PROBLEMATIC ISSUES RELATED TO THE PHENOTYPIC CHARACTERISTICS, PERSISTENCE, AND PROGRESSION OF CHRONIC ENDOMETRITIS A CRITICAL REVIEW

***OLIKO MURGHULIA**, PhD Student,[1] **GEORGE TEVDORASHVILI**, MD, PhD, Prof.,[2]*
***NINO TEVZADZE**, Resident,[3] **GEORGE BURKADZE**, Prof.[4]*
[1] Tbilisi State Medical University
[2] Tbilisi State Medical University, Department of Obstetrics and Gynaecology
[3] Tbilisi State Medical University, Department of Anatomical and Clinical Pathology
[4] Tbilisi State Medical University, Head of the Department of Molecular Pathology, Tbilisi, Georgia

SUMMARY

Chronic endometritis is defined as mild persistent inflammation of the endometrium, characterized histologically by inflammatory cells in the endometrial stroma, including plasma cells, lymphocytes, eosinophils, and even lymphoid follicles. Diagnosing chronic endometritis is difficult for a variety of reasons. Most patients are asymptomatic, and ultrasound features are nonspecific. Microbiological examination is often not informative because most pathogens are non-cultivable. A hysteroscopy can diagnose chronic endometritis by detecting specific endometrial changes, such as focal or diffuse hyperemia, stromal edema, and micro polyps. Histopathological identification of plasma cells in endometrial biopsy specimens is considered the gold standard for the diagnosis of chronic endometritis. There is a hypothesis that chronic endometritis may be related to endometriosis, although studies in this direction are very scarce. There are different opinions about the persistence and progression of chronic endometritis, which require further research.

Keywords: chronic endometritis; plasma cells.

First published: Georgian Scientists vol. 6 N3, 2024 | 99. https://doi.org/10.52340/gs.2024.06.03.10

Chronic endometritis is defined as mild persistent inflammation of the endometrium, characterized histologically by the presence of inflammatory cells in the endometrial stroma, including plasma cells, lymphocytes, eosinophils, and even lymphoid follicles.[1,2] Other histologic features include superficial edema, increased stromal density, and asynchronous differentiation of the endometrial epithelium and stroma.

The cause of the existing inflammatory process is not entirely known. Some cases of chronic endometritis show regression after antibiotic therapy, suggesting an infectious etiology.[3] The cases resistant to antibiotics might be due to another etiological agent. So far, the presence of plasma cells in the endometrial stroma is considered the most important diagnostic criterion.[4-6] A plasma cell is a differentiated form of a B lymphocyte that can produce immunoglobulins or antibodies and is, therefore, involved in humoral immunity. Chronic endometritis is often associated with infertility, endometriosis, implantation disorders, spontaneous abortion, and obstetric complications such as preeclampsia and premature birth. It is also connected to neonatal diseases in premature infants, including periventricular leukomalacia.[1,2]

Inflammatory processes damaging the endometrial cavity can affect the implantation of a fertilized egg, potentially resulting in infertility or spontaneous abortion.[3,4] Physiologically, the endometrial stroma contains various immunocompetent cells, including natural killer (NK) cells, macrophages, T cells, and neutrophils. The composition and number of these cells vary throughout the menstrual cycle.[7] While the leukocyte content does not exceed 10% of stromal cells during the proliferative and early secretory phases, their number increases dramatically starting in the mid-secretory phase. It remains elevated during the late secretory phase and early pregnancy. The cycle-dependent variation of these cell subpopulations is crucial for the implantation process. Studies reveal that the leukocyte population in the endometrium of women experiencing recurrent spontaneous abortions is notably different from that in women who achieve full-term pregnancies. Within the immune cell population present in the endometrial stroma, an increased number of NK cells and plasma cells is associated with recurrent spontaneous abortion, implantation failure, or in vitro fertilization failure. Generally, the causes, as mentioned earlier, often include anatomical defects of the uterus, parental karyotype abnormalities, and blood clotting disorders such as protein C deficiency, Factor V Leiden mutation, and antiphospholipid syndrome. Nonetheless, in 50% of cases involving miscarriage and failed in vitro fertilization, the underlying cause remains unexplained and may be related to chronic endometritis.

Infertility is a relatively common gynecological issue, with its frequency gradually increasing in recent years. Studies have revealed that the incidence of chronic endometritis among infertile patients ranges from 0.2% to 46%. Studies have also established that the frequency of chronic endometritis in cases of implantation defects following in vitro fertilization is 14%, and the overall implantation rate after endometritis treatment is 11.5%, which is notably low compared to cases where the presence of endometritis is not confirmed (32,7 %).[8]

It is important to emphasize that implantation occurs due to a complex interaction between the blastocyst and the endometrium.[9] Numerous signaling pathways are involved in this connection, and the endometrium must be suitable for successful implantation. Both embryonic and endometrial factors can easily disrupt this unstable balance. In this case, chronic endometritis is one factor that prevents successful implantation in the endometrium. According to some per-

spectives, inflammation is merely the trigger for a much more intricate and organized sequence of events. The altered secretion of cytokines and chemokines leads to changes in the leukocyte population. These modifications, in turn, affect the contractility of the uterus and the function of the endometrium in the process of decidualization and vascularization. Autophagy plays a crucial role and is essential for successful implantation.

As previously mentioned, the etiology of this pathology remains a subject of controversy. The literature presents conflicting data regarding endometrial cultures. In women with chronic endometritis, the endometrial microbiome is characterized by the presence of the following bacteria: Lactobacillus, Enterobacter, Pseudomonas, and Gardnerella, with their respective proportions being 33.21%, 7.17%, 7.32%, and 6.91%. Also noteworthy are E. faecalis, Streptococcus spp., Staphylococcus spp., Mycoplasma spp., and others.[10] Gardnerella is not detected in control samples of healthy women. Similarly, another study identified Bifidobacterium, Prevotella, and Gardnerella in infertile women with chronic endometritis, whereas these were not observed in infertile women without chronic endometritis.[11] Other research has reported significantly elevated levels of Gardnerella, Klebsiella, and Streptococcus in endometrial biopsies from infertile patients.[12]

The results of traditional methods for obtaining endometrial cultures depend on the specific laboratory performing the analysis. Additionally, the literature reveals that contamination with vaginal and endocervical contents cannot be entirely excluded, even when employing specialized devices intended to minimize contact with the vagina and cervix.

Finally, demonstrating an infectious agent in the endometrial cavity does not necessarily indicate its pathogenicity. Collecting an endometrial sample to detect infectious agents in the endometrial cavity is an invasive technique that can sometimes be difficult and painful. To avoid the necessity of obtaining endometrial specimens in suspected or confirmed cases of chronic endometritis, the compatibility of vaginal and endocervical cultures with infectious agents present in the endometrium was investigated. In the studies mentioned, it was revealed that the concordance of positive vaginal and endocervical cultures with endometrial cultures depends on the specific infectious agent. The overall concordance for endocervical canal samples is 48.3%, which varies significantly depending on the organism. For instance, there was no concordance between endocervical and endometrial cultures in cases where Staphylococcus spp. was present in the endometrium. In contrast, concordance was 100% and 58.3% for C. trachomatis and U. urealyticum cases. For vaginal samples, the overall concordance was 50.2%, ranging from 0% for Staphylococcus spp—cases to 16.7% for C. trachomatis cases and 48.8% for U. urealyticum cases. Therefore, neither vaginal nor endocervical cultures can be considered reliable predictors of endometrial microbial culture.

In patients with implantation defects and repeated spontaneous abortions, the presence of endometrial polyps is a common pathology alongside endometritis. Blood vessels play an essential role in the inflammatory process and, at the same time, are also the primary morphological component of polyps, with large-caliber blood vessels observed in the functional layer. According to some studies, signs of active endometritis have been detected in cases of polyps. In the part of the polyps, no vascular changes were detected in the presence of endometritis; only the presence of a vascular axis was noted. However, the incidence of polyps and endometritis cases was significantly higher than cases without vascular changes. This observation supports the hypothesis that

endometrial polyps may be part of a spectrum of changes contributing to chronic endometritis. Infertile women with endometrial polyps exhibit elevated levels of cytokines, particularly interferon-γ, suggesting an inflammatory etiology. According to these studies, the localized growth of the endometrium, which characterizes polyps, may be secondary to an inflammatory reaction. Furthermore, these studies have demonstrated that vascular changes significantly increase the risk of developing new vascular axes and polyps. According to this scenario, polyps are not the direct cause of infertility but are instead a consequence of vasculopathy, which is attributable to an underlying inflammatory or autoimmune etiology. This may account for the high rate of successful pregnancies observed following polypectomies.

There is a hypothesis that chronic endometritis may be related to endometriosis, although studies exploring this connection are minimal. Existing research indicates that the frequency of chronic endometritis is significantly higher in patients with endometriosis compared to those without endometriosis. Specifically, chronic endometritis was observed in 52.94% of cases with endometriosis. Chronic endometritis was noted in 40.0% of grade I endometriosis cases, 50.0% of grade II endometriosis cases, 70.0% of grade III endometriosis cases, and 46.7% of grade IV endometriosis cases. These indicators do not correlate with the stage, and deep endometriotic lesions may not be associated with chronic endometritis. According to these results, chronic endometritis may represent an independent complication of endometriosis or may be involved in its pathogenesis, given that endometritis is observed in the early stages of endometriosis.

Plasma cells in the eutopic endometrium play a role in eliminating bacteria and newly formed neoplastic cells. However, chronic endometritis may lead to tumor development. In general, endometriosis is not classified as a tumor but resembles the process of tumor metastasis. Several studies have indicated that tumors may exploit the plasticity of immune cells to their advantage. The overproduction of early inflammatory mediators (such as IL-12, TNF, and reactive oxygen species) activates the adaptive immune response to eliminate tumor cells. However, the same process can also facilitate neoplastic transformation. Chronic endometritis in the uterine cavity may contribute to transforming normal eutopic endometrium into endometriotic tissue, which can invade the pelvic cavity.

Studies have established that endometriosis and chronic endometritis have a similar immune background. For example, an unusual infiltration by B cells and endometrial stromal plasma cells (ESPCs) has been described during both processes in parallel with the local production of some proinflammatory cytokines, such as IL-6 and TNF-α. IL-6 is known to act as a differentiation factor for immature B cells. At the same time, TNF-α induces estrogen biosynthesis locally in endometrial glandular cells, which transforms endometrial cells into a proliferative phenotype and may contribute to the development of endometrial polyposis.[13] At the same time, overexpression of specific immunoglobulins (IgG1, IgG2, and possibly IgA) is frequently observed in the eutopic endometrium when endometriosis and chronic endometritis coexist. This overexpression is attributed to the increased production of immunoglobulins by endometrial stromal plasma cells.[14]

An increased inflammatory response could potentially be associated with the onset and progression of both diseases.

The role of the microbiome in tumor progression has been most thoroughly studied in colorectal carcinomas, where dysbiosis results in a reduction of regulatory commensal species and an

ensuing inflammatory process.[15] Endometrial cancer is similarly associated with proinflammatory conditions. Several studies have investigated the microbial environment of the uterus and its role in tumor development. Considering the inflammatory profile of endometrial tumors, it is suggested that a microbial component is also involved in malignant processes.[16]

Chronic endometritis is challenging to diagnose for a variety of reasons.[10, 13] Most patients are asymptomatic, and ultrasound features are nonspecific. Microbiological examination is often uninformative because most pathogens are not culturable. Additionally, during the collection of endometrial samples, it is impossible to prevent contamination of the material with cervical and vaginal flora. Chronic endometritis can be diagnosed through hysteroscopy by identifying specific endometrial changes, such as focal or diffuse hyperemia, stromal edema, and micropolyps.[10] However, the accuracy of this diagnostic method is contingent upon the operator's experience.[17]

Histopathologically identifying plasma cells in endometrial biopsy specimens is considered the gold standard for diagnosing chronic endometritis.[18] However, identifying plasma cells by conventional tissue staining alone is challenging. There is an apparent lack of standardized methods for histological evaluation of plasma cell infiltrates, although several options have been proposed in the literature.[1,2] According to some authors, the presence of one or several plasma cells in endometrial biopsies is sufficient to confirm the diagnosis of chronic endometritis. In contrast, others believe a specific number of plasma cells is required for this diagnosis. Additionally, plasmacytes are typically large cells with a high nuclear-to-cytoplasmic ratio, basophilic cytoplasm, and a "clock-face" pattern of heterochromatin in the nucleus.[19] These morphologic features of plasma cells are not always evident upon microscopic examination, as plasma cells often resemble endometrial stromal fibroblasts and mononuclear leukocytes.[17] Immunohistochemical study using the plasmacytic marker CD-138 (also known as syndecan-1, a transmembrane type heparan sulfate proteoglycan) is currently the most reliable and time-efficient diagnostic method. CD-138 immunostaining is more sensitive and specific than routine hematoxylin and eosin staining (sensitivity: 100% vs. 75%; specificity: 100% vs. 65%) and is characterized by less interobserver variability (93% vs. 47%).

Despite these advantages, this research method should be used cautiously, as CD-138 is also expressed on the plasma membrane of endometrial epithelial cells, which may lead to false-positive results.[19] Consequently, searching for new methods to detect plasma cells remains justified. Multiple myeloma antigen 1 (MUM-1) is a protein typically expressed in plasma cells and activated B and T cells. MUM-1 is essential at certain stages of B-cell development, including differentiating mature B cells into antigen-producing plasma cells. Considering the need for additional staining techniques, it is possible to test the utility of MUM-1 immunohistochemistry for identifying endometrial plasma cells.

Chronic endometritis can also disrupt the hormonal profile of the endometrium. Changes in the number and ratio of estrogen and progesterone receptors, along with other endometrial pathologies, can contribute to infertility, amenorrhea, and menstrual disorders. In one study, the expression of estrogen and progesterone receptors in endometrial glands and stromal cells was significantly higher in chronic endometritis cases than in the control group. Failure to decrease the expression of hormone receptors indicates a defect in endometrial maturation and the subsequent inability to support blastocyst implantation. Consequently, inflammation, especially diffuse inflammation, inhibits the expression of estrogen receptors in the endometrial glands and

stromal cells. Since the number of progesterone receptors depends on the expression of estrogen receptors, this disruption impairs the optimal conditions for the implantation of a fertilized egg.

The mean expression index of Ki-67 in endometrial glandular and stromal cells is significantly higher in chronic endometritis compared to the control group, similar to the pattern observed with hormone receptors. Therefore, the expression of Ki-67 correlates with the index of hormone receptor expression, i.e., with delayed maturation of the endometrium.[20]

As mentioned earlier, the persistence and progression of chronic endometritis are associated with infertility, endometriosis, and atypical hyperplasia of the endometrium. However, opinions on these issues differ, highlighting the need for further research.

REFERENCES

1. Farghali MM, Abdelazim I, El-Ghazaly TE. Relation between chronic endometritis and recurrent miscarriage. *Menopausal Review.* 2021; 20(3): 116–121.
2. Song D, Feng X, Zhang Q, Xia E, Xiao Y, Xie W, et al. Prevalence and confounders of chronic endometritis in premenopausal women with abnormal bleeding or reproductive failure. *Reprod Biomed Online* [Internet]. 2018 Jan 1 [cited 2024 Jul 24]; 36(1): 78–83. Available from: https://pubmed.ncbi.nlm.nih.gov/29111313/
3. García-Velasco JA, Budding D, Campe H, Malfertheiner SF, Hamamah S, Santjohanser C, et al. The reproductive microbiome - clinical practice recommendations for fertility specialists. *Reprod Biomed Online* [Internet]. 2020 Sep 1 [cited 2024 Jul 24]; 41(3): 443–453. Available from: https://pubmed.ncbi.nlm.nih.gov/32753361/
4. Kaku S, Kubo T, Kimura F, Nakamura A, Kitazawa J, Morimune A, et al. Relationship of chronic endometritis with chronic deciduitis in cases of miscarriage. *BMC Womens Health* [Internet]. 2020 Jun 1 [cited 2024 Jul 24]; 20(1). Available from: https://pubmed.ncbi.nlm.nih.gov/32487112/
5. Xu Y, Mei J, Diao L, Li Y, Ding L. Chronic endometritis and reproductive failure: Role of syndecan-1. *Am J Reprod Immunol* [Internet]. 2020 Sep 1 [cited 2024 Jul 24]; 84(3). Available from: https://pubmed.ncbi.nlm.nih.gov/32329146/
6. Kimura F, Takebayashi A, Ishida M, Nakamura A, Kitazawa J, Morimune A, et al. Review: Chronic endometritis and its effect on reproduction. *J Obstet Gynaecol Res* [Internet]. 2019 May 1 [cited 2024 Jul 24]; 45(5): 951–960. Available from: https://pubmed.ncbi.nlm.nih.gov/30843321/
7. Santoro A, Travaglino A, Inzani F, Angelico G, Raffone A, Maruotti GM, et al. The Role of Plasma Cells as a Marker of Chronic Endometritis: A Systematic Review and Meta-Analysis. *Biomedicines.* 2023 Jun 15; 11(6): 1714.
8. Chen YQ, Fang RL, Luo YN, Luo CQ. Analysis of the diagnostic value of CD138 for chronic endometritis, the risk factors for the pathogenesis of chronic endometritis and the effect of chronic endometritis on pregnancy: a cohort study. *BMC Womens Health.* 2016 Dec 5; 16(1): 60.
9. Buzzaccarini G, Vitagliano A, Andrisani A, Santarsiero CM, Cicinelli R, Nardelli C, et al. Chronic endometritis and altered embryo implantation: a unified pathophysiological theory from a literature systematic review. *J Assist Reprod Genet.* 2020 Dec 6; 37(12): 2897–911.
10. Moreno I, Cicinelli E, Garcia-Grau I, Gonzalez-Monfort M, Bau D, Vilella F, et al. The diagnosis of chronic endometritis in infertile asymptomatic women: a comparative study of histology,

microbial cultures, hysteroscopy, and molecular microbiology. *Am J Obstet Gynecol.* 2018 Jun; 218(6): 602.e1-602.e16.

11. Liu Y, Ko EYL, Wong KKW, Chen X, Cheung WC, Law TSM, et al. Endometrial microbiota in infertile women with and without chronic endometritis as diagnosed using a quantitative and reference range-based method. *Fertil Steril.* 2019 Oct; 112(4): 707-717.e1.
12. Moreno I, Garcia-Grau I, Perez-Villaroya D, Gonzalez-Monfort M, Bahçeci M, Barrionuevo MJ, et al. Endometrial microbiota composition is associated with reproductive outcome in infertile patients. *Microbiome* [Internet]. 2022 Dec 1 [cited 2024 Jul 24]; 10(1). Available from: https://pubmed.ncbi.nlm.nih.gov/34980280/
13. Cicinelli E, Bettocchi S, de Ziegler D, Loizzi V, Cormio G, Marinaccio M, et al. Chronic Endometritis, a Common Disease Hidden Behind Endometrial Polyps in Premenopausal Women: First Evidence From a Case-Control Study. *J Minim Invasive Gynecol* [Internet]. 2019 Nov 1 [cited 2024 Jul 24]; 26(7): 1346–1350. Available from: https://pubmed.ncbi.nlm.nih.gov/30708117/
14. Shen M, O'donnell E, Leon G, Kisovar A, Melo P, Zondervan K, et al. The role of endometrial B cells in normal endometrium and benign female reproductive pathologies: a systematic review. *Hum Reprod Open* [Internet]. 2022 [cited 2024 Jul 24]; 2022(1). Available from: /pmc/articles/PMC8825379/
15. Buchta Rosean C, Feng TY, Azar FN, Rutkowski MR. Impact of the microbiome on cancer progression and response to anti-cancer therapies. In 2019. p. 255–294.
16. Walther-António MRS, Chen J, Multinu F, Hokenstad A, Distad TJ, Cheek EH, et al. Potential contribution of the uterine microbiome in the development of endometrial cancer. *Genome Med.* 2016 Dec 25; 8(1): 122.
17. Cicinelli E, Vitagliano A, Kumar A, Lasmar RB, Bettocchi S, Haimovich S, et al. Unified diagnostic criteria for chronic endometritis at fluid hysteroscopy: proposal and reliability evaluation through an international randomized-controlled observer study. *Fertil Steril.* 2019 Jul; 112(1): 162-173.e2.
18. Li Y, Xu S, Yu S, Huang C, Lin S, Chen W, et al. Diagnosis of chronic endometritis: How many CD138 + cells/HPF in endometrial stroma affect pregnancy outcome of infertile women? *Am J Reprod Immunol.* 2021 May 24; 85(5).
19. Parks RN, Kim CJ, Al-Safi ZA, Armstrong AA, Zore T, Moatamed NA. Multiple Myeloma 1 Transcription Factor Is Superior to CD138 as a Marker of Plasma Cells in Endometrium. *Int J Surg Pathol.* 2019 Jun 28; 27(4): 372–379.
20. Mishra K, Wadhwa N, Guleria K, Agarwal S. ER, PR and Ki-67 expression status in granulomatous and chronic non-specific endometritis. *J Obstet Gynaecol Res.* 2008 Jun 23; 34(3): 371–378.