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Immune and Extracellular Matrix Dynamics in Chronic Endometritis: Insights into Endometrial Homeostasis and Fibrogenic Niche Evolution

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Abstract

Background: Chronic endometritis (CE), a condition involving abnormal immune responses in the endometrial microenvironment, is associated with infertility and implantation failure. Yet, the underlying regulatory mechanisms of autoimmunity and tissue homeostasis are not fully understood. Identifying molecular and biological markers that characterize the pathogenesis and morphogenesis of CE could help predict disease progression and risk. This study aims to explore the molecular and biological underpinnings of CE across varying levels of severity.

Methods and Results: This prospective observational comparative study included 53 women of reproductive age with abnormal uterine bleeding and a history of RPL. All women were presented with CE, which was histologically verified. Endometrial samples were obtained via Pipelle biopsy using a Pipelle vacuum syringe during the mid-proliferative phase (days 7-10 of the menstrual cycle). The biopsies underwent pathomorphological analysis following modified Noyes criteria for CE confirmation. Based on CE severity, participants were categorized into three groups: Group 1 (mild inflammation), Group 2 (moderate inflammation), and Group 3 (severe inflammation).

In our study, mild CE was characterized by an adaptive immune response, with increased CD8+T-cell density and overexpression of CD38+, CD68+, and CD163+. Collagen-producing cell activity was moderately elevated, accompanied by a relative increase in reticular fibers. In moderate CE, the microenvironment displayed a reactive immune response, evidenced by overexpression of pro-inflammatory markers CD38+ and CD68+, alongside impaired fibrillogenesis. Macrophage activity shifted toward an M2 anti-inflammatory response with higher CD163+ expression. In severe CE, severe inflammation correlated with an upregulated expression and cooperative interaction of pro-inflammatory markers (CD8+, CD68+, CD38+) and an abundance of type III collagen-rich reticular fibers against a backdrop of mitigated CD163+ expression. This advanced severity suggested a decline in anti-inflammatory regulation and an excessive deposition of ECM components.

Conclusion: The findings show that CE severity is modulated by a complex interplay of immune and cellular factors, typically with progressive ECM fibrous formation and collagen and reticular fiber ratio shifts. The severity of inflammation aligns with elevated pro-inflammatory factors (CD8+, CD68+, and CD38+) and reduced anti-inflammatory CD163+ expression, particularly in severe CE. These molecular and cellular insights elucidate the significance of biomarkers for diagnosing and stratifying CE severity, providing a basis for targeted interventions to mitigate CE progression.(**International Journal of Biomedicine. 2024;15(1):119-128.**)

Keywords: chronic endometritis • immunity • collagen • inflammatory markers • extracellular matrix

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Abbreviations

CE, chronic endometritis; **IHC**, immunohistochemistry; **ECM**, extracellular matrix; **RPL**, recurrent pregnancy loss; **NK**, natural killer.

Introduction

The endometrium is a highly specialized morphofunctional tissue that undergoes dynamic cyclic changes every 28 to 30 days, regulated by complex neuroimmunoendocrine and molecular mechanisms.[1]This continuous remodeling, involving over 400 cycles of regeneration, differentiation, rejection, and rapid repair, is driven by ovarian steroid hormones, primarily 17β-estradiol and progesterone, which orchestrate shifts in cellular composition and immune cell activities within the endometrial microenvironment.[2] These precisely coordinated changes at the tissue, cellular, and molecular levels are essential to maintain endometrial receptivity, facilitating embryo implantation, chorion development, and early embryogenesis.[3]

The physiological immune landscape of the uterus includes both resident cells (T and B cells) and migratory immune cells (macrophages, neutrophils, dendritic cells, mast cells, and natural killer cells), which interact with various factors, including hormones, microbiota, and external antigens, all while preserving harmony with resident commensal organisms.[2] Disruptions in endometrial receptivity, particularly during the implantation window (days 19 to 22 of a 28-day cycle), or asynchrony in fertilized egg interactions, have been associated with infertility, miscarriage, and low success rates in in vitro fertilization.[2] Among the leading causes of endometrial infertility—repeated implantation failures in IVF and unexplained recurrent pregnancy loss (RPL)—chronic endometritis (CE) is a primary contributor, with prevalence rates between 15.0% and 57.5%.[4-8] Chronic endometritis represents a localized inflammatory condition characterized by superficial mucosal stromal edema, increased stromal density due to leukocyte infiltration, and the presence of plasma cells within the stroma.[9] Its impact on endometrial function is profound, as the inflammation disrupts receptor expression, compromises blood flow, and leads to fibrosis within the endometrial stroma, all contributing to impaired endometrial receptivity.[10] Diagnosis of CE is based on histological examination and IHC detection, specifically identifying≥5 CD138+ plasma cells per high-power field at ×400 magnification, alongside cultures from the uterine cavity.[8,11,12] However, the absence of standardized hysteroscopic and histopathological criteria for diagnosing CE highlights the need to identify molecular and cellular markers that reflect the biological characteristics of CE-affected tissue, improving early detection and enabling targeted therapies.[13]

Recent research demonstrated the importance of immune dysregulation within the endometrial microenvironment in the pathogenesis and progression of CE.[2] A healthy endometrium must foster an environment that promotes immune tolerance, as the embryo is recognized as a semi-allogenic entity at the maternal interface.[14] The precise mechanisms by which immune cells, primarily macrophages, dendritic cells, T cells, and NK cells, constituting 30% to 40% of decidual cells, coordinate to support implantation and embryonic development remain an area of active investigation.[15] In CE, abnormal immune activation affects resident and migratory immune cells and disrupts interactions with stromal fibroblasts, impairing endometrial receptivity and compromising embryo implantation potential.[16-18] Emerging evidence reveals altered immune cell profiles in CE, with increased B cells, activation of proinflammatory cytokines, and abnormal expression of paracrine mediators, adhesion molecules, and chemokines.[3] Investigation into mature T-cell populations in CE, distinguished by surface markers and function (CD8+ cytotoxic, CD4+ helper, and others without specific clusters), is essential to elucidate CE's immune dysregulation.[19] Cytokine and chemokine profiles further elucidate immune interactions, as CD4+ T cells recognize HLA class II molecules to coordinate immune responses, and CD8+ T cells exhibit cytolytic activity during the proliferative menstrual phase via apoptosis induction.[20,21]

Macrophages play critical roles in both innate and adaptive immunity. M1 macrophages are involved in pathogen phagocytosis, antigen presentation to Th1 cells, and pro-inflammatory signaling through IL-12, IL-23, and nitric oxide synthesis.[22,23] Conversely, M2 macrophages support angiogenesis, tissue remodeling, and Th2-type anti-inflammatory responses by secreting IL-10.[24-26] While CD68, a transmembrane glycoprotein receptor, is broadly expressed across immune cells, including NK cells, dendritic cells, and basophils, it is a general marker for lysosomal-associated proteins rather than being specific to macrophages.[27] High CD163+ expression in macrophages indicates anti-inflammatory activity, contributing to tissue repair by removing cellular debris and producing IL-10.[28] Other immune cells, such as those expressing CD38, are involved in regulating immune response across cell types, including macrophages, NK cells, and T and B lymphocytes.[29] B lymphocytes are essential for the humoral immune response, facilitating antigen recognition, T-cell regulation, and innate immune signaling. The unique immune activation profile in the endometrial microenvironment of CE encompasses resident and migratory immune cells and stromal fibroblasts, all contributing to the immune imbalance.

Collagen, a primary component of ECM, is vital in providing the structural integrity needed for successful embryo implantation.[30] It imparts tensile strength and elasticity to the tissue, enduring external physical stresses and internal inflammatory responses.[31] In the endometrium, collagen fibers consist of tightly packed fibrils, exhibiting distinctive structural properties essential for cellular attachment, migration, and signaling, which are critical for endometrial repair and regeneration.[32,33] Fibrils are formed through the fusion of thinner structures and reticular fibers, which are closely associated with collagen and form a fine subepithelial network, providing mechanical support within ECM.[33,34] The collagen expression and metabolism changes can lead to immune dysregulation and tissue dysfunction, affecting cell adhesion, migration, and signaling pathways.[35] Continued

investigation into collagen metabolism and immune cell dynamics in CE is needed to clarify CE's pathogenesis and morphogenesis and facilitate identifying potential therapeutic targets.

This study aims to explore the molecular and biological underpinnings of CE across varying levels of severity.

Materials and Methods

Study Design

This prospective observational comparative study was conducted at the Department of Pregnancy Pathology and Gynecology, Perinatal Center, City Clinical Hospital, named after Academician G.M. Savelyeva, Moscow Department of Health. A total of 53 women of reproductive age with abnormal uterine bleeding and a history of RP were included, all with histologically verified CE. For comparison, a control group of 10 women with a regular menstrual cycle, intact reproductive function, and an endometrial assessment showing no abnormalities were selected before undergoing surgery for uterine fibroids.

Exclusion criteria encompassed patients with reproductive system malignancies, current pregnancy or lactation, and histories of cesarean section or myomectomy. Based on CE severity, participants were categorized into three groups: Group 1 (mild inflammation) (n=18), Group 2 (moderate inflammation) (n=16), and Group 3 (severe inflammation) (n=19).

Endometrial Biopsy and Histological Evaluation

Endometrial samples were obtained via Pipelle biopsy using a Pipelle vacuum syringe during the mid-proliferative phase (days 7-10 of the menstrual cycle). The biopsies underwent pathomorphological analysis following modified Noyes criteria for CE confirmation.

Tissue Processing and Staining

The biopsy specimens, retained from diagnostic procedures, were fixed in buffered 4% formaldehyde and embedded in paraffin. Paraffin-embedded sections (2.5 μm thick) were then deparaffinized using xylene and rehydrated with graded ethanol following standard protocols.[36] The histological evaluation included staining with hematoxylin and eosin and Mallory staining, followed by IHC to assess CD138+plasma cell expression, fibrosis, and morphometric parameters of arterioles such as lumen diameter (D) and area (S).[37]

CE severity

CE severity was classified based on histological staining and morphometric analysis:

Mild: No fibrosis, arteriolar lumen area of 150-449 μm² and diameter of 10.0-19.9 μm, with ≤1 CD138+ plasma cell.

Moderate: Presence of fibrosis, arteriolar lumen area of 100-149μm² and diameter of 8.0-9.9 μm, with 2-3 CD138+ plasma cells.

Severe: Presence of fibrosis, arteriolar lumen area of ≤99 μm² and diameter of ≤7.9 μm, with ≥4 CD138+plasma cells.

Immunohistochemical examination

For IHC analysis, antigen retrieval was performedwas on deparaffinized sections by heating in R-UNIVERSAL Epitope Recovery Buffer (Aptum Biologics Ltd., Southampton, UK) at 95°C for 30 minutes.[36] Following antigen retrieval and peroxidase quenching (when necessary), primary antibodies were applied overnight at 4°C. A list of primary and secondary antibodies is provided in Table 1 and Table 2, respectively. Secondary goat, anti-mouse, or anti-rabbit antibodies (AmpliStain™ anti-Rabbit 1-Step HRP; SDT GmbH, Baesweiler, Germany) were applied for target detection with the DAB Peroxidase Substrate Kit (Vector Laboratories, Burlingame, CA, USA), according to the manufacturer's guidelines (Table 2).

Table 1.

Primary antibodies used in this study.

Antibodies	Host	Catalogue. No.	Dilution	Manufacturer
CD38	Rabbit monoclonal	ab108403	1:500	AbCam, UK
CD8	Rabbit monoclonal	ab101500	1:100	AbCam, UK
CD20	Rabbit monoclonal	ab166865	1:100	AbCam, UK
CD163	Rabbit recombinant monoclonal	ab182422	1:500	AbCam, UK
CD68	Mouse recombinant monoclonal	ab955	1:3000	AbCam, UK

Table 2.

Secondary antibodies and other reagents.

	Source	Dilution	Label
Goat anti-mouse IgG Ab (#ab97035)	AbCam, United Kingdom	1/500	Cy3
Goat anti-mouse IgG (#Ab150113)	AbCam, United Kingdom	1/200	AF488
Goat anti-rabbit IgG (#Ab97075)	AbCam, United Kingdom	1/200	Cy3
Goat anti-rabbit IgG (#ab150077)	AbCam, United Kingdom	1/200	AF488
AmpliStain™ anti-Mouse 1-Step HRP (#AS-M1-HRP)	SDT GmbH, Baesweiler, Germany	ready-to-use	HRP
AmpliStain™ anti-Rabbit 1-Step HRP (#AS-R1-HRP)	SDT GmbH, Baesweiler, Germany	ready-to-use	HRP
4',6-diamidino-2-phenylindole (DAPI, #D9542-5MG)	Sigma, Hamburg, Germany	5 μg/mL	w/o
VECTASHIELD®® Mounting Medium (#H-1000)	Vector Laboratories, Burlingame, CA, USA	ready-to-use	w/o
DAB Peroxidase Substrate Kit (#SK-4100)	Vector Laboratories, Burlingame, CA, USA	ready-to-use	DAB
Silver impregnation (#21-026)	Biovitrum, ErgoProduction LLC, Russia	ready-to-use	w/o
Picro Sirius Red Stain Kit (#ab150681)	AbCam, United Kingdom	ready-to-use	w/o
Mayer's hematoxylin (#HK-G0-DL01)	Biovitrum, ErgoProduction LLC, Russia	ready-to-use	w/o

AF-Alexa Fluor

For immunofluorescence analysis, CD38, CD138, CD163, and CD68-bound primary antibodies were visualized using Cy3-conjugated secondary antibodies (Dianova, Hamburg, Germany, and Molecular Probes, Darmstadt, Germany), following standardized single-labeling protocols.[36] For histochemical analysis of the fibrous ECM, silver impregnation staining was conducted, enabling assessment of collagen fiber maturity and the detection of types I and III collagen.[38-44]

Immunostaining Controls

Control assays included substituting primary antibodies with matching IgG isotypes or excluding primary and/or secondary antibodies. The staining specificity was verified by omitting primary antibodies or substituting them with IgG of the same species and concentration, confirming the specificity of immunohistochemical signals.

Image Acquisition

Microscopy was performed using a ZEISS Axio Imager. Z2 microscope equipped with a Plan-Apochromat 150×/1.35 Glyc DIC Corr M27 objective and ZEISS Axiocam 712 color digital camera. Images were processed using Zen 3.0 Light Microscopy Software, Bundle Intellesis & Analysis, and Zen Module Z Stack Hardware (Carl Zeiss Vision, Jena, Germany) and were prepared at 300 DPI for submission.

Quantitative Image Analysis

Quantitative image analysis was conducted using QuPath software. Planimetric analysis included the quantification of CD38+ and CD8+ cells, as well as positively stained immune and other endometrial cells. Similarly, QuPath was used to evaluate fibrous structures stained by silver impregnation, isolating mature collagen (red-yellow) and reticular fibers (black or dark brown) across the entire tissue section.

Statistical analysis was performed using the statistical software package SPSS version 22.0 (SPSS Inc, Armonk, NY: IBM Corp). The normality of the distribution of continuous variables was tested by the Shapiro-Wilk test. The results are presented as median (Me) and interquartile range (IQR [Q1; Q3]). The Mann-Whitney U test and Kruskal-Wallis test were used, respectively, to compare differences between 2 and 3 or more independent groups. A probability value of $P<0.05$ was considered statistically significant.

Ethical Considerations

The study was carried out in accordance with the Helsinki Declaration of the World Medical Association (1964, ed. 2013) and approved by the institutional ethics committee. All participants provided written informed consent.

Results

The mean age range for women with RPL was comparable across groups, spanning 25 to 43 years: Group I had a mean age of 32.4 [28.8; 36.2] years, Group II 33.5 [23.6; 42.5] years, and Group III 32.7 [25.5; 38.6] years. Similarly, the comparison group showed no significant age differences, averaging 32.0 [26; 39] years.

The histopathological analysis identified CE by lymphoplasmacytic infiltrates encircling uterine glands and vasculature, with lymphocyte clusters and exfoliated epithelial cells observed in individual glandular lumens. Dense stromal architecture included fibroblast-like cells arranged in spiral formations around endometrial glands. Fibrotic changes in the vascular walls and stromal regions were further confirmed via Mallory staining. The glandular epithelium showed variable alignment with menstrual cycle phases, often displaying non-cyclic indifferent epithelium, while some glands indicated early proliferative phase characteristics.

The presence of specific immune markers, such as rare CD138+plasma cells, validated CE diagnoses. These markers, localized outside fibrosis zones during extended inflammation, highlighted the utility of immune profiling and Mallory staining in CE detection.

Immunohistochemical data (Figure 1) indicated leukocyte subpopulations in CE cases of varying severity and in the comparison group. CD38+leukocytes were most abundant in severe CE cases (Figure 1f-i), contrasting with other inflammation types (Figure 1b-e) and the scarcity of plasma cells in the comparison group (Figure 1a). Severe inflammation corresponded with CD38+cell migration into the endometrial stroma (Figure 1f) and increased interactions between plasma cells and stromal elements, leading to exoenzyme transfer to neighboring cells (Figure 1f-i).

In healthy endometria, cytotoxic CD8+ lymphocytes were minimal (Figure 1j), contrasting with elevated CD8+ cell counts in CE groups, showing diffuse (Figure 1n) or localized periglandular distributions (Figure 1p) in the stroma. CE severity was correlated with increased CD8+ clustering, forming cell groups and facilitating active immunogenesis (Figure 1m, o, p, q). Mild and moderate CE cases had less prominent CD8+cell increases, maintaining diffuse or localized patterns (Figure 1l, m).

Endometrial macrophage infiltration with CD68+cells varied across CE groups, depending on inflammation morphology (Figure 2c-i), with severe CE displaying the highest CD68+ expression (Figure 2f-i) relative to the comparison group (Figure 2a,b). Severe CE also showed macrophage migration to basal epithelial layers (Figure 2g), perivascular regions (Figure 2h), and stromal loci (Figure 2i), with increasing CD68+cell prevalence correlating with inflammation severity.

CE-related lymphocyte distribution imbalances also involved phenotypic shifts in M2 macrophages. These included differences in cytoplasmic extensions, with M2 cells (CD68+CD163+) having thin extensions, facilitating apoptotic cell clearance at extended stromal distances (Figure 2s-u). The M1 phenotype (CD68+CD163-) displayed more restricted cytoplasmic reach, affecting spatial immune interactions in CE (Figure 2r).

The escalation of CE severity increased CD163+macrophage accumulation in the endometrium (Figure 2k-q). As with M1 macrophages, the intra-organ population of CD68+CD163+ M2 macrophages formed intra-endometrial clusters, demonstrating extensive cellular interactions and long cytoplasmic processes over distances of more than 50 μm. Sparse M2 macrophages in the control endometrium are shown in Figure 2j. These features likely

promote functional M2 macrophage networks essential for endometrial homeostasis, potentially facilitating chronic inflammatory transformations within the stromal matrix.

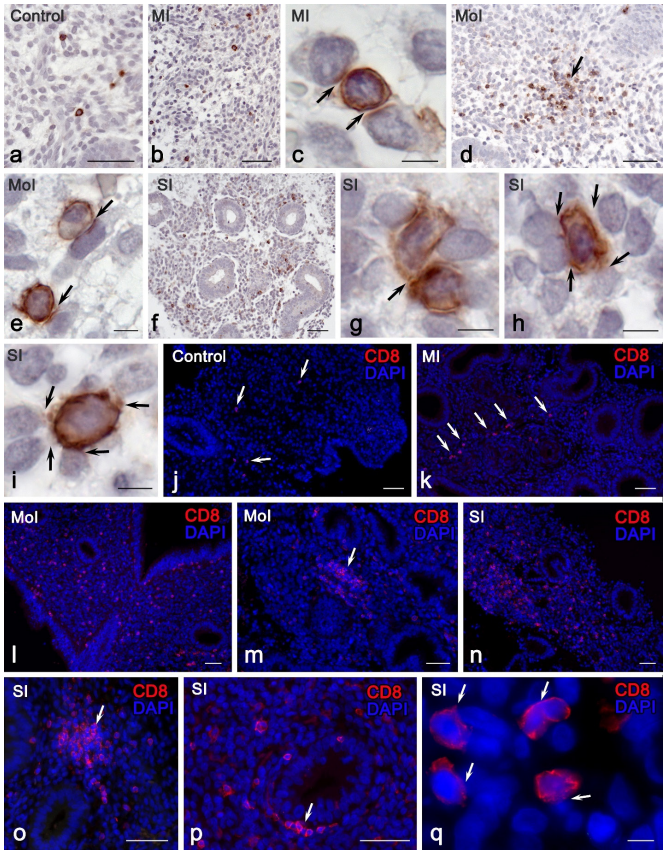

Figure 1. *Localization and distribution of CD38+ leukocytes and CD8+ cytotoxic lymphocytes in endometrial tissue with varying CE severity. Immunohistochemical staining for CD38+ leukocytes (a-i) and CD8+ cytotoxic lymphocytes (j-q). Observations: CD38+ leukocytes:*
(a) Minimal infiltration of CD38+ plasma cells in stromal regions.
(b) Increased CD38+ cell migration within the stroma.
(c) Interaction of a CD38+ cell with two stromal cells (arrow).
(d-e) Formation of local clusters of CD38+ cells in moderate CE; stromal cell interactions are shown (arrows).
(f-i) Elevated density of CD38+ leukocytes in severe CE, demonstrating intercellular interactions (g) and transfer of secretory molecules to adjacent cells (i, arrows).
CD8+ cytotoxic lymphocytes:
(j) Baseline levels of CD8+ lymphocytes in control endometrial stroma (arrow).
(k) Localized areas of CD8+ hyperexpression (arrow).
(l-m) Distribution of CD8+ natural killer (NK) cells in mild CE with diffuse (l) and focal (m) patterns.
(n-q) Progressive CD8+ cell accumulation in severe CE with expansive or focal localization in stromal (o, arrow) or periglandular (p, arrow) regions, showing colocalization with other cell types (q, arrow).
Scale: Panels c, e, g, h, i, q at 5 μm; others at 50 μm.
Groups: Control group and CE groups classified as MI (mild), MoI (moderate), and SI (severe).

Comparatively, the endometrium in the control group was characterized by a structured collagen framework, predominated by mature ECM fibers (Figure 3a-c). Silver staining revealed a mostly longitudinal collagen orientation, with transverse fibrils and rare reticular fibers confirmed under polarized microscopy (Figure 3c). Mild CE increased fibrous matrix components, particularly Type I collagen, with elevated reticular fiber loci emerging (Figure 3d-g). Transitioning from mild to severe CE prompted extensive fibrillogenesis (Figure 3h), proliferating mature collagen, and Type III-rich reticular fibers (Figure 3i-k).

In severe CE, collagen and reticular fiber volumes increased substantially, with a higher reticular fiber proportion among mature and immature collagen profiles (Figure 3l-r). A notable CE-associated pattern included intensified collagen fibrillogenesis within fibrotic areas, observable through silver impregnation and polarization microscopy (Figure 3l-q), suggesting persistent fibrotic niche morphogenesis. This structural alteration could contribute to the progression of stromal fibrosis in the endometrium, as indicated by dense mature collagen and emerging reticular fiber clusters (Figure 3p-r).

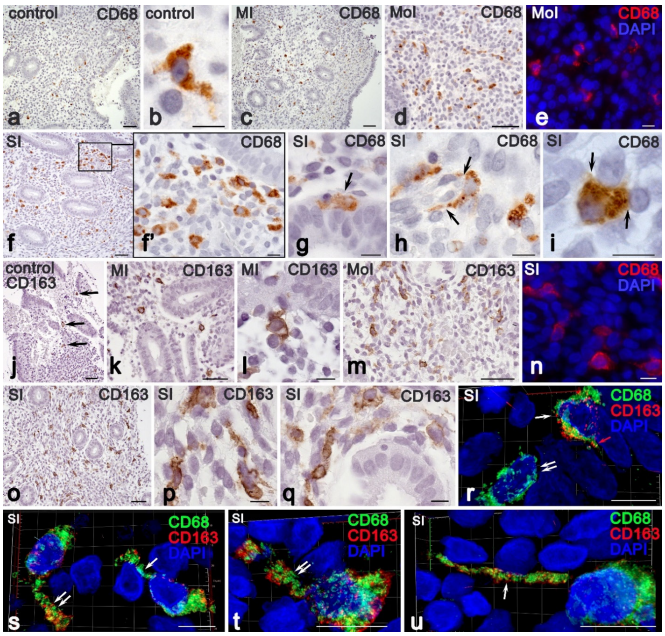

Figure 2. *Spatial distribution of macrophage subtypes (Type 1 and Type 2) in CE of differing severities. Methods: Immunohistochemical staining of CD68 (M1) and CD163 (M2) macrophages.*
Observations: CD68+ Type 1 Macrophages:
(a) Limited macrophage presence in control endometrial tissue.
(b) Intracellular distribution of CD68+ markers in macrophages.
(c-f) Incremental increase in CD68+ macrophages in mild (c), moderate (d, e), and severe CE (f).
(g-i) Migration of macrophages to basal epithelial layers (g), perivascular zones (h), and interstitial stromal cells (i, arrows).
CD163+ Type 2 Macrophages:
(j) Sparse M2 macrophages in control endometrium (arrow).
(k-q) Rising M2 macrophage density from mild (k, l) to moderate (m) and to severe CE (n-q), reflecting inflammatory progression.
(r-u) 3D models of type 2 and type 1 macrophages in advanced chronic endometritis.
(r) Type 2 macrophage (CD68 + 163 + phenotype, arrow) has a thin cytoplasmic process (red arrow) as opposed to type 1 macrophage (CD68+163- phenotype, double arrow) (Supplementary 1).
(s-t) Various variants of thin (arrow) and wide (double arrow) cytoplasmic processes of type 2 macrophages (Supplementary 2, Supplementary 3).
(u) A narrow cytoplasmic process of macrophage type 2 extending a considerable distance from the mother cell in the endometrial stroma (arrow) (Supplementary 4).
Scale: Panels b, e, f', g-i, n, p-u at 10 μm; others at 50 μm.
Groups: Control and CE severity groups (MI, MoI, SI).

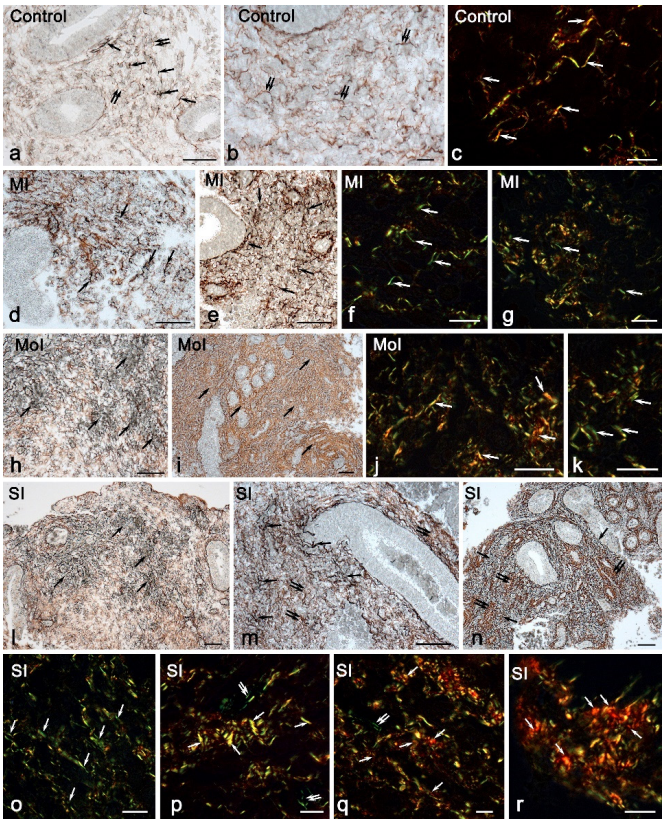

Figure 3. *Collagen fiber composition in endometrial extracellular matrix with increasing CE severity. Silver impregnation for (a-b, d-e, h-i, l-n), picrosirius red staining with polarized microscopy for the rest. Observations: Control: (a-b) Mature collagen predominance (arrow), interspersed with reticular fibers (double arrow). (c) Abundant type I collagen in the endometrial stroma (red-yellow, arrow). Mild CE (d-g) Mild CE shows elevated collagen levels with type III collagen (arrow). Moderate CE: (h-k) Increased fibrous matrix intensity; active collagen fibrillogenesis (h, arrow), areas with high content of mature collagen fibers (i,j, arrow), and type III-rich reticular fibers (k, arrow). Severe CE: (l-n) Collagen-rich stroma (double arrow) with reticular fiber loci (arrow). (o) Tissue microenvironment with a predominance of immature collagen fibers with a high content of type III collagen (green, arrow). (p-r) High-density mature fibers with type I collagen (red-yellow, arrow) in the presence of reticular fibers containing type III collagen (green, double arrow).*
Groups: Control and CE groups by severity (MI, MoI, SI).

CE groups (Figure 4) exhibited elevated immune cell subtype expression and ECM collagen content (mature and reticular), with values significantly exceeding those in the control group across CE severity levels ($P<0.05$).

The results of median immune cell expression (Figure 5) describe the increased CD8, CD38, CD68, CD163, and CD20 populations within CE-diagnosed endometrial stroma. Mann-Whitney U tests revealed significant differences in immune marker levels, with moderate CE showing notable increases in CD38+ macrophages, CD8+ T cells, and CD163+ cells compared to mild CE ($P<0.05$). Typically, the CD20 population was stable across CE groups, while severe CE cases showed markedly higher pro-inflammatory CD68+, CD38+, and CD8+ T-cell levels than other groups ($P<0.05$). Anti-inflammatory CD163+ expression was the highest in mild CE, distinguishing it from moderate and severe cases ($P<0.05$).

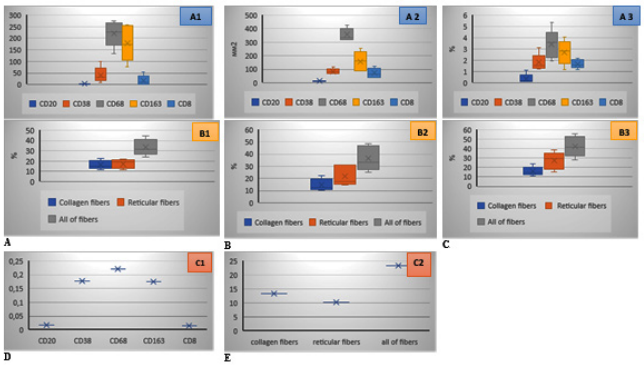

Figure 4. *Comparative expression of immune cells and fibrous matrix in CE groups. Immune markers and collagen fiber content (mature and reticular) in CE display significant increases compared to the control group across all severity groups ($P<0.05$).*
A) Mild CE: immune markers (A1), extracellular matrix fibers (B1).
B) Moderate CE: immune markers (A2), extracellular fibers (B2).
C) Severe CE: immune markers (A3), matrix fibers (B3).
D-E) Control group: immune marker (C1), extracellular matrix fibers (C2).

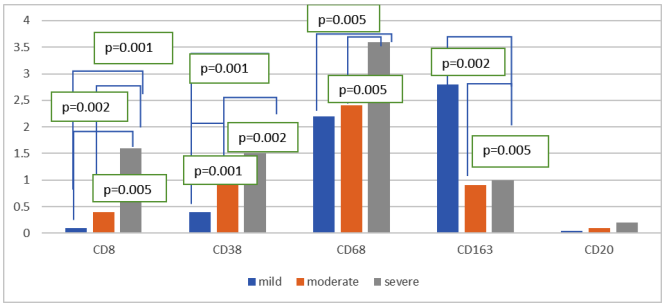

Figure 5. *Median immune cell expression of CD8, CD38, CD68, CD163, and CD20 in CE groups. Median immune cell percentages (CD8, CD38, CD68, CD163, CD20) across endometrial stroma in CE groups show significant intergroup differences.*

Reticular and collagen fiber composition (Figure 6) was consistent between mild and moderate CE, with severe CE showing significant reticular fiber increases relative to collagen fibers ($P=0.02$). Severe CE cases showed maximal reticular fiber content alongside minimal mature collagen ($P<0.05$), with pronounced fibrotic changes and elevated collagen fiber levels evident in severe and moderate CE but not in mild cases or the control group ($P<0.05$).

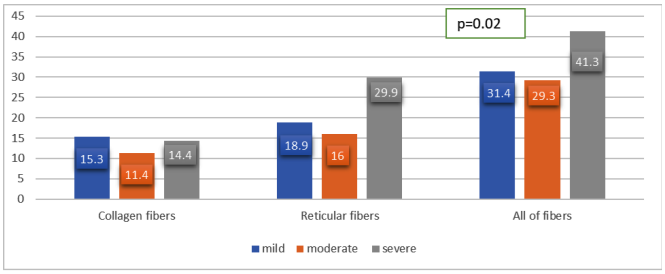

Figure 6. *Median expression of extracellular matrix fibrous component across CE groups. Median collagen fiber content analysis in CE groups indicates significant intergroup differences in fibrous matrix content.*

Discussion

Local Endometrial Homeostasis in Mild CE

In cases of mild CE, the endometrial dysfunction is largely influenced by the adaptive immune response, maintaining homeostasis by forming lymphoid aggregates enriched with CD8+ T cells, CD38+ leukocytes, and M2 macrophages expressing CD68+. This immune response leads to the transformation of macrophages into an anti-inflammatory M2 phenotype characterized by the overexpression of CD163+. This indicates a potential inhibition of tissue remodeling and local homeostasis regulation amidst mild inflammation.[47,48] The structural integrity of the endometrium during mild inflammation is supported by a maintained metabolism of collagen fibers, accompanied by a moderate increase in reticular fiber expression.[49]

This pattern of fiber expression signifies an adaptive remodeling of ECM and regulation of cellular functions. The morphofunctional changes in mild CE are driven by the specific immune microenvironment and intercellular cooperation, facilitating ECM remodeling. This adaptive reaction is comparable to processes during the implantation period, where numerous decidual macrophages migrate to the trophoblast and spiral artery invasion zones.[22]

Local Endometrial Homeostasis in Moderate CE

In moderate CE, disrupting biological functions impairs cell proliferation, migration, and cell-adhesive interactions. Activation of M1 macrophages results in the secretion of proinflammatory cytokines, angiogenic, and growth factors, which are detrimental to embryo attachment, leading to a decrease in cytotoxic CD8+ T-cell populations. The creation of an unfavorable microenvironment for embryo implantation in moderate CE is driven by Th1 proinflammatory responses, in contrast to the increased presence of CD163+ macrophages.[26]

CD68+leukocyte infiltration in the endometrium moderately increases in moderate CE, compared to mild inflammation, with CD38+ expression also showing a comparative increase relative to the control group. Elevated CD38+ expression correlates with disruptions in NAD+ metabolism, crucial for energy metabolism, DNA repair, and redox potential.[50,51] The positive moderate association between CD68+ expression and CD38+ leukocyte infiltration suggests that CD38+ expression modulates the tissue microenvironment, promoting a proinflammatory immune response aligned with CE severity.

Significant changes in the immune landscape disrupt communication and signaling between stromal cells, including fibroblasts, endothelial, and immune cells. Dysregulation in the cellular immune response, particularly the imbalance between NK cells and T cells, is crucial for endometrial receptivity and is associated with RPL. An elevated proinflammatory Th1 cytokine profile and Th17 phenotype are linked to recurrent implantation failures.[52] Increased endometrial immune cell infiltration induced by CE fosters a cytotoxic environment, leading to abnormal metabolic pathways, impaired protein and lipid synthesis, and inadequate endometrial receptivity, thus hindering trophoblast proliferation and embryo acceptance.

The increased collagen and reticular fiber content reflects disrupted ECM remodeling due to relative tissue ischemia. Abnormal formation of fibrous components, mediated by an inflammatory microenvironment and proinflammatory signaling molecules, particularly lipopolysaccharides from gram-negative bacteria, compromises stromal-epithelial cell communication. The results suggest a reactive nature of moderate inflammation, with a maintained capability to switch macrophage activity to the M2 anti-inflammatory type (increased CD163+ expression), indicating potential tissue repair and regulation.[53-55]

Local Endometrial Homeostasis in Severe CE

Severe CE is marked by intensified inflammatory changes, with elevated expression and interaction of proinflammatory immune cells, including CD8+, CD68+, and CD38+ cells. The Th1/Th2 balance shifts towards Th1, mediated by M1 macrophages, negatively impacting immune tolerance at the fetus-maternal interface even during early pregnancy. Increased CD38+ expression correlates with mitigated NAD+ metabolism, a disrupted immune landscape, and enhanced anti-inflammatory CD163+ M2 macrophage infiltration alongside abundant CD8+ cells.

Severe CE-induced immune imbalance causes tissue damage, disrupted homeostasis, and compromised metabolic shifts towards M2 macrophage activity and an anti-inflammatory, proangiogenic environment. Abnormal intercellular cooperation results in specific molecular and biological effects, leading to significant disorganization of collagen and reticular fibers, altered collagen protein biosynthesis, and impaired fibrillogenesis amidst fibrosis and inflammation. The disrupted ECM homeostasis, cyclic endometrial restoration, and regeneration lead to a hypoxic microenvironment and dissociation of immunocompetent cells from the fibroblastic cluster. Disruption of cellular-stromal interaction is due to loss of cell function and metabolic regulation in severe CE, exacerbated by immune dysfunction.[53-55]

The reduction and fragmentation of reticular fibers weaken the elastic framework, deteriorate microcirculation, and cause ischemia, strong epithelial adhesion, basement membrane degradation, and abnormal endothelial cell proliferation and migration towards angiogenic factors. Abnormal ECM remodeling in severe CE results in pathomorphic features characteristic of severe inflammation, driven by immune infiltration impacting cellular proliferation and apoptosis. These findings suggested the formation of a specific tissue microenvironment in severe CE that promotes fibrillogenesis and fibrous structure formation amidst progressive endometrial fibrosis. Identified mechanisms of local homeostasis disturbance in severe CE are induced by complex interactions of endometrial lymphocyte subpopulations, resulting in ECM remodeling dominated by type III collagen and reticular fibers, affecting intercellular cooperation.[53-55]

Conclusions and Future Directions

The data presented in this study enhance our understanding of endometrial homeostasis at varying levels of

CE activity, highlighting the significant role of immune cells and ECM in female reproductive health. Our findings provide insights into the intricate intercellular communication system among immune cells and their microenvironment, reflecting the functional state of the comprehensive regulatory system within the endometrium. The regulation of CE expression involves a complex interplay of immune and cellular factors, including interactions between stromal and epithelial cells, hormone levels, cytokines, and growth factors.

The severity of inflammatory changes in the uterine mucosa correlates with the levels of CD8+, CD68+, CD163+, and CD38+ in the endometrial stroma. Chronic inflammation leads to an excessive formation of fibrous components within ECM, with the extent of fibrosis varying according to the activity level of CE. Mainly, severe and moderate CE exhibit pronounced fibrous changes in the endometrial stroma, characterized by an increased accumulation of mature collagen and reticular fibers, compared to mild CE and control groups.

The intensity of chronic inflammation is directly associated with the reticular fiber content, which is highest in cases of severe CE. Inflammatory changes in the endometrium result in altered cytological and histotopographic profiles of immunocompetent cells, leading to the formation of local pathological clusters with active intercellular signaling and proinflammatory characteristics. These changes synchronize the activity of these cells as the disease progression occurs. Development and evolution of fibrogenic niches within the endometrium, marked by excessive ECM biogenesis in localized areas, serve as crucial diagnostic features and prognostic markers. These niches also guide personalized therapy for CE. The pathological changes observed in specific endometrial regions can spread to larger areas, exacerbating the severity and duration of inflammation and associated clinical manifestations. Our findings on the structural and metabolic changes in collagen and reticular fibers of the ECM under inflammatory conditions describe varying degrees of endometrial biodegradation. The content of reticular fibers serves as an indicator of fibrous change activity, making it a valuable prognostic criterion for assessing CE progression.

Further studies are needed to elucidate the specific mechanisms by which immune cells and ECM components interact to regulate endometrial homeostasis in CE. Investigating the signaling pathways and molecular interactions involved in developing fibrogenic niches could provide underlying mechanisms for the disease's pathology and progression. Additionally, exploring the potential therapeutic targets within these pathways may lead to developing more effective personalized treatment strategies. Longitudinal studies examining the progression of CE and its impact on reproductive outcomes will be essential in validating the prognostic markers identified in this study. Further research into the role of hormonal regulation and its interaction with immune responses in the endometrium could also explore new avenues for therapeutic modalities. Finally, applying advanced imaging and molecular techniques will enhance our ability to monitor and assess ECM remodeling and immune cell dynamics in real time, providing a more comprehensive understanding of CE and its implications for female reproductive health.

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Competing Interests

The authors declare that they have no competing interests.

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