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THE FIELD OF MEDICINE

THESIS

SUMMARY

Immunophenotyping of endometriosis with CD45 and CD8 biomarkers, using artificial intelligence

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THESIS CONTENT

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INTRODUCTION

Endometriosis is the most common benign pathology of women, located in the peritoneum and pelvic organs, represented by the appearance of islands of functional endometrial tissue with other localization than the uterine cavity.

Endometriosis is influenced by estrogen and is specific to women at reproductive age. It is difficult to diagnose this pathology as its signs and symptoms are unspecific. The most common symptoms are dysmenorrhea, chronic pelvic pain, dyspareunia and infertility, but there are patients in whom the symptoms are absent. The treatment of endometriosis can be medical, surgical or both. Whatever treatment we choose, the risk of recurrence is the same. Anti-inflammatory drugs and hormone therapy may be combined in the treatment of endometriosis.

Laparoscopy is the gold standard in diagnosing endometriosis, although is an invasive method.

The aim of this paper is testing with immunomarkers the endometriosis located in different tissues: ovarian, peritoneal, striated muscle (right abdominal muscles), smooth muscle (myometrium) and analysis the inflammatory response to the presence endometriosis.

The inflammation of the parietal endometriosis has been very little studied clinically, imagistically and morphopathologically.

We didn't find data in the literature on the immunohistochemical study with CD45 and CD8 of abdominal wall endometriosis, which makes this thesis unique.

The thesis consists of two parts: the general part and the special part.

GENERAL PART

In the general part I included theoretic informations about the anatomy of the genital organs, about endometriosis, immunology and artificial intelligence.

Endometriosis is a fascinating pathology that we strive to understand. Molecular techniques are beginning to clarify the level of knowledge of the disease: from pathogenesis to staging. The claim that endometriosis is a precursor to cancer is reinforced by the fact that mutations found in cancers associated with endometriosis are also found in adjacent endometriosis.

Recent genetic studies suggest that infiltrative pelvic endometriosis is a benign neoplasm that spreads locally rather than metastasizes. Further studies are needed to elucidate the various aberrations that occur in this phenotype.

Endometriosis affects the woman of reproductive age. This ectopic tissue can be found at any level, but most often it will be found in the pelvis, and less often, in the lungs or brain. Adenomyosis is the location of endometrial tissue in the uterine muscle.

The pathogenesis of endometriosis is still less known, despite the fact that recently a lot of information has been accumulated about this condition. However, there are some classical theories that provide explanations for the pathogenesis of endometriosis, to which is added the possibility of associating immunological, genetic and hormonal factors.

a) The theory of retrograde implantation, initially proposed by Sampson, suggested that, during menstruation, there are refluxes of tubal endometrial fragments from the uterine cavity to the peritoneal cavity and neighboring structures, where they will implant and evolve under the stimulus hormonal, similar to the endometrium present in the uterine cavity.

b) The metaplasia of the celomic epithelium, claims that the mechanism of endometriosis occurrence is the endometrioid transformation of the peritoneal, ovarian and uterine serous mesothelium, under the influence of unidentified factors, possibly hormonal or infectious. The theory is logical, because both the endometrium and the peritoneum derive from the same embryological precursor, the coelomic epithelium. However, the theory could not be supported by clinical or experimental arguments.

c) The induction theory is based on the two theories above, considering that an unknown biochemical factor released by menstrual blood and endometrial cells destroyed by macrophages can induce the transformation of undifferentiated peritoneal cells into endometrial tissue. This theory is supported by experiments performed on rabbits, but did not materialize in humans.

d) Metastatic theory. While the above theories may argue for the occurrence of endometriosis in common implantation sites, it has been difficult to explain the existence of ectopic foci in less accessible sites, such as: umbilicus, pelvic lymph nodes, ureters, rectovaginal septum, intestinal wall, lung, pleura, endocardium and limbs. Therefore, embolization of menstrual fragments could occur hematogenously or lymphatically.

The immune system is represented by all the body's defense mechanisms against pathogens and non-self structures.

In this study, I used monoclonal antibodies from mice, and the species on which the study was performed is human, not being studied in other species. The isotype is IgG2a / kappa, located at the cell membrane, and the epitope or antigen is CD45RO.

CD45 is an enzyme called common leukocyte antigen. CD45 belongs to the enzyme group Protein-Tyrosine-Phosphatases (PTP) which are molecules that regulate a variety of cellular processes such as: cell growth, differentiation, mitotic cycle and oncogenesis. CD45 is a transmembrane protein present in multiple isoforms in all differentiated hematopoietic cells (except erythrocytes and plasma cells) and is also an essential regulator of T and B cell antigens.

CD8 is a lymphocyte marker that recognizes the antigen only if it is associated with MHC class I molecules. As a structure it is a heterodimer with two chains, alpha and beta, of 210 amino acids each. Each chain has three segments: transmembrane, intracytoplasmic and extracellular. It is a defining marker for cytotoxic T lymphocytes.

Today, artificial intelligence occupies an important part of our lives, it is a growing field, which is constantly developing and which is introduced in most daily activities. In addition, it continues to capture many areas of health, making huge progress especially in the analysis of medical imaging, such as radiography, computed tomography, magnetic resonance imaging, digital tomosynthesis of the breast, positron emission tomography or retinal imaging.

Computer programs developed for the automatic analysis of digitized images are an interdisciplinary bridge with major impact in research.

Digital pathology includes the acquisition, management, distribution and interpretation of anatomical and pathological information, including sections on slides, in the digital environment. Digital sections are created by scanning slides and thus obtaining high-resolution images that can be viewed on a computer or any monitor, including personal devices such as a telephone.

The process of digitization of the pathology has been due to the development and improvement of several systems in the last decade, which gives a multitude of imaging data to pathologists or researchers. The base of artificial intelligence is machine learning, which consists of different models and statistical methods based on a vast set of data. First, different mathematical models were used as machine learning tools, such as supervised and unsupervised learning, randomforest, grouped algorithms or component analysis. Of course, there are many other tools used in imaging analysis, all of which help the pathologist establish the final diagnosis.

PERSONAL CONTRIBUTION
OBJECTIVES

1. The correlation between symptoms and:
- the environment
- parity
- localization of endometriosis
- hemoglobin and hematocrit values
- the value of leukocytes
- platelet count

2. The correlation between the location of endometriosis and:
- parity
- hemoglobin and hematocrit values
- the value of leukocytes
- platelet count

3. The correlation between the expression of CD45 and CD8 biomarkers and:
- localization of endometriosis
- symptoms
- hemoglobin and hematocrit values
- the value of leukocytes

MATERIAL AND METHOD

Eligibility criteria

- The cases registered in the Obstetrics and Gynecology Clinic within the County Emergency Clinical Hospital „St. Andrei" from Constanţa who went surgery.
- Histopathologically confirmed cases with endometriosis.
- Cases with immunohistochemical examination consisting of a mandatory panel of immunomarkers (CD45, CD8).

Exclusion criteria

- The cases with other type of malignant or benign tumor than endometriosis.
- Cases with incomplete immunohistochemical panel.

The analyze of the histopathological material was performed macroscopically and microscopically.

The immunohistochemical study was performed on the paraffin blocks from which the sections necessary for the classical histopathological processing with hematoxylin-eosin were performed. By sectioning them at the microtome, at three levels, were obtained serial sections with a thickness of 4μ which were applied on slides treated with adhesive (polylysine).

For the quantification of immunomarkers we used digital pathology. We used the HURON Tissue Escope 4000XT blade scanner with software system for capturing and storing images resulting from the CEDMOG research center of Ovidius University in Constanţa. This method allows complete scanning of immunohistochemical preparations and then analysis of digital images using the QuPath computer platform for automatic quantification of the CD8 and CD45 index, using virtual microscopy. We analyzed each section using the QuPath software.

The experimental data were processed using the IBM SPSS Statistics statistical processing program 23. We used nonparametric tests for The analysis of biomarkers expression.

RESULTS

1. Patients in rural areas have more frequently associated the association of pelvic pain symptoms with metrorrhagia.
2. There is a relationship of dependence (statistically significant) between the types of symptoms and parity.
3. No nulliparous patient had metrorrhagia without association with pelvic pain and no nulliparous patient had parieto-abdominal pain.
4. Patients diagnosed with adenomyosis had metrorrhagia or the association of metrorrhagia with pelvic pain more frequently (statistically significant).
5. All patients with abdominal wall endometriosis had pain in the abdominal wall.
6. Most patients with pelvic endometriosis had pelvic pain (statistically significant).
7. Analyzing the types of symptoms, it is found that there are statistically significant differences depending on the location of endometriosis.
8. There are statistically significant differences between the types of symptoms depending on the average age values of the patients.
9. Patients with pelvic pain and those with abdominal wall pain were younger than patients with metrorrhagia - as a single symptom or in combination.
10. There are statistically significant differences between patients' mean hemoglobin values depending on symptoms.
11. Patients with metrorrhagia or the combination of the two symptoms, metrorrhagia and pelvic pain, had a lower (statistically significant) hemoglobin value than those with abdominal wall pain or those who had only pelvic pain.
12. There are statistically significant differences between the mean values of the hematocrit depending on the symptoms.
13. Patients with metrorrhagia and the associaton of the two symptoms, metrorrhagia and pelvic pain, had lower values of hematocrit (statistically significant) than those with abdominal wall pain or those with pelvic pain.
14. There were no statistically significant differences between mean platelet counts depending on symptomatology.
15. There were no statistically significant differences between mean leukocyte values depending on symptomatology.
16. All patients with abdominal wall endometrioma were multiparous, and most patients with pelvic endometriosis were nulliparous, while those with adenomyosis were mostly multiparous. On the other hand, most nulliparous patients were diagnosed with pelvic endometriosis, and of the multiparous patients, most had adenomyosis. Between the location of endometriosis and parity there is a statistically significant relationship of dependence.
17. We observed statistically significant differences, in general, between the mean values of hemoglobin and hematocrit depending on the location of endometriosis. In particular, patients with adenomyosis were more anemic. Thus, they had a statistically lower hemoglobin value than the patients with pelvic or abdominal wall endometriosis and had a statistically lower hematocrit than the patients with abdominal wall endometrioma.
18. There were no statistically significant differences between mean platelet or leukocyte values depending on the location of endometriosis.
19. The CD45 biomarker was statistically significantly more intensely expressed intraepithelially in the wall endometrioma compared to the other two locations.

20. The intraepithelial CD45 biomarker was statistically significantly correlated with symptomatology. Thus, CD45 was statistically significantly more intensely expressed intraepithelial in patients who presented with abdominal wall pain or the association of pelvic pain with metrorrhagia.
21. The intraepithelial CD45 biomarker had, in anemic patients, a statistically significant distribution, in general, correlated with the localization of MS. CD45 was statistically significantly more intensely expressed intraepithelially in anemic patients with wall endometrioma than in patients with adenomyosis or pelvic endometriosis.
22. In patients with normal hemoglobin value, the intraepithelial CD45 biomarker also had a statistically significant distribution, generally correlated with endometriosis localization. CD45 was statistically significantly more intensely expressed intraepithelially in patients diagnosed with wall endometriosis than in those diagnosed with pelvic endometriosis.
23. The distribution of intraepithelial CD45 values was the same in patients with anemia compared to patients with normal hemoglobin values for the three locations of MS.
24. In both patients with leukocytosis and those with normal leukocyte values, intraepithelial expression of the CD45 biomarker was statistically significantly correlated, in general, with the location of EM. CD45 was statistically significantly more intensely expressed intraepithelially in patients diagnosed with wall endometriosis than in patients with adenomyosis or pelvic endometriosis.
25. The distribution of intraepithelial CD45 values was the same in patients with leukocytosis compared to patients with normal leukocyte values for the three locations of MS.
26. The CD45 biomarker was (statistically significant) more intensely expressed stromally in wall endometriosis compared to the other two locations.
27. The CD45 biomarker was (statistically significant) more intensely expressed stromal in patients who had abdominal wall pain compared to other types of symptoms.
28. The CD45 biomarker was (statistically significant) more intensely expressed stromally in anemic patients with wall endometriosis than in patients with adenomyosis or pelvic endometriosis.
29. In patients with normal hemoglobin value, the expression of the stromal biomarker CD45 differed statistically significantly depending on the location of endometriosis - more intensely expressed at the parietal level than the other two locations.
30. The CD45 biomarker was (statistically significant) more intensely expressed stromally in patients with leukocytosis diagnosed with wall endometriosis than in patients with adenomyosis or pelvic endometriosis.
31. The CD45 biomarker was more intensely expressed stromally in patients with abnormal leukocyte values diagnosed with wall endometriosis than in patients with adenomyosis or pelvic endometriosis.
32. There were no significant differences in intraepithelial CD8 biomarker expression depending on the location of endometriosis.
33. There were no significant differences in intraepithelial CD8 biomarker expression depending on symptomatology.
34. There were no significant differences in intraepithelial CD8 biomarker expression depending on hemoglobin value and endometriosis location.
35. There were no significant differences in intraepithelial CD8 biomarker expression depending on leukocyte value.
36. There were generally no significant differences in the analysis of median values of stromal CD8 biomarker depending on the location of endometriosis but there was an uneven distribution

of the marker; thus, the distribution is statistically significantly different between the adenomyosis and the abdominal wall.

37. There were no significant differences in the expression of the stromal CD8 biomarker depending on the symptomatology.

38. There were no significant differences in median values of stromal CD8 biomarker depending on hemoglobin value and endometriosis location. On the other hand, there were statistically significant differences in the distribution of these values among patients with adenomyosis and pelvic endometriosis.

39. There were no significant differences in stromal CD8 biomarker expression depending on leukocyte value.

CONCLUSIONS

1. The symptoms studied (pelvic / parietal pain, metrorrhagia or a combination them) were statistically significantly correlated with age, environment of origin, parity, location of endometriosis, hemoglobin and hematocrit values. We did not identify such correlations with platelet or leukocyte counts.
2. The location of endometriosis was correlated with (apart from the symptomatology, described above) parity, hemoglobin and hematocrit values.
3. The CD45 biomarker in the intraepithelial and stromal component was more intensely expressed in patients who had abdominal wall endometriosis, compared to those with pelvic endometriosis or adenomyosis (regardless of hemoglobin or leukocyte value). Biomarker expression was statistically significantly correlated with symptomatology.
4. The expression of the CD8 biomarker in the intraepithelial and stromal component did not show significant differences, correlated with the symptomatology or the value of leukocytes. Although there were no statistically significant differences in the median values of this expression depending on the location of endometriosis or hemoglobin, the distribution of values of that expression was statistically significant, depending on the location of endometriosis (between adenomyosis and abdominal wall) or hemoglobin. (between adenomyosis and pelvic endometriosis).
5. Inflammation is much more intense in parietal endometriosis, supported by the increased presence of CD45-labeled T lymphocytes at this level.
6. The CD8 biomarker (which marks cytotoxic T lymphocytes) was less expressed compared to CD45, which supports the invasive nature of endometriosis.