably induces MMP-2 expression (Esposito, Parrila, De Mauri et al., 2005). Interestingly, in actively proliferating cells, HGF does not prevent fibrosis but promotes TGFβ and TIMP-2 synthesis, activating myofibroblast, further promoting the accumulation of extracellular matrix molecules and fibrosis (Esposito, Parilla, Cornacchia et al., 2009).

In the study, HGF positively correlated with the VEGF findings, which is possibly related to an enhanced blood vessel remodelation in adhesion

development. This is supported by the fact that HGF has angiogenic properties. Moreover, this factor can work together with VEGF, therefore promoting neovascularisation and decreasing local hypoxia (Sulpice et al., 2009).

A moderately strong correlation observed between HGF and TNFα, IL-6, IL-8, IL-10, depicts HGF involvement in pro- and anti-inflammatory factor regulation. In the case of inflammation, TNFα induces HGF secretion in fibroblasts (Bigatto et al., 2015). Also, IL-6 signaling pathways promote HGF production that ensures an increased production of the anti-inflammatory cytokine IL-10, therefore decreasing inflammation. HGF is regarded as a strong anti-inflammatory factor (Coudriet et al., 2010), because, for example, monocytes produce a considerably higher IL-10 when stimulated with HGF (Chen P. et al., 2014).

In almost half of the adhesion group specimens, at least a moderate number of FGF-2 positive structures were detected, indicating a possible indirect role in the pathogenesis of this disease. This is approved also in other author's studies, outlining that the amount of FGF-2 increases in case of inflammation, because inflammatory cytokines induce FGF-2 synthesis and release (Zittermann & Issekutz, 2006). Additionally, the broad role of this factor has been pointed to, reinforced by the correlation observed between FGF-2, its receptor FGFR1 and inflammation mediators, for example, IL-4, IL-7, IL-8 and IL-10. Therefore, it has been approved that the pathogenesis of intraabdominal adhesions is possibly related to changes in the FGF-2/FGFR1 signaling pathway, which can favour the development of inflammation, as well as disturb vascularisation, tissue growth, and remodelation. A statistically significant moderately tight correlation was detected between a patient's age and FGF-2, indicating an increasing impact on the adhesion tissues. This factor was detected in several cells, broadening the understanding about the functions of FGF-2, because it is known that FGF-2

induces genes linked to inflammation expression in endotheliocytes (Presta et al., 2009).

FGF-2 supports wound healing and reduces scarring, through inhibiting the differentiation of myofibroblasts (Wang P. et al., 2017). Besides the inhibition of activated myofibroblasts, FGF-2 also inhibits myofibroblast precursors and connective tissue fibroblasts (Dolivo et al., 2017). The ability of FGF-2 to prevent fibrosis in the liver, lungs, blood vessels and eyes are delineated in literature and the role of TGFβ signal inhibition in this effect is also ascertained (Koo et al., 2018). Interestingly, in one-third of the analysed specimens, there were very few, occasional or no FGF-2 positive structures detected. This shows that the statistically significant decrease in FGF-2 positive cells in the adhesion specimens is responsible for fibrosis to occur.

FGF-2 has a high affinity for binding FGFR1 (Itoh & Ornitz, 2004). Until now there have been no studies describing the FGFR1 findings in adhesion tissues. A statistically significant positive correlation between FGF-2 and FGFR1 findings was observed; however, it has to be noted that there were significantly more FGFR1 positive structures seen in the study group. Therefore, it could be assumed that the less detected FGF-2 and more prominent FGR1 finding points out a compensatory receptor stimulation in response to the lacking same factor in adhesion tissues. This innovative finding is significant because it expresses the direct stimulation of the main connective tissue producing cells – fibroblasts.

A high FGFR1 expression in healthy tissues is normally seen in endotheliocytes (Sun H. et al., 2017); however, a significantly lower FGFR1 finding was observed in these cells when compared to the control group, and therefore assume that in adhesions these cells do not react adequately and that the disturbances in the FGFR1 signaling pathway cause the apoptosis of endotheliocytes (Sun H. et al., 2017).

It is depicted in literature that endotheliocytes and mesotheliocytes of the peritoneum produce VEGF (Basbug et al., 2011). It was as well observed in the adhesion specimens; however, a statistically significant difference to the control group was not detected, outlining the insignificance of the factors produced by these cells in the morphopathogenesis of adhesions.

Interestingly, however, there are conflicting findings on VEGF's role in tissues. One study describes that VEGF can even decrease the formation of adhesions by stimulating fibrinolysis and plasminogen activator activity, and also inhibiting TGF-β and its promoting effects on fibrosis (Barcz et al., 2012). This relation might explain the negative correlation observed between VEGF and TGF-β, possibly expressing a compensatory mechanism in the development of adhesions. Marked neoangiogenesis was detected and it was considered that VEGF might be indirectly involved in the vasculature changes. This is supported by the fact that VEGF is the main mediator in angiogenesis, regulating vessel remodelation in case of tissue injury and inflammation (Wu et al., 2010).

There is data on VEGF increase in adhesion being the central factor in the development of adhesions (Bi et al., 2017). The increase of VEGF is characterised as a compensatory mechanism, regulating angiogenesis and promoting nutrients and oxygen to injured tissues (Rout et al., 2000). When analysing the VEGF finding in several cells, significantly more VEGF positive macrophages in the adhesion group were found. Considering this finding and the VEGF positive cell count altogether, the role of hypoxia in the development of intraabdominal adhesions is conceivable. It is known that VEGF production in macrophages occurs during hypoxia (Harmey et al., 1998). During hypoxia and inflammation it is IL-10 that stimulates the increase of VEGF in macrophages (Wu et al., 2010). The observed positive correlation between IL-10 and VEGF supports this relation.

In the current study, a statistically significant positive correlation between VEGF and HBD-2 findings was observed. It has been discovered that HBD-2 can increase VEGF synthesis and, like VEGF, promote the migration and proliferation of endotheliocytes as well as capillary formation during inflammation (Baroni et al., 2009). HBD-2 increases the synthesis of pro-inflammatory cytokines, for example, IL-6, IL-8, TNFα, through these inducing the synthesis of fibrosis promoting factors, as VEGF (Tiriveedhi et al., 2014). Therefore, it is feasible that the observed correlations between pro-inflammatory cytokines (IL-6, IL-8, TNFα) and VEGF are significant in the morphopathogenesis of adhesions. This finding might be of importance pointing out increased collagen production in inflamed tissues and angiogenesis responding to the VEGF, produced by all cells in adhesions.

The innervation in intraabdominal adhesions has not been investigated much so far, and the role of PGP 9.5 in intraabdominal tissues remains unclear. The results of the present study, in some cases, showed moderate to numerous PGP 9.5 positive nerve fibers; however, overall few to a moderate number of PGP 9.5 positive structures were observed. PGP 9.5 is necessary for axon stability and its loss causes axon degeneration and neuronal cell death (Bishop et al., 2016; Guglielmotto et al., 2017). A decrease in PGP 9.5 is a characteristic finding in ischemic injury (Guglielmotto et al., 2017). Therefore, it cannot be excluded that decrease in PGP 9.5 in intraabdominal adhesions is due to tissue hypoxia. Additionally, it is noteworthy that a decrease in PGP 9.5 induces protein accumulation, promoting the development of inflammatory, degenerative processes (Lima et al., 2012).

PGP 9.5 is the dominating protein in the brain, composing up to 5 % of all neuronal proteins there. In smaller amounts, it is found in gonad cells and under certain circumstances also in other cells, for example, in fibroblasts during wound healing. (Bishop et al., 2016). In scientific literature, PGP 9.5 is reported

to be broadly expressed in human myofibroblasts, promoting fibrosis. PGP 9.5 positive, fibroblast-like cells were found in hepatic fibrosis specimen (Wilson et al., 2015). The obtained data point out that also fibroblasts and macrophages secrete PGP 9.5, and there is a statistically significant link between PGP 9.5 and the fibrosis promoting TGFβ, as well as MMP-2 un TIMP-2 – factors responsible for connective tissue matrix remodelation. This finding is innovative as it has so far not been described in the literature about adhesions.

The synthesis of PGP 9.5 is promoted by such inflammatory cytokines as IFN-γ and TNFα (Ichikawa et al., 2010; Gu et al., 2018), but the increase in PGP 9.5 promotes the TNFα regulated inflammation (Guglielmotto et al., 2017). A positive, moderately tight correlation between TNFα and PGP 9.5 indicates that, possibly, TNFα induces nerve fiber growth in intraabdominal adhesions and PGP 9.5 is crucial for maintaining the inflammation. We can present unique data on a moderately tight correlation between PGP 9.5, IL-6 and IL-8, as well as a weak correlation with IL-4 and IL-7, proving PGP 9.5 involvement in regulation of inflammatory processes in intraabdominal adhesions.

Human endotheliocytes and smooth muscle cells in the blood vessel wall produce PGP 9.5; therefore, it is believed that PGP 9.5 is partly involved in vascular remodelation (Guglielmotto et al., 2017). This could explain the moderately tight correlation between PGP 9.5 and factors responsible for vascular remodelation, such as VEGF and HGF.

In experiments with mice, it has been established that CgA accelerates wound healing, keratinocyte migration and proliferation in the skin (Curnis et al., 2012). It is reported that the CgA levels in plasma correlate with the extent of hepatic fibrosis (El-Fatah et al., 2016). However, publications about the significance of CgA in fibrotic processes are sporadic and the functional role of CgA in intraabdominal adhesions is still unclear. Overall, occasional CgA positive cells were observed, with no significant difference compared to the

controls, which indicates that this factor has no significant role in pathogenesis of adhesions. However, the Italian investigator Angelo Corti reported that CgA regulates the formation of stroma in tumour tissues, promoting extracellular matrix protein production in fibroblasts (Corti, 2010). As observed in the study, there is a statistically significant moderately strong correlation between a patient's age and CgA. It cannot be excluded that CgA promotes a gradual change in fibroblast phenotype in the case of adhesions.

The development of adhesions is a complicated process that involves the induction of an inflammatory response and cytokine production (Schanaider et al., 2016; Zhang H. et al., 2016). Pro- and anti-inflammatory cytokines broadly interact with fibrinolytic processes and promote remodelation of the extracellular matrix. Disturbances in the control of extracellular matrix remodelation can promote the development of adhesions after peritoneal injury (Cheong et al., 2002). Until now only a few inflammatory cytokines have been investigated in adhesions, but a systematic analysis on pro- and anti-inflammatory cytokines and their relative distribution in intraabdominal adhesions are lacking.

IL-1 is the main inflammatory cytokine that primarily promotes the inflammatory response (Garlanda et al., 2013). It is thought that IL-1 promotes adhesions, increasing fibrin accumulation and inhibiting fibrinolysis (Maciver et al., 2011). An early IL-1 increase is an important marker in the process of postoperative adhesion development (Saba et al., 1998). Simultaneously, another paper states that IL-1 signaling releases fibroblast MMPs, which has a role in tissue destruction (Rajshankar et al., 2012). In the study, few to moderate number of IL-1 positive cells were observed. Compared to control group tissues, the IL-1 finding in intraabdominal adhesions tissues was significantly lower; therefore, an ineffective systemic inflammation process has been detected and/or concluded that IL-1 has a minor role in adhesions.

Moreover, statistically significant correlations between IL-1 and other pro- and anti-inflammatory interleukins (IL-4, IL-6, IL-7, IL-10) were observed, pointing out an interaction of pro- and anti-inflammatory cytokines in the morphopathogenesis of intraabdominal adhesions. It is known and have been stated as important that IL-1 induces the synthesis of the inflammatory cytokine IL-6 in fibroblasts, endotheliocytes, and monocytes (Tosato & Jones, 1990), as well as induce the synthesis of IL-7, together with TNFα (Weitzmann et al., 2000). IL-10 inhibits inflammation, mainly through blocking the activation of pro-inflammatory signaling pathways and reducing the synthesis of pro-inflammatory mediators, such as IL-1, IL-6, IL-8, IFN-γ and TNFα (Mion et al., 2014).

In the present study, statistically significant differences in IL-10 findings between study and control groups were not detected. However, the overall moderate to numerous positive IL-10 cell count and their interaction with other pro-inflammatory mediators implies of an ongoing local protection process. Moreover, this is supported in other publications where a more pronounced IL-10 finding was observed in fibroblasts found in adhesions, compared to peritoneal fibroblasts (Saed et al., 2001).

It was observed that the IL-10 finding negatively correlates with TGFβ findings, which is crucial in the development of adhesions, because in such a way IL-10 decreased the development of connective tissue in the case of adhesions. This is supported in other publications where IL-10 potential to decrease the synthesis of TGFβ and inhibit fibrosis is reported (Zheng W. et al., 2005). In addition, it is reported that IL-10 inhibits MMP-2 and TIMP-1 expression in hepatic fibrosis in mice (Chou et al., 2006). Moreover, other authors have approved that the IL-10 induced reduction in TGFβ and MMP-2 expression levels markedly decreased the development of fibrosis in several organs, thereof in the heart, lungs, liver, and kidneys (Onishi et al., 2015). When analysing the

link between IL-10 and MMP-2 and TIMP-2, a moderately tight correlation was observed with TIMP-2, again the correlation with MMP-2 was weak, proving of a disbalance between MMP-2/TIMP-2.

IL-4 supports wound healing and tissue remodeling processes, it is the main stimulator of an anti-inflammatory response in macrophages (Wang & Joyce, 2010). IL-4 induces changes in the macrophage morphology, meaning that IL-4 stimulates the formation of macrophage aggregates or the development of giant multinuclear cells (Binder et al., 2013). Interestingly, also in the current study, in almost 20 % of the cases, giant multinuclear cells were observed. Overall, significantly less IL-4 positive cells in the adhesion group were observed, compared to the control group. The lack of IL-4 in case of adhesions, could point out changes in humoral response and be the initiator of giant epithelioid cell formation.

A moderately tight positive correlation between IL-4 and MMP-2 and a weak positive correlation between the IL-4 the TIMP-2 findings in intraabdominal adhesions were detected. Until now, there are no publications about the link between IL-4 with matrix remodeling factors in adhesions; however, the interaction of these factors has been observed in wound healing and skin remodelation processes. It is proven that IL-4 positively correlates with MMP-2 in those patients who develop a local wound infection (Nessler et al., 2014). MMPs and TIMPs are regulated though similar cytokines (IL-1, IL-4, IL-6, TNFα) and growth factors (HGF, TGFβ), but IL-4 inhibits MMPs synthesis in macrophages, not affecting TIMPs synthesis (Sun J., 2010). Another publication claims that IL-1 and TNFα do not affect biosynthesis of TIMP-2, but IL-4 stimulates TIMP-2 expression in skin fibroblasts. Therefore, IL-4 promotes synthesis of extracellular matrix proteins not only in normal wound healing but also in the case of pathological fibrosis (Ihn et al., 2002). It has been assumed that the lack of IL-4 and a disbalance in MMP-2/TIMP-2 maintains the adhesion

process, and the tissue disbalance is additionally pointed out by the formation of epithelioid cells.

IL-8 is one of the most meaningful cytokines, promoting neutrophil leukocyte activation and their migration to the place of inflammation (Henkels et al., 2011). When evaluating the correlations between IL-8 and other interleukins (IL-6, IL-7, IL-10) in the adhesion specimens in the current study, moderately strong statistically significant correlations were observed, proving the interaction between the cytokines regulating inflammation. This finding is consistent with other author's findings reporting the stimulation of IL-8 secretion through other pro-inflammatory cytokines. IL-6 can initiate and promote IL-8 secretion from mast cells (McHale et al., 2018). Moreover, other authors have proven that pro-inflammatory cytokines in combination with HGF increase synthesis of IL-8 even more. HGF in interaction with IL-1 induces IL-8 secretion from fibroblasts (Unger & McGee, 2011). Hypoxia induces the expression of HGF and IL-8 (Le et al., 2012). In the present study, a moderately strong correlation between IL-8 and HGF was observed; however, a statistically significant correlation was neither detected between HGF and IL-1, nor IL-1 and IL-8. Overall, in the adhesion group, a few or few to moderate number of IL-8 positive structures were observed, which was significantly less than in the control group. Such a finding indicates a possible delay in neutrophil leukocyte chemotaxis in case of adhesions, which prolongs the course of inflammation. This is indirectly supported by the finding of inflammatory cell infiltration and the occurrence of epithelioid cells in adhesion tissues.

Overall, in the study, a moderate to numerous IL-6 positive structures were observed, but a statistically significant difference between the groups was not detected. Possibly, IL-6 can maintain tissue inflammation; however, this cytokine is non-specific in the pathogenesis of intraabdominal adhesions. An increase of IL-6 has been observed in the peritoneal fluid in patients after

abdominal surgery (Jin et al., 2016). The main activators of IL-6 expression are IL-1 and TNFα (Schmidt-Arras & Rose-John, 2016). This fact explains the complex coherencies observed the present study – a positive correlation between IL-6 and TNFα, as well as IL-6 and IL-1 immunoreactive structures.

IL-7 promotes the activation of fibroblasts and macrophages (Bikker et al., 2012). An increase in IL-7 plasma levels promotes monocyte and macrophage migration through the endothelium (Li R. et al., 2011). It has been described in literature that IL-7 stimulates the secretion of other cytokines, such as IFN-γ, TNFα and IL-10 (Li H. et al., 2015). The present study demonstrated similar results –a positive correlation between IL-7 and IL-1, IL-4, IL-8, and IL-10 was observed. Overall, moderate to numerous IL-7 positive cells in adhesion tissues were observed, with no statistically significant difference compared to the control group. However, the correlation between IL-7 and other pro- and anti-inflammatory cytokines does not exclude the possibility that this factor is significant in the case of intraabdominal adhesions. Moreover, it must be taken into account that the amount of IL-7 is possibly impacted by the number of TGFβ positive structures that were markedly increased in the adhesion tissues. This conclusion in the study is similar to the findings in the Swedish study group, who concluded that TGFβ inhibits IL-7 production (Thang et al., 2010).

In the present study, a statistically significant difference between the TNFα immunoreactive structures in the adhesions and control groups was not detected; however, the dominance of moderate numbers of TNFα positive structures makes it possible that this factor is involved in pathogenesis of intraabdominal adhesions, and its decrease might have an impact on the development of adhesions. Markedly more TNFα is seen in the fibroblasts in adhesions when compared to peritoneal fibroblasts (Ambler et al., 2012). In an experiment with rats, the decrease of TNFα was linked to a decrease in adhesion development (Bayhan et al., 2016). When TNFα activity was suppressed in the

rats after appendix abrasion, macroscopically adhesions were less frequently observed; however, in histological findings no statistically significant differences were detected between study and control groups (Kurukahvecioglu et al., 2007).

Evaluating the HBD-2 findings in adhesions tissues, significantly less HBD-2 positive mesotheliocytes compared to the control group were noticed. However, considering that the difference was small and overall in adhesions tissues there were moderate to numerous numbers of HBD-2 positive structures with no statistical difference compared to the control group, possibly this antimicrobial peptide is not specific in the morphopathogenesis of adhesions, but rather for inflammation maintenance. This is supported by the observed moderately strong positive correlation between HBD-2 and factors regulating inflammation (IL-6, IL-10 un TNFα). Other authors' data on HBD-2 findings in intraabdominal adhesions are missing; however, our results are consistent with HBD-2 functions previously described in other tissues. The production of HBD-2 is stimulated by pro-inflammatory cytokines and pathogenic bacteria (Habil et al., 2014). Additionally to the antimicrobial functions, this peptide has an impact on epithelial and inflammatory cells, promoting their proliferation, cytokine production and their migration to the place of inflammation (Tewary et al., 2013).

Interestingly, in the present study statistically significant positive correlations were observed between HBD-2, MMP-2, and TIMP-2. Until now there are no published data about the interaction of these factors; however, it has been detected that in human conjunctival tissues fibroblast HBD-2 selectively increases the expression of MMP-2 and TIMP-2 expression, promoting cell migration in case of wound healing and extracellular matrix remodelation (Li J. et al., 2006). Taking into account that the correlation between HBD-2 and MMP-2 was strong, but between HBD-2 and TIMP-2 moderately strong, it cannot be excluded that HBD-2 also promotes the disbalance between MMP-2/TIMP-2, increasing extracellular matrix synthesis and the development of adhesions.

In the study, variable immunohistochemical MMP-2 findings were detected, but overall there were few to moderate numbers of MMP-2 positive structures observed. No statistically significant difference was distinguished between the study and control groups. However, in the adhesion tissues, statistically significant moderately strong correlations between MMP-2 and several factors regulating inflammation, such as IL-4, IL-6, IL-7, IL-8, were observed indicating the inflammation-promoting impact of MMP-2 in the morphopathogenesis of intraabdominal adhesions, which is indirectly supported by the inflammation observed in adhesions. The obtained data are consistent with the other authors' findings regarding the fact that inflammation as a process is relying on multiple factors, thereof MMPs expression and their proteolytic activity, localisation and the substrate availability. MMPs can either promote or prevent inflammation through their proteolytic impact to pro-inflammatory cytokines (Manicone & McGuire, 2008). MMPs regulates the synthesis of growth factors and cytokines, such as IL-1, IL-6, TNFα (Ma et al., 2014).

A connection between MMP-2 and VEGF in adhesion tissues was observed, which indicates that MMP-2 has an angiogenesis promoting impact, supported by the neoangiogenesis observed. Other authors as well have described an interaction between MMPs and VEGF (Belotti et al., 2003; Partyka et al., 2012; Araújo et al., 2015). MMP-2 stimulates angiogenesis and increases VEGF secretion (Zheng H. et al., 2006). It is known that a prolonged MMP-2 expression promotes an increase in vasculature density, but its deficit causes vascular instability and a decrease of vascularisation (Trivedi et al., 2016). TIMP-2 can diminish the expression of VEGF, by inhibiting MMPs (Li M. et al., 2014). In the current study, a similarly tight correlation between MMP-2 and VEGF as well as between TIMP-2 and VEGF was observed, pointing out the significance of MMP-2/TIMP-2 balance for regulation of VEGF and angiogenesis. Considering that significantly less TIMP-2 in the adhesion specimens was

observed, this finding also supports the dominating impact of MMP-2 on the stimulation of angiogenesis.

In the study statistically significant correlations between MMP-2 findings and TIMP-2 were observed; however, the count of TIMP-2 positive cells were smaller in the adhesions group. Possibly, this decrease in TIMP-2 observed in adhesions is a significant factor in morphopathogenesis of adhesions, because it promotes the formation of excess tissue. This is supported by the characteristic morphological findings with a large accumulation of collagen fiber bundles and chaotically located tight connective tissue bundles. Also, other authors in their studies describe that the balance between MMPs and TIMPs expression regulates normal wound healing. Changes in expression levels can interrupt the healing process or cause the formation of excess tissue, for example, the development of peritoneal adhesions (Chegini et al., 2002). The imbalance between MMPs and TIMPs is linked to diseases, characterised by fibrosis (Dohi et al., 2015).

TIMP-2 has a dual impact on MMP-2: in low concentrations, it activates MMP-2 precursors, but in high concentrations completely inhibits this reaction (Dohi et al., 2015). Overall, moderate number of TIMP-2 positive structures were observed; however, in the adhesion group, significantly less TIMP-2 positive endotheliocytes and fibroblasts were observed, again pointing out the disbalance between MMP-2/TIMP-2. The disproportionateness between TIMP-2 and MMP-2 proves of fibrosis promoting environment in the case of peritoneal adhesions. The increased MMP-2 activity and decreased TIMP-2 expression significantly increases the relation between MMP-2/TIMP-2, promoting fibrosis (Chen X. et al., 2014). TIMP-2 deficit promotes monocyte/macrophage invasion (Di Gregoli et al., 2016) and the synthesis and accumulation of collagen (Dohi et al., 2015).

In the present study, moderately strong correlations between TIMP-2 and IL-1, IL-6, IL-8, IL-10, TNFα was observed. It has to be taken into account that

in the adhesion group tighter correlation was observed between inflammatory cytokines and TIMP-2, but not MMP-2, pointing out the MMP-2/TIMP-2 disbalance even more, thus promoting fibrosis. The interaction of the inflammation-promoting cytokines and TIMP-2 in adhesions has so far not been reported, therefore our findings are unique. However, the interaction of these factors has been investigated in other tissues, where an increase of TIMP-2 was observed under the impact of inflammatory cytokines (Watanabe et al., 2002). It has been reported that TNFα and IL-1β are involved in regulation of TIMPs (Li Y. et al., 1999). IL-6 induces the secretion of TIMP-2, boosting the MMP/TIMP disbalance (Badr, 2016).

The study presents complex morphopathogenetic processes of intraabdominal adhesion development in infants which is the first time analysis of the sorts. In the intraabdominal adhesion tissues an increased TGFβ finding, a decreased HGF finding and a disbalance in FGF-2/FGFR1 and MMP-2/TIMP-2 was observed, overall facilitating fibrosis, tissue remodelation disorders and adhesion development. In the adhesion tissues, neoangiogenesis and an increase in VEGF positive macrophages are characteristic. Possibly, hypoxic injury caused the observed decrease in PGP 9.5 positive structures. The decrease in IL-1, IL-4, and IL-8 are the most significant changes in inflammation regulating cytokines in the pathogenesis of intraabdominal adhesions. The inflammation in intraabdominal adhesions is also linked to the increase of the antimicrobial peptide HBD-2, promoting the maintenance of inflammation.

Therefore, all together it can be concluded that intraabdominal adhesions in children are characterised by increased TGFβ, VEGF, FGFR1 and decreased FGF-2, HGF, PGP 9.5, IL-1, IL-4, IL-8, TIMP-2 findings. The most significant aspects promoting and maintaining adhesion development in children are related to changes in extracellular matrix remodelation, neoangiogenesis and the maintenance of prolonged inflammation.

4. CONCLUSIONS

1. Neonatal adhesions are characterised by unspecific changes, such as fibrosis and neoangiogenesis, as well as changes specific to chronic inflammation, such as mesotheliocytes and fibroblasts of modified shape, due to a phenotypic change in the cell, as well as giant cells.

2. A statistically significant increase in TGFβ containing structures, relative to the decrease of HGF containing structures in adhesion tissues, gives evidence of the growth/regenerative potential of loose connective tissue and their promotive role in the pathogenesis of inatraabdominal adhesions. The decreased in FGF-2 and more pronounced FGFR1 finding, proves a compensatory receptor stimulation in response to the lack of the same factor, in case of adhesions.

3. The statistically significant increase in VEGF positive macrophages in intraabdominal adhesions, indicates tissue ischemia and the stimulation of neoangiongenesis. VEGF also correlates with IL-10 and MMP-2, promoting the synthesis of growth factors and endotheliocyte proliferation in chronic inflammation, again underlining VEGF's promoting role in the formation of adhesions.

4. The statistically significant decrease in PGP 9.5 positive structures proves a hypoxia induced prolonged injury that is attempted to be compensated through a stable TNFα expression, producing a statistically significant correlation between PGP 9.5 and TNFα. The innovative finding of PGP 9.5 in fibroblasts and macrophages, in correlation with TGFβ, MMP-2 and TIMP-2, promotes fibrosis.

5. Similar findings of CgA in adhesion and control groups shows that this factor has no specific role in morphopathogenesis of adhesions. However, its statistically significant correlation with a patient's age indicates a shift of fibroblasts phenotype in neonatal intraabdominal adhesions.

6. In intraabdominal adhesions, there is an unpronounced IL-1 and marked IL-10 finding, indicating the dominating local tissue protection reaction in adhesions. However, the statistically significant correlation between IL-1 and interleukins IL-4, IL-6, IL-7, proves of its role in inducing pro-inflammatory cytokines in case of adhesions, in a prolonged period. Additionally, the decrease in IL-4 positive structures could be the direct cause for the formation of giant cells, but the decrease of IL-8 positive structures could explain a delayed chemotaxis of inflammatory cells and the prolongation of the inflammatory process itself. Finally, IL-7 underlies the inhibitory impact of TGFβ in the morphopathogenesis of adhesions, therefor maintaining the tissue inflammation.

7. The relatively similar amount of HBD-2 positive structures in intraabdominal adhesions and healthy tissues, as well as the statistically significant correlation between HBD-2 and pro-inflammatory/anti-inflammatory cytokines indicates on this antimicrobial peptide's unspecific role in the inflammatory response. However, the correlation between HBD-2 and MMP-2 and TIMP-2 could indicate an HBD-2 induced disbalance in tissue remodelation factors and increased synthesis of extracellular matrix and adhesion formation.

8. There is a marked decrease of tissue inhibitor of metalloproteinase-2 (TIMP-2) in intraabdominal adhesions, meanwhile the expression of tissue degenerative enzymes (MMP-2) does not considerably differ from the control group. The disbalance between MMP-2 and TIMP-2 proves the decrease in remodelation processes and increased fibrosis.

9. Intraabdominal adhesions are characterised by an increasing TGFβ, VEGF, FGFR1 expression and decreased FGF-2, HGF, PGP 9.5, IL-1, IL-4, IL-8, TIMP-2 expression, indicating the role of these factors in the pathogenesis of adhesions. The most significant changes are observed in the remodelation of the extracellular matrix, promotion of neoangiongenesis, the maintenance of a prolonged atypical decompensating inflammation and the emergence of a

peculiar *circulus vitiosus*, promoting fibrosis based on a decompensated tissue inflammatory response. Therefore, the most significant markers in the prognosis and diagnosis of adhesions in newborns are TGFβ, VEGF, HGF, IL-1 and IL-4.

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LIST OF PUBLICATIONS

Scientific papers (6)

1. **Augule A**., Pilmane M., Ābola Z., Volrāts O. 2015. Basic Fibroblast Growth Factor (bFGF), Fibroblast Growth Factor Receptor 1 (FGFR1), Transforming Growth Factor Beta (TGF-β) and Chromogranin A (CgA) Appearance in Congenital Intra-abdominal Adhesions in Children under One Year of Age. *British Journal of Medicine & Medical Research*. 10 (11): 1–9.
2. **Junga A**., Pilmane M., Ābola Z., Volrāts O. 2018. Interleukin-1, 4, 6, 7, 8, 10 (IL-1, 4, 6, 7, 8, 10) Appearance in Congenital Intra-abdominal Adhesions in Children under One Year of Age. *Applied Immunohistochemistry & Molecular Morphology*. 26 (9): 664–669.
3. **Junga A**., Pilmane M., Ābola Z., Volrāts O. 2018. Iekaisuma procesu regulējošo citokīnu sastopamība intraabdominālu saaugumu audos bērniem līdz gada vecumam (Eng. Inflammatory process cytokine incidence in intraabdominal adhesion tissues in children under one year of age). *RSU Zinātniskie raksti 2017*. 154–165.
4. **Junga A**., Pilmane M., Ābola Z., Volrāts O. 2018. Tumour Necrosis Factor alpha (TNFα), Protein Gene Product 9.5 (PGP 9.5), Matrix Metalloproteinase-2 (MMP-2) and Tissue Inhibitor of Metalloproteinase-2 (TIMP-2) Appearance in Congenital Intra-Abdominal Adhesions in Children under One Year of Age. *Archives of Medical Science*. Accepted for publication on 19 March 2018.
5. **Junga A**., Pilmane M., Ābola Z., Volrāts O. 2018. The Distribution of Vascular Endothelial Growth Factor (VEGF), Human Beta-Defensin-2 (HBD-2) and Hepatocyte Growth Factor (HGF) in Intra-Abdominal Adhesions in Children under One Year of Age. *The World Scientific Journal*. Article ID 5953095, 7 pages.

6. **Junga A.**, Pilmane M., Ābola Z., Volrāts O. 2019. The Morphopathogenetic Aspects of Intraabdominal Adhesions in Children under One Year of Age. *Medicina (Kaunas)*. 55(9): 556.

Abstracts and presentations in international conferences (8)

1. **Augule A**., Pilmane M., Ābola Z., Volrāts O. Basic fibroblast growth factor (bFGF) and fibroblast growth factor receptor 1 (FGFR1) appearance in congenital intra-abdominal adhesions in children under one year of age, Rīga Stradiņš University Student international conference "Health and Social Sciences" abstract, Riga 2015, abstract book, 309–310. (Oral presentation, 2nd place).
2. **Junga A**., Pilmane M., Ābola Z., Volrāts O. A correlative comparison of the epidemiologic profile and the morphologic finding in children with congenital intra-abdominal adhesions, The 8th Baltic morphology scientific conference: Interdisciplinary nature of contemporary morphology abstract, Vilnius 2015, abstract book, 46. (Oral presentation).
3. **Junga A**., Pilmane M., Ābola Z., Volrāts O. Interleukin-1, 4, 6, 7, 8, 10 (IL-1, 4, 6, 7, 8, 10) appearance in congenital intraabdominal adhesions in children under one year of age, European Paediatric Surgeon's Association 17th European Congress abstract, Milano 2016, abstract book, 154. (Poster presentation).
4. **Junga A**., Pilmane M., Ābola Z., Volrāts O. The occurrence of inflammation regulating cytokines in congenital intraabdominal adhesions in children under 1 year of age, European Paediatric Surgeon's Association 18th European Congress abstract, Limassol 2017, abstract book, 205. (Poster presentation).
5. **Junga A**., Pilmane M., Ābola Z., Volrāts O. The occurrence of fibrosis modulating factors, transforming growth factor beta and chromogranin A in

intraabdominal adhesions in infants, IX Baltic Morphology abstract, Tartu 2017, abstract book, 54. (Oral presentation).

6. **Junga A**., Pilmane M., Ābola Z., Volrāts O. Tumour necrosis factor α (TNFα), protein gene product 9.5 (PGP 9.5) and extracellular matrix remodeling factor appearance in congenital intra-abdominal adhesions in children under one year of age, World Summit on Pediatrics 4th Edition abstract, Madrid 2018, abstract book, 95. (Poster presentation).
7. **Junga A**., Pilmane M., Ābola Z., Volrāts O. Vascular endothelial growth factor (VEGF), human beta-defensin-2 (HBD-2) and hepatocyte growth factor (HGF) appearance in congenital intra-abdominal adhesions in children under one year of age, XXVI International Symposium on Morphological Sciences abstract, Prague 2018, abstract book, 32. (Poster presentation).
8. **Junga A.**, Pilmane M., Ābola Z., Volrāts O. The significance of growth factors, degenerating enzymes, inflammatory and antimicrobial factors in the morpho-pathogenesis of intraabdominal adhesions in infants, Rīga Stradiņš University International Conference on Medical and Health Care Sciences "Knowledge for Use in Practice" abstract, Riga 2019, abstract book, 563. (Oral Presentation).

Abstracts and presentations in local conferences in Latvia (5)

1. **Augule A**., Pilmane M., Ābola Z., Volrāts O. Bāziskā fibroblastu augšanas faktora (bFGF), fibroblastu augšanas faktora receptora 1 (FGFR1), transformējošā augšanas faktora beta (TGF-β) un hromogranīna A (CgA) ekspresija iedzimtu intraabdominālu saaugumu gadījumos bērniem līdz gada vecumam (Eng. Transforming growth factor beta (TGF-β), basic fibroblast growth factor (bFGF), fibroblast growth factor receptor 1 (FGFR1) and chromogranin (CgA) incidence in congenital intra-abdominal adhesions in

children under one year of age), XX Students' Conference on Morphological Science abstract, Riga 2015, abstract book, 9–10. (Oral presentation, 1st place).

2. Volrāts O., **Augule A**., Pilmane M., Ābola Z. Transforming growth factor beta (TGF-β), basic fibroblast growth factor (bFGF) and fibroblast growth factor receptor 1 (FGFR1) appearance in congenital intra-abdominal adhesions in children under one year of age, annual Rīga Stradiņš University Scientific Conference 2015 abstract, abstract book, 236. (Poster presentation).
3. **Junga A**., Pilmane M., Ābola Z., Volrāts O. Interleikīnu 1, 6, 7, 10 sastopamība un relatīvais sadalījums iedzimtu intraabdominālu saaugumu gadījumos bērniem līdz gada vecumam (Eng. Interleukin-1, 6, 7, 10 appearance and relative distribution in congenital intraabdominal adhesions in children under one year of age), annual Rīga Stradiņš University Scientific Conference 2016 abstract, abstract book, 153. (Oral presentation).
4. **Junga A**., Pilmane M., Ābola Z., Volrāts O. Iekaisuma procesu regulējošo citokīnu sastopamība intraabdominālu saaugumu audos bērniem līdz gada vecumam (Eng. Inflammatory process cytokine incidence in intraabdominal adhesion tissues in children under one year of age), annual Rīga Stradiņš University Scientific Conference 2017 abstract, abstract book, 182. (Poster presentation).
5. **Junga A**., Pilmane M., Ābola Z., Volrāts O. Iekaisuma procesu regulējošo citokīnu, fibrozi modulējošo faktoru, transformējošā augšanas faktora β un hromogranīna A sastopamība intraabdominālu saaugumu audos bērniem līdz gada vecumam (Eng. Inflammatory process cytokine, basic fibroblast growth factor, transforming growth factor beta and chromogranin incidence in intraabdominal adhesion tissues in children under one year of age), annual Rīga Stradiņš University Scientific Conference 2018 abstract, abstract book, 146. (Oral presentation).

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