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Altered Immune Cell Profiles in the Follicular Fluid of Patients with Poor Ovarian Response According to the POSEIDON Criteria

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Objective: This study aims to investigate alterations in immune cell counts within preovulatory follicles of patients with poor ovarian response (POR) during assisted reproductive technology (ART), classified according to the POSEIDON criteria.

Methods: This single-centre cross-sectional study included 543 women undergoing IVF/ICSI treatment, selected based on specific inclusion and exclusion criteria: 292 with normal ovarian response and 251 with poor response. Follicular fluid (FF) was collected on the day of oocyte retrieval and analysed by flow cytometry to determine the proportions of macrophages (Mφs), M1 and M2 Mφs, T cells (CD4 and CD8 T cells), dendritic cells (DCs), including type 1 conventional dendritic cells (cDC1) and type 2 conventional dendritic cells (cDC2), and neutrophils. Multivariable logistic regression assessed the relationship between immune cell counts and POR, Pearson correlation determined associations with the number of retrieved oocytes, and receiver operating characteristic (ROC) curves evaluated the predictive power of immune cell counts for POR.

Results: Immune cells accounted for 52.57% (±23.90%) of the total cell population in the follicular microenvironment, which was approximately equal to that of granulosa cells, with Mφs being the most abundant, followed sequentially by T cells, DCs, and neutrophils. In patients with POR, overall Mφs infiltration in the follicular microenvironment decreased, whereas M1 and M2 polarization increased. T cell infiltration increased, with a decrease in the CD4/CD8 ratio. Both cDC1 and cDC2 were significantly elevated. Moreover, multivariable logistic regression revealed that the total macrophage count, CD4 T cell count, and cDC2 count were independent predictors of POR. Notably, cDC2 showed the largest area under the ROC curve, suggesting its strong potential as a biomarker for predicting POR.

Conclusion: The proportion of immune cells in preovulatory follicles were significantly altered in patients with POR. These findings suggest that immune cell dynamics in the follicular microenvironment may play a crucial role in determining ovarian response and prognosis, indicating that targeted immunomodulatory strategies could be considered in future therapeutic approaches.

Keywords: follicular fluid, immune cells, macrophages, T cells, dendritic cells, number of oocytes retrieved, low prognosis

Introduction

Approximately 10–15% of couples of childbearing age worldwide experience infertility, for which assisted reproductive technology (ART) is the main treatment method.[1] With ART, the likelihood of a live birth is correlated with the number of oocytes retrieved. Poor ovarian response (POR) is characterized by a poor response to standard ART regimens,[2] resulting in an insufficient number of retrieved oocytes, a increased cancellation rate, and a decreased live birth rate.[3–5] The incidence of POR after ovarian stimulation is approximately 5.6–35.1%,[6] and as the number of individuals facing infertility increases, the proportion of individuals with POR is also rising, posing great challenges for clinicians.[7]

To better stratify individuals with POR and facilitate assisted pregnancy counselling, researchers proposed the Patient-Oriented Strategy Encompassing the Individualized Oocyte Number (POSEIDON) criteria in 2016[8] to identify patients with a poor response and thus classified 'low-prognosis' patients. The POSEIDON criteria categorize patients into four groups on the basis of age, biomarkers and functional markers, specifically anti-Müllerian hormone (AMH) and

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antral follicle count (AFC), as well as the number of retrieved oocytes. The goal of these criteria is to precisely identify patients with a low prognosis and tailor treatment strategies accordingly. Patients meeting the POSEIDON criteria are presumed to have a poorer prognosis after ART than normal responders with the same ovarian reserve. Therefore, identifying strategies to improve the clinical outcomes of patients meeting the POSEIDON criteria remains one of the foremost issues in ART research.

Immune cells and immune cell-derived cytokines play key roles in regulating the occurrence of various events in the ovary,[9] including gonad formation, tissue reorganization during ovulation, folliculogenesis, follicle growth, oocyte maturation, ovulation, and corpus luteum formation and regression.[10–15] The follicular microenvironment, which contains immune cells and intrafollicular granulosa cells (GCs), controls oocyte maturation and subsequent ovulation. Related events (such as extracellular matrix remodeling, chemotaxis, vasomotion, and oocyte–cumulus complex formation) are regulated by a cytokine-mediated inflammatory response driven by lymphocytes, granulocytes, and macrophages (Mφs).[11] Moreover, GCs in ovulatory follicles appear to exhibit properties of innate immune cells.[16]

Changes in the follicular immune microenvironment, including alterations in Mφs and T cells, are significant contributors to infertility,[17] which has direct implications for oocyte quality[18–21] and embryo implantation,[22,23] further posing the challenges to achieve successful ART outcomes. Specifically, polycystic ovary syndrome (PCOS), as a main cause of anovulatory infertility, is closely associated with immune dysregulation. Studies have shown that the follicular fluid (FF) of individuals with PCOS is markedly proinflammatory, with elevated cytokine levels,[24,25] which significantly correlated with the number of D3 good-quality embryos and the good-quality blastocyst rate[18] during ART. Additionally, dysregulation of T lymphocytes and antigen-presenting cells is observed in the FF of individuals with PCOS,[26,27] and M2 Mφs phenotype polarization ameliorates inflammatory in PCOS.[28] Furthermore, diminished ovarian reserve (DOR), as an expected POR, is a significant obstacle in in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), leading to poor reproductive outcomes. Aging and apoptotic GCs accumulation, along with Mφs phagocytosis dysfunction, contribute to ovarian aging in DOR. Impaired Mφs function leads to insufficient clearance of aging cells, increasing DOR risk.[29] Moreover, an abnormal proportion of CD8 T cells and increased levels of CCL5 and interferon-γ (IFN-γ) may disrupt the immune balance in the FF,[30] affecting the growth of GCs and further promoting the progression of DOR.

The relationships between immune cells in the FF and low prognosis in patients are not yet known. Given the critical role of immune cells in shaping the follicular microenvironment and the lack of an effective and widely accepted treatment strategy for POR has been established, investigating the distribution and function of immune cells in patients with poor prognosis under the POSEIDON criteria could provide valuable insights for the development into new therapeutic strategies. This study serves as an exploratory investigation aimed at identifying potential patterns and associations between immune cell dynamics and POR. As a preliminary study, it is limited by a relatively small sample size and the absence of longitudinal data, which may restrict the generalizability of the findings. The results are intended to be used to generate hypotheses and provide initial insights, which may guide the design of more comprehensive and larger-scale studies in the future. By identifying how specific immune cell dynamics contribute to the challenges of POR, this study aims to lay the foundation for the development of potential immunotherapy approaches that may improve ART outcomes and offer better support for patients with POR.

Materials and Methods

Ethical Approval and Consent to Participate

All the experimental protocols in this study were conducted in accordance with the principles of the Declaration of Helsinki and were approved by the Ethics Committee of The First Affiliated Hospital of Kunming Medical University. Informed written consent was obtained from each patient prior to enrolment in this study.

Study Population and Design

This was a prospective cross-sectional study involving women with infertility. Infertile patients who received IVF/ICSI treatment during the study period were potentially eligible for participation in this study. Patients were excluded if:1) they had a chromosomal abnormality; 2) they were diagnosed with cancer, endometriosis (EM), an immune disorder (such as

systemic lupus erythematosus) or a chronic metabolic condition (such as diabetes); or 3) pregnancy loss had occurred ≥ 2 times. Only samples from the first oocyte retrieval cycle were included in this study.

All patients underwent a standardized ovarian stimulation protocol, oocyte retrieval, fertilization, embryo transfer, and luteal support. Patients underwent ovarian stimulation protocols according to their age and ovarian reserve (evaluated on the basis of AMH concentration and AFC). Follicle development was monitored via transvaginal ultrasound, and reproductive hormone levels were measured. When two to three follicles reached 18 mm in diameter, final oocyte maturation was triggered by administration of human chorionic gonadotropin (Gn). Transvaginal ultrasound-guided oocyte retrieval was performed 34–36 hours after triggering maturation. All follicles with diameters of ≥10 mm were aspirated. Fertilization was achieved via either conventional IVF or ICSI, depending on the patient's condition. For a detailed description of these protocols, please refer to our previous publication.[31]

The patients' demographic and clinical characteristics were documented, including the number of oocytes retrieved, age, body mass index (BMI), AFC, AMH, type of infertility, controlled ovarian hyperstimulation (COH) protocol used, and levels of sex and thyroid hormones, were recorded. The levels of sex hormones, including baseline oestradiol (E2), baseline progesterone (P), baseline testosterone (T), baseline luteinizing hormone (LH), baseline follicle-stimulating hormone (FSH) and prolactin (PRL), were measured via an electrochemiluminescence immunoassay (ECLIA) on a Cobas e601 analyser (Roche, Switzerland). The levels of thyroid hormone levels, including thyroid-stimulating hormone (TSH), free triiodothyronine (FT3) and free thyroxine (FT4), were measured via ECLIA on an i2000 SR analyser (Abbott, United States). All blood samples were acquired after overnight fasting for at least 8 hours.

Definition and Classification of POR Under the POSEIDON Criteria

The participants were categorized into a normal-prognosis group (control group: C1, C2) and a low-prognosis group (POR group: G1, G2, G3, G4) according to the POSEIDON criteria. Specifically, participants with a normal prognosis were classified by age according to the following detailed criteria: Group C1: age < 35 years, AMH ≥ 1.2 ng/mL, AFC ≥ 5, and number of oocytes retrieved in the present IVF cycle > 9 and Group C2: age ≥ 35 years, AMH ≥ 1.2 ng/mL, AFC ≥ 5, and number of oocytes retrieved in the present IVF cycle > 9. Furthermore, participants with a low prognosis were classified by age and ovarian reserve function according to the following POSEIDON stratification. Group G1: age < 35 years, AMH ≥ 1.2 ng/mL, AFC ≥ 5, and number of oocytes retrieved in the present IVF cycle ≤ 9; Group G2: ≥ 35 years, AMH ≥ 1.2 ng/mL, AFC ≥ 5, and number of oocytes retrieved in the present IVF cycle ≤ 9; Group G3: age < 35 years; AMH < 1.2 ng/mL or AFC < 5; and Group G4: age ≥ 35 years; AMH < 1.2 ng/mL or AFC < 5.

Patients aged ≥35 years were considered to constitute the ageing groups (Groups C2, G2, and G4), and patients aged <35 years were considered to constitute the young groups (Groups C1, G1, and G3). Women with normal ovarian reserve function in the low-prognosis group were classified as the NOR group, and those with reduced ovarian reserve function were classified as the DOR group.

Flow Cytometric Analysis of Immune Cells in the FF

FF was collected from the follicle of each participant on the oocyte retrieval day, with attention given to minimizing potential blood contamination. Depending on the condition of the sample, red blood cell lysis solution (Solarbio, China) was applied to lyse erythrocytes when necessary. The FF sediment was centrifuged (400×g, 10 min) to obtain the sediment and then digested with 2% hyaluronidase (BioFroxx, Germany) for 10 min. Then, the cell suspension was filtered through a 200-mesh filter and centrifuged (400 × g, 5 min) to remove the cell supernatant. Single-cell suspensions were blocked with nonspecific Fc receptor blocking solution (Human TruStain FcX™, Biolegend, Cat #: 422301) and labelled with a specific conjugated monoclonal antibody. To identify immune cell types, existing literature indicates that preovulatory follicles primarily contain GCs, Mφs, T cells, dendritic cells (DCs), and neutrophils.[9,32–34] Based on previous studies, appropriate flow cytometry markers were selected for each immune cell type.[27,34–38]

An 8-colour panel for analysis of Mφs and T cells (PerCP-conjugated anti-CD45, Biolegend, Cat #:368506; APC-Cy7-conjugated anti-CD11b, Biolegend, Cat #: 301306; PE-conjugated anti-CD163, Proteintech, Cat #: PE-65169; BV510-conjugated anti-CD86, Biolegend, Cat #: 305432; APC-conjugated anti-CD3, Biolegend, Cat #: 300312; BV421-conjugated anti-CD4, Biolegend, Cat #: 317434; FITC-conjugated anti-CD8, Biolegend, Cat #: 300906; and PE-Cy7-conjugated anti-

CD69, Elabscience, Cat #: E-AB-F1138H) was developed for the detection of Mφs and T cells, and a 6-colour panel (PerCP-conjugated anti-CD45, Biolegend, Cat #: 368506; APC-Cy7-conjugated anti-CD11b, Biolegend, Cat #: 301306; FITC-conjugated anti-CD11c, Proteintech, Cat #:FITC-65086; PE-Cy7-conjugated anti-CD1c, Biolegend, Cat #: 331516; PE-conjugated anti-CD141, Biolegend, Cat #: 344104; and APC-conjugated anti-CD182 (CXCR2), Biolegend, Cat #: 320710) was designed for the simultaneous detection of DCs and neutrophils. After the cells were washed with PBS 3 times, data were acquired on a flow cytometer (BD Fortessa, United States) and analysed with FlowJo software.

Definition of Immune Cells in the FF

We defined CD45+ cells as immune cells, CD45+CD11b+ cells as Mφs; CD45+CD11b+CD86+ cells as M1 Mφs; CD45+CD11b+CD163+ cells as M2 Mφs; CD45+CD3+ cells as T cells, CD45+CD3+CD4+ cells as CD4+ T cells (T helper cells, Th cells); CD45+CD3+CD8+ cells as CD8+ T cells (cytotoxic T cells, Tc cells); CD45+CD3+CD69+ cells as activated T cells; CD11C+CD141+ cells as type 1 conventional dendritic cells (cDC1s); CD11C+CD1C+ cells as type 2 conventional dendritic cells (cDC2s), and CD11b- CD182+ cells as neutrophils. CD45- cells were defined as GCs (Figure 1a).

Data and Statistical Analysis

Following an established protocol, flow cytometry data were processed with FlowJo™ 10.0. The data were finally analysed with SPSS 23.0 and GraphPad Prism 9.0. The distribution of the data was first assessed via the Kolmogorov–Smirnov test. For comparisons between two groups, if the data followed a Gaussian distribution, results are presented as means ± SDs, and Student's *t*-test was applied (using the paired sample *t*-test for paired samples). For comparisons across multiple groups, one-way ANOVA was used. If the data were not normally distributed, results are presented as medians (interquartile range, IQR), and nonparametric tests were employed (the Mann–Whitney test for two groups or the Kruskal–Wallis test for three or more groups).

Pearson correlation analysis was used to assess the relationship between the number of retrieved oocytes and immune cell proportions. The Pearson correlation coefficient (r), ranging from −1 to +1, indicates correlation strength and direction: r>0 suggests a positive correlation, r<0 a negative one, and values closer to 0 imply little to no linear relationship. The closer the absolute value of r is to 1, the stronger the correlation.

A logistic regression model was used to determine how variations in immune cell counts influence the probability of POR. Potential confounding variables included age, BMI, AMH, AFC, COH protocol used, basal LH level, basal FSH level, basal P level, basal E2 level, basal T level, TSH level, FT3 level, FT4 level, total immune cells count, total Mφs count, M1 Mφs count, M2 Mφs count, total T cell count, CD4 T cell count, CD8 T cell count, CD69+ T cell count, cDC1 count,cDC2 count and neutrophils count. All odds ratios (ORs) were reported as OR with 95% confidence interval (CI). The area under the curve (AUC) for each immune cell type was determined using receiver operating characteristic (ROC) curve analysis to evaluate the diagnostic accuracy of these immune cells in predicting POR. The AUC value represents the ability of the immune cell type to correctly classify patients as having a normal or poor ovarian response, with higher AUC values indicating better discriminatory power. Specifically, an AUC of 0.5 suggests no discriminative ability, whereas an AUC close to 1.0 indicates excellent accuracy. This analysis allowed us to quantify the predictive potential of each immune cell type, providing valuable insights into their clinical relevance as biomarkers for POR.

A P value of <0.05 was considered to indicate statistical significance. For comparisons between two groups, "ns" denotes no significant difference ($P > 0.05$), "*" indicates $P \leq 0.05$, "**" denotes $P \leq 0.01$, "***" denotes $p \leq 0.001$ and "****" denotes $p \leq 0.0001$. Multiple comparisons were visualized using alphabetical letters, with distinct letters (eg, "a" vs "b") indicating significant differences and identical letters denoting non-significance among groups (eg, "a" or "ab"). When missing data were <5%, they were excluded from the analysis without imputation. When missing data exceeded 5%, multiple imputation was used to replace missing values for continuous variables.

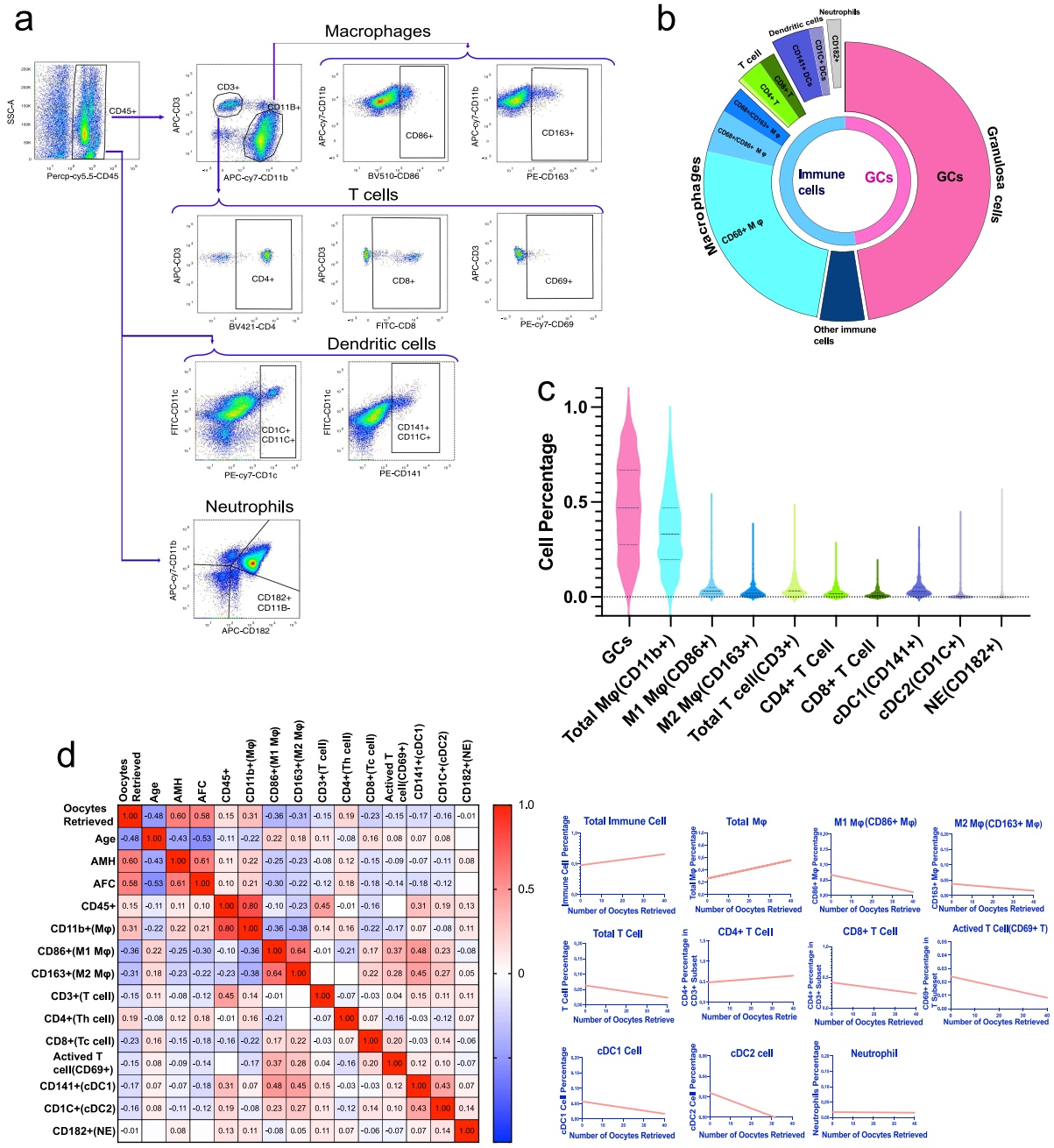

Figure 1 Comprehensive analysis of immune cell populations and their association with the number of retrieved oocytes in the FF. (**a**) Flow cytometric gating strategy for identifying immune cell populations in FF. The initial gating was applied to identify CD45+ immune cells from the total cell population. Further analysis was conducted to differentiate specific immune subtypes: Macrophages (Mφs): Gated as CD11b+ cells, followed by subsequent gating to identify M1 Mφs (CD86+) and M2 Mφs (CD163+). T Cells: Identified as CD3+ cells, which were further divided into CD4 T cells (CD3+ CD4+) and CD8 T cells (CD3+ CD8+). Additionally, activated T cells were identified as CD3+ CD69+. Dendritic Cells (DCs): Gated as CD11c+ cells, with further characterization to identify cDC1 (CD141+) and cDC2 (CD1c+). Neutrophils: Identified as CD11b- CD182+. The flow cytometric plots show representative data for each immune cell subtype, providing a detailed overview of immune cell infiltration and polarization states in preovulatory follicles. (**b**) Distribution of immune cell types and GCs in the follicular microenvironment. The proportion of immune cells (blue area) was approximately equal to that of GCs (pink area). Immune cells were further categorized into macrophages, T cells, DCs, and neutrophils. Among Mφs, M1 (CD86+) and M2 (CD163+) subsets were identified, although the classic M1 and M2 polarized subtypes accounted for relatively small proportions. The chart illustrates the relative proportions of these immune cell subsets in comparison to GCs. (**c**) Violin plot showing the proportions of different cell types in the follicular microenvironment. GCs and various immune cell populations are represented. The plot highlights the distinct distribution of each cell type, with GCs and Mφs showing the highest abundance, followed by other immune cell types.NE, neutrophils. (**d**) Correlation analysis between immune cell subsets, clinical parameters, and ovarian response. The left panel displays a heatmap illustrating the Pearson correlation coefficients(r) among key clinical parameters and immune cell types in the follicular microenvironment. Variables include oocyte retrieval count, age, AMH, AFC, and various immune cell subsets. Positive correlations are represented in red, while negative correlations are represented in blue. The intensity of the color indicates the strength of the correlation. The right panel contains line charts depicting the relationship between the number of retrieved oocytes and the proportions of different immune cell subsets. Correlations were observed for several immune cell types, with positive and negative trends depending on the cell type.

Results

Baseline Characteristics and Cell Profile of the Included Participants

Flow cytometry analysis was performed following the established protocol to quantify the proportions of specific immune cell populations (Figure 1a). A total of 543 patients who met the inclusion criteria were enrolled in this study; with 292 patients in the control group (292/543, 53.8%) and 251 patients in the POR group (251/543, 46.2%). Compared with those in the control group, women in the POR group presented lower AMH levels, AFC, baseline FSH, total immune cell counts, total Mφs counts, and Th cell count. In contrast, they presented higher counts of M1 Mφs, M2 Mφs s, total T cells, Tc cells, cDC1 cells, and cDC2 counts (Table 1 and Figure 2a).

In the FF of patients from all groups, GCs constituted 47.42% ± 23.90% of the total cell population, whereas immune cells made up 52.57% ± 23.90% of total cells, indicating an approximately equal distribution between GCs and immune cells. Among the immune cell populations, Mφs were the most prevalent, followed by T cells, cDC1, cDC2, and neutrophils (Figure 1b and c).

Table 1 Characteristics of the Included Participants

Clinicopathological Factor	Overall	Control	POR	P
No. of patients	543	292	251	
Age, years, mean (SD)	33.23±5.44	31.38±4.28	35.61±5.69	0.000**
BMI, kg/m^2, median (IQR)	22.31 (20.26–24.88)	22.50 (20.07–25.24)	22.31 (20.44–24.78)	0.608
AMH, ng/mL, median (IQR)	2.96 (1.38–5.00)	4.11 (2.87–6.83)	1.32 (0.67–2.65)	0.000**
AFC, n, median (IQR)	12 (7–19)	16 (11–24)	7 (4–12)	0.000**
Basal E2, pg/mL, median (IQR)	56.00 (36.20–133.00)	54.14 (36.2–129.25)	58.33 (36.50–143.20)	0.592
Basal P, ng/mL, median (IQR)	0.26 (0.18–0.58)	0.26 (0.18–0.59)	0.26 (0.18–0.52)	0.677
Basal LH, IU/L, median (IQR)	5.24 (3.25–8.56)	5.50 (3.29–8.65)	5.01 (3.25–8.38)	0.804
Basal FSH, IU/L, median (IQR)	6.42 (4.85–8.49)	5.97 (4.44–7.50)	7.29 (5.32–10.53)	0.000**
Basal PRL, uIU/mL, median (IQR)	16.05 (12.32–22.43)	16.55 (12.65–23.16)	15.82 (11.82–21.42)	0.158
Basal T, pmol/L, median (IQR)	0.28 (0.20–0.37)	0.31 (0.21–0.41)	0.24 (0.16–0.38)	0.067
TSH, μIU/mL, median (IQR)	2.24 (1.58–3.06)	2.16 (1.48–2.93)	2.30 (1.63–3.18)	0.060
FT3, pg/mL, median (IQR)	4.87 (4.49–5.25)	4.89 (4.52–5.26)	4.84 (4.46–5.24)	0.366
FT4, ng/mL, median (IQR)	16.74 (15.09–18.35)	16.85 (15.26–18.54)	16.58 (1.92–18.14)	0.201
Oocytes retrieved, n, median (IQR)	10 (5–16)	16 (12–20)	4 (1–7)	0.000**
Type of infertility, n (%)				0.000**
Primary (n/N)	277 (277/543, 51.01%)	172 (172/292, 58.90%)	105(105/251, 41.83%)	
Secondary (n/N)	266 (266/543, 48.99%)	120 (120/292, 41.10%)	146 (146/251, 58.16%)	
Ovarian stimulation protocol, n (%)				0.000**
Long protocol (n/N)	130 (130/543, 29.94%)	98 (98/292, 33.56%)	32 (32/251, 12.75%)	
Antagonist protocol (n/N)	242 (242/543, 44.57%)	160 (160/292, 54.79%)	82 (82/251, 32.67%)	
PPOS protocol (n/N)	101 (101/543, 18.60%)	34 (34/292, 11.64%)	67 (67/251, 26.69%)	
Mild stimulation protocol (n/N)	55 (55/543, 10.13%)	0 (0/292, 0%)	55 (55/251, 21.9%)	
Other protocol (n/N)	15 (15/543, 2.39%)	0 (0/292, 0%)	15 (15/251, 5.98%)	
Fertilization, n (%)				
IVF (n/N)	430 (430/543, 79.19%)	230 (230/292, 78.77%)	200 (200/251, 79.68%)	0.439
ICSI (n/N)	113 (113/543, 20.81%)	62 (62/292, 21.23%)	51 (51/251, 20.32%)	
Proportion of GCs, (%), mean (SD)	47.42±23.90	44.49±22.35	50.84±25.21	0.002**
Proportion of Immune cells, (%), mean (SD)	52.57±23.90	55.51±22.35	49.16±25.21	0.002**
Proportion of total Mφs, (%), mean (SD)	33.30 (19.93–47.18)	37.72 (24.43–51.91)	25.75 (15.02–41.93)	0.000**
[a] Proportion of M1 Mφs, (%), median (IQR)	11.20 (6.52–20.8)	9.05 (5.84–14.68)	16.60 (8.45–34.40)	0.000**
[a] Proportion of M2 Mφs, (%), median (IQR)	8.12 (4.38–14.3)	6.91 (3.69–11.65)	9.80 (4.91–19.30)	0.000**
Proportion of T cells, (%), median (IQR)	3.66 (2.16–6.88)	3.31 (2.13–5.98)	4.04 (2.18–8.00)	0.041*

(*Continued*)

Table 1 (Continued).

Clinicopathological Factor	Overall	Control	POR	P
[b] **Proportion of Th cells, (%), median (IQR)**	55.90 (45.80–62.50)	58.70 (51.32–66.00)	50.30 (42.80–59.40)	0.000**
[b] **Proportion of Tc cells, (%), median (IQR)**	37.30 (29.60–45.20)	35.10 (27.08–42.18)	39.8 (32.80–49.90)	0.000**
[b] **Proportion of activated T cells, (%), median (IQR)**	1.80 (0.54–4.41)	1.80 (0.63–5.32)	2.00 (0.48–4.15)	0.589
Proportion of cDC1 cells, (%), median (IQR)	3.29 (1.99–5.56)	3.15 (1.89–5.01)	4.64 (2.63–7.46)	0.016*
Proportion of cDC2 cells, (%), median (IQR)	0.69 (0.37–1.50)	0.51 (0.29–0.88)	1.15 (0.51–2.45)	0.000**
Proportion of Neutrophils, (%), median (IQR)	0.31 (0.11–1.24)	0.32 (0.16–1.20)	0.30 (0.11–1.36)	0.741

Notes: [a] indicates the proportion of this cell subtype within the macrophage population, while [b] indicates the proportion of this cell subtype within the T cell population. Bold text represents primary variable categories or major headings, distinguishing the main sections of the data. If data followed a Gaussian distribution, data were presented as mean ± SD. If data were not normally distributed, data were presented as median (interquartile range). A P value ≤ 0.05 was considered statistically significant (* for P ≤ 0.05; ** for P ≤ 0.01). **Abbreviations**: IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; POR, poor ovarian response; PPOS, progestin primed ovarian stimulation; BMI, body mass index; AFC, antral follicle account; AMH, anti-Müllerian hormone; luteinizing hormone, LH; follicle-stimulating hormone, FSH; progesterone, P; estradiol, E2; testosterone, T; prolactin, PRL; thyroid stimulating hormone, TSH; free triiodothyronine, FT3; free thyroxine, FT4; IQR, interquartile range; SD, standard deviation.

Correlation Between Immune Cell Proportions and Oocyte Retrieval

Based on Figure 1d, the correlation heatmap demonstrates the relationships between clinical parameters (the number of oocytes retrieved, age, AMH, and AFC) and immune cell subsets in the FF. The proportions of total immune cells, Mφs, and CD4+ T cells showed a positive correlation with the number of retrieved oocytes, indicating that a higher presence of these immune cells is associated with an increased number of oocytes. Conversely, the proportions of M1 Mφs, M2 Mφs, total T cells, CD8+ T cells, activated T cells, cDC1, and cDC2 were negatively correlated with the number of retrieved oocytes, suggesting that higher levels of these immune cell types are linked to a lower number of retrieved oocytes. The line plots on the right further illustrate these correlations, highlighting the different trends between immune cell proportions and oocyte retrieval outcomes.

Specific Immune Cell Counts are Independent Key Factors Associated with POR

Various confounding factors were considered to explore the associations between different types of immune cell counts and poor response. The model included age, BMI, AMH levels, the AFC, basal gonadal hormone (E2, P, LH, FSH,T, PRL) levels, thyroid hormone levels (TSH, FT3, FT4) levels, COH protocol used, the Mφs count, T cell count, DC count and their subtypes count, as well as the neutrophil count. After adjusting for confounding factors, we identified the total Mφs count, CD4 T cell count, and cDC2 count as key factors influencing poor prognosis among immune cells, as detailed in Table 2.

An increase in the total Mφs count (OR, 0.000; 95% CI: 0.000–0.044) and CD4 T cell count (OR, 0.007; 95% CI: 0.000–0.783) was significantly negatively associated with a decreased risk of POR, with elevated numbers of these cells corresponding to a reduced likelihood of POR occurrence. In contrast, the cDC2 count demonstrated a strong positive association with POR, as a higher cDC2 count was linked to an increased probability of POR. Although the confidence interval was broad with a high upper limit (OR,1.535E+25; 95% CI: 13.790–1.708E+49), suggesting considerable variability in the effect of the cDC2 cell count on POR, the significant P value confirmed the statistical robustness of this result.

cDC2 in FF as Biomarkers for Predicting Low Prognosis

Figure 2b presents a comparison of the AUCs for the count of each immune cell type. Among these, the cDC2 count had the highest AUC, reaching 0.7015, indicating that is a strong ability to distinguish between patients with a poor prognosis and those with a normal prognosis. The Youden Index was used to calculate the cut-off value, and the result revealed that when the proportion of cDC2s exceeded 0.89% of the total cell population, the patient was classified as having a POR.

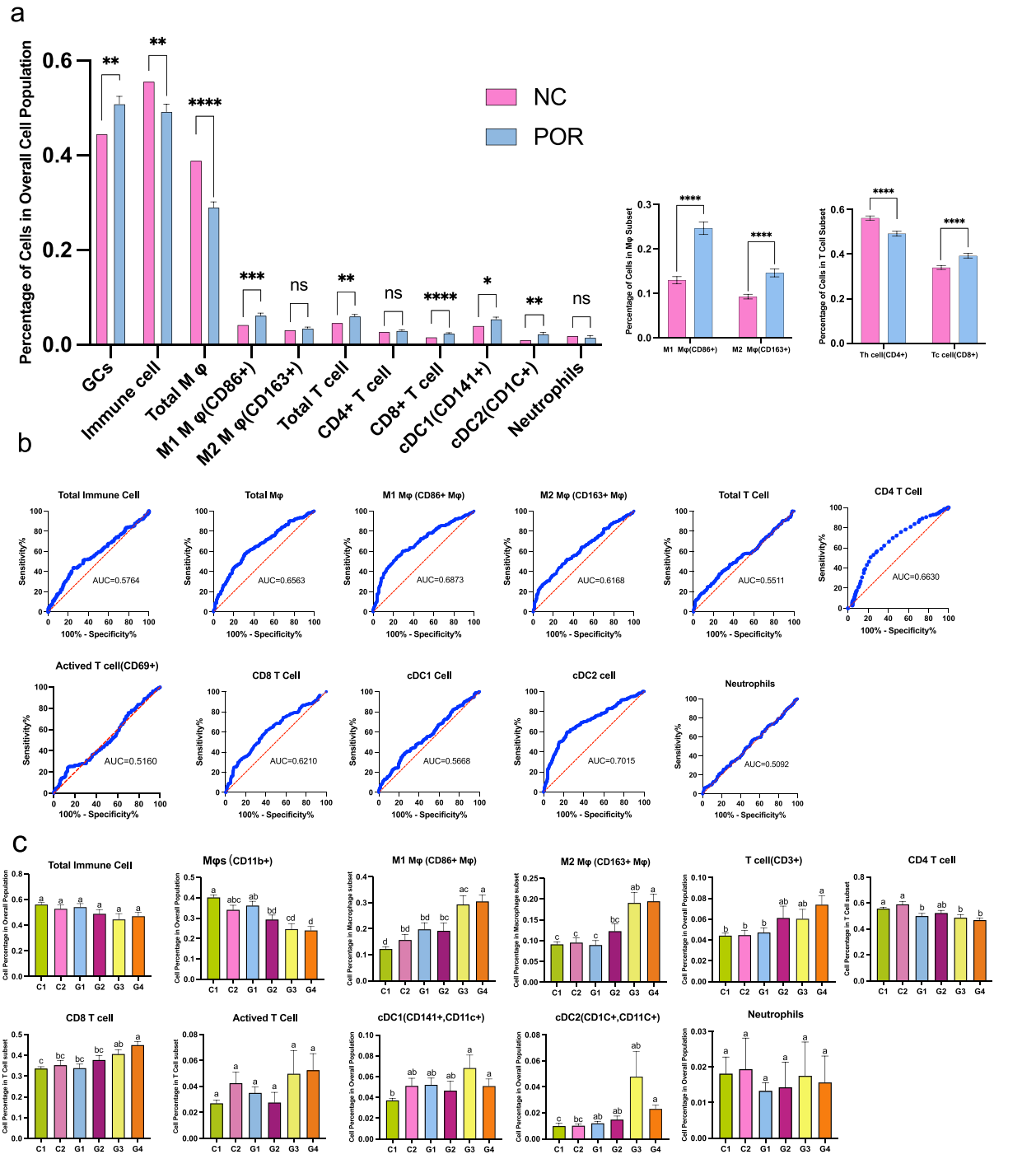

Figure 2 Immune cell distribution comparison between POR and control groups, and evaluation of immune cell subsets as predictors of POR in the FF. (**a**) Comparison of immune cell and GC proportions between normal prognosis (NC) and poor ovarian response (POR) groups. The left bar chart shows the percentage of various cell types within the overall follicular cell population. Pink bars represent the NC group, and blue bars represent the POR group. Statistically significant differences between groups are indicated by asterisks: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, ns = not significant. The right panels provide a more detailed view of the relative distribution within specific immune cell populations. The first panel illustrates the proportions of M1 and M2 Mφs within the total macrophage population, while the second panel shows the proportions of T helper (Th, CD4+ T) cells and cytotoxic (Tc, CD8+ T) cells within the total T cell population. Significant differences between the NC and POR groups are highlighted in both panels. (**b**) Receiver operating characteristic (ROC) curves and area under the curve (AUC) values for different immune cell subsets in predicting poor ovarian response (POR). The ROC curves evaluate the diagnostic accuracy of various immune cell types. The AUC values quantify each cell subset's ability to distinguish between POR and non-POR cases, with higher AUC values indicating better discriminatory power. Notably, cDC2 cells demonstrated the highest AUC (0.7015), indicating their potential as a biomarker for predicting POR. (**c**) Proportions of different immune cell types across groups classified by ovarian response according to the POSEIDON criteria. Groups are labelled as C1, C2, G1, G2, G3, and G4, representing varying prognoses based on ovarian reserve and age. Different letters above the bars denote statistically significant differences between groups ($p < 0.05$), with shared letters indicating no significant difference.

Table 2 Associations Between POR and the Counts of Different Types of Immune Cells

Variables	B	S.E.	Wald	P	Exp(B) (95% CI)
Age	0.120	0.046	6.972	0.008**	1.128 (1.031–1.233)
AMH	−0.535	0.129	17.179	0.000**	0.586 (0.455–0.754)
AFC	0.059	0.043	1.924	0.165	1.061 (0.976–1.153)
BMI	−0.046	0.054	0.710	0.400	0.955 (0.859–1.062)
Basal E2 level	−0.003	0.001	9.686	0.002**	0.997 (0.995–0.999)
Basal P level	−0.130	0.059	4.758	0.029*	0.878 (0.782–0.987)
Basal LH level	−0.022	0.026	0.712	0.399	0.979 (0.931–1.029)
Basal FSH level	0.138	0.063	4.742	0.029*	1.148 (1.014–1.299)
Basal T level	−0.429	1.223	0.123	0.726	0.651 (0.059–7.157)
TSH	0.044	0.113	0.149	0.699	1.045 (0.837–1.305)
FT3	−0.045	0.217	0.004	0.834	0.956 (0.624–1.463)
FT4	−0.031	0.040	0.609	0.435	0.969 (0.897–1.048)
COH protocol	0.811	0.291	7.780	0.005**	2.251 (1.273–3.980)
Immune cell count	3.054	1.989	2.356	0.125	21.195 (0.429–1046.053)
Total M φ count	−8.345	2.666	9.800	0.002**	0.000 (0.000–0.044)
[a]M1 M φ count	1.601	1.558	1.056	0.304	4.960 (0.234–105.090)
[a]M2 M φ count	−1.516	3.421	0.196	0.658	0.220 (0.000–179.463)
Total T cell count	2.517	6.652	0.143	0.705	12.391 (0.000–5,697,031.8)
[b]CD4 T cell count	−5.003	2.428	4.247	0.039*	0.007 (0.000–0.783)
[b]CD8 T cell count	−3.618	2.503	2.091	0.148	0.0276 (0.000–3.618)
Activated T cell count	−6.247	5.051	1.530	0.216	0.002 (0.000–38.557)
cDC1 count	7.808	5.662	1.902	0.168	2461.417 (0.037–162,595,158)
cDC2 count	57.993	28.250	4.214	0.040*	1.535E+25 (13.790–1.708E+49)
NE count	15.453	13.608	1.290	0.256	5,144,538 (0.000–1.97E+18)

Notes: [a] indicates the proportion of this cell subtype within the macrophage population, while [b] indicates the proportion of this cell subtype within the T cell population. Bold text represents primary variable categories or major headings, distinguishing the main sections of the data. A P value ≤ 0.05 was considered statistically significant (* for P ≤ 0.05; ** for P ≤ 0.01).
Abbreviations: POR, poor ovarian response; BMI, body mass index; AFC, antral follicle account; AMH, anti-Müllerian hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; P, progesterone; E2, estradiol; T, testosterone; PRL, prolactin; TSH, thyroid stimulating hormone; FT3, free triiodothyronine; FT4, free thyroxine.

Mφs Distribution in Patients with Low Prognosis According to the POSEIDON Criteria

Overall, the proportion of total Mφs (as a percentage of the total cell population) progressively decreased as the prognosis worsened, whereas the proportions of M1 and M2 Mφs (as percentages of the total Mφs population) increased. In younger individuals (Figure 2c) aged under 35 years, the total Mφs count declined as the prognosis worsened, a trend that was similarly observed in older individuals aged 35 years and above. The total Mφs proportion was significantly greater in the younger cohort with adequate ovarian reserve and favourable prognosis (the C1 group) than in the other groups and was lowest in the older cohort with reduced ovarian reserve and poor prognosis (the G4 group). The trends for the numbers of M1 and M2 Mφs were consistent, as they gradually increased with age, peaking in the older groups with diminished ovarian function (Figure 2c). Additionally, we observed a significant increase in the number of M1 Mφs in the POR group with normal ovarian function, a pattern that was not observed for the number of M2 Mφs.

T-Cell Distribution in Patients with a Low Prognosis According to the POSEIDON Criteria

The total T cell count (as a percentage of the overall cell population) tended to increase as the prognosis worsened, with a particularly pronounced increase in the G4 group, which comprised patients over 35 years of age with insufficient ovarian reserve. The proportion of CD4 T cells (as a percentage of total T cells) was lower in groups with a poorer prognosis (the G1, G2, G3 and G4 groups) than in those with a favourable prognosis. In contrast to the trend observed for

the proportion of CD4 T cells, the proportion of CD8 T cells (as a percentage of total T cells) was greater in the poor prognosis groups, with groups of patients with DOR (the G3 and G4 groups) exhibiting the highest proportion of CD8 T cells. Consequently, the CD4/CD8 ratio gradually increased as the prognosis worsened. The number of activated T cells was slightly elevated in patients with DOR patients and a poor prognosis, but this increase did not reach statistical significance. These findings are detailed in Figure 2.

DC Distribution in Patients with Low Prognosis According to the POSEIDON Criteria

Patients with a poor prognosis and DOR (the G3 and G4 groups) showed a significant increase in the proportions of both cDC1s and cDC2s (relative to the overall cell population), particularly in comparison to the younger control group with normal ovarian function. Among these groups, the younger group with DOR (the G3 group) presented the greatest proportion of DCs, followed by the older group with poor ovarian function (the G4 group). In contrast, the younger control group with adequate oocyte yield and normal ovarian function (the C1 group) presented the lowest proportion of DC cells. Compared with the proportion of cDC1s, the proportion of cDC2s exhibited more pronounced changes among individuals with POR. These findings are detailed in Figure2.

Neutrophil Distribution in Patients with Low Prognosis According to the POSEIDON Criteria

As shown in Figure 2, the neutrophil count exhibited a slight but nonsignificant decreasing trend in patients with low prognosis.

Discussion

In this study, we focused on the differences in immune cell populations between patients with low prognosis and patients with normal prognosis according to the POSEIDON criteria who were seeking infertility treatment. COH constitutes a very important part of ART. Through controlled ovulation stimulation, women with infertility can produce sufficient high-quality oocytes, which are used for subsequent embryo culture and to induce pregnancy. Patients with ovarian hyporesponsiveness respond poorly to Gn during ovulation hyperstimulation, resulting in a low number of retrieved oocytes and a high cycle cancellation rate. Although the molecular mechanisms driving the poor response to COH are largely unknown, most related studies have focused on cumulus cells because of their close connection with oocytes. In the follicle, bidirectional communication between oocytes and GCs is critical for oocyte development and maturation.[39] Studies have shown obvious changes in the GCs of people with POR, such as abnormal mitochondria-related changes,[40] altered miRNA expression,[41] allelic combinations of FSH receptor (FSHR) gene polymorphisms,[42] changes in the levels of Notch signalling proteins,[43] and changes in the levels of the androgen receptor and FSHR.[44]

Our research revealed that the proportions of immune cells and nonimmune cells (GCs) in FF were almost equal; Mφs accounted for the largest proportion, followed by T cells and DCs. The homeostatic immune microenvironment established by such a large proportion of immune cells is crucial for the normal functioning of the ovary. On the one hand, alterations in immune cell populations may affect oocyte development through the action of these immune cells on GCs. A single-cell landscape study[34] conducted in 2022 revealed strong crosstalk between immune cells and GCs involving cytokine receptor interactions, cell adhesion molecules, chemokine signalling pathways and epidermal growth factor receptor (EGFR) tyrosine kinase pathways. In addition, GC-derived miRNAs can regulate Mφs polarization.[45] On the other hand, immune cells may also exert effects through secreted cytokines. Studies have shown that elevated levels of CCL5, IFN-γ and IL-2 may change the immune balance in FF and impair the growth of GCs, which in turn drives adverse IVF outcomes.[30,46] This important role of immune cells should be discussed earnestly, but whether ovarian reactivity to COH is related to the immune microenvironment has not been studied. Our study addressed this question by analysing immune cell profiles in the FF of patients with POR.

Role of Mφs in Follicle Development

Ovarian Mφs are able to regulate cellular proliferation, differentiation and apoptosis and influence steroid production, vascularization and tissue remodelling during follicle growth, ovulation and luteinization.[15] In our study, the total number of Mφs was found to gradually decrease with age and ovarian function. This result aligns with previous research. In a mouse model of reproductively aging, the degree of Mφs infiltration decreased.[47] The loss of Mφs infiltration can impair fertility, as Mφs pyroptosis shifts the immunoregulatory environment of young ovary towards a proinflammatory state characteristic of ageing ovaries. This remodeling further drives stromal cell senescence and accelerates reproductive decline.[48] Through logistic regression analysis, we further confirmed that Mφs serve as protective factors for ovarian follicles and their responsiveness to COH.

Numerous previous biological studies have established a classical polarization model for Mφs, classifying them as either proinflammatory M1 Mφs or reparative M2 Mφs. Ovulation-induced local inflammation drives the selective activation of surrounding primordial follicles, and this function was found to be related to infiltrating Mφs in ovulatory follicles and dynamic changes in the two populations of polarized Mφs, ie, M1 and M2 Mφs.

Mφs play pivotal roles in both physiological and pathological processes in female reproduction, establishing themselves as key players in these dynamics processes. The roles of M1 and M2 Mφs function during follicular development remain debated. M1 Mφs can support vascular growth, follicle development,[49] luteolysis,[50] and primordial follicle activation[51] but may also impair oocyte quality, increase the number of atretic follicles, and reduce the number of growing follicles.[52] M2 Mφs, on the other hand, promote luteinization,[53] progesterone production, and follicle dormancy[51] while also potentially promoting extracellular matrix (ECM) deposition and fibrosis.[54] Additionally, M2 Mφs can increase the ovarian reserve by reducing GCs apoptosis,[55] increasing the number of growing follicles to improve oocyte quality,[51] increasing AMH and oestrogen levels, and reducing the number of atretic follicles. Clearly defining the overall effects of M1 and M2 Mφs in the follicular microenvironment on follicle development is challenging. The numbers of M1 and M2 Mφs in ovarian EM patients are significantly greater than those in controls,[56] contributing to a proinflammatory and profibrotic environment. The roles of M1 and M2 Mφs in EM remain debated: some studies suggest a shift from M1 to M2 Mφs in both eutopic and ectopic endometrium,[57] whereas others note a predominance of M1 Mφs[58,59] or argue against a strict M1/M2 division, highlighting the presence of complex, mixed phenotypes.[60] Nonetheless, Mφs are central to EM pathogenesis, showing decreased phagocytic capacity,[61] increased inflammatory cytokine secretion, and promoting vascularization and adhesion of ectopic tissue,[62] and sustained chronic inflammation in the peritoneal microenvironment.[63] In PCOS, which is characterized by systemic low-grade inflammation and chronic ovarian inflammation,[64,65] the number of M1 Mφs is elevated in peripheral blood and ovarian tissue.[65–67] Excess androgens, insulin resistance, and lipid metabolism disorders intensify inflammation, driving M1 Mφs formation, which further increases androgen secretion and exacerbates metabolic abnormalities, impairing follicular maturation and leading to ovarian dysfunction.[66]

Our study found that as prognosis worsened, the total Mφs count decreased, diminishing the ability of Mφs to play an essential supportive role in follicular development. This alteration in the follicular microenvironment may impede folliculogenesis, thereby contributing to a poor response to Gn. Concurrently, the proportions of M1 and M2 Mφs relative to total Mφs population progressively increased, a pattern resembling that observed in ovarian EM. This shift likely reflects persistent chronic inflammation within the ovary associated with ageing and declining ovarian function, signalling an imbalance in Mφs homeostasis and a compensatory response by the immune system to ovarian senescence. Although further evidence is needed to substantiate this hypothesis, our findings suggest that the reduced response to COH in patients with POR may be closely linked to abnormalities in the proportion and polarization of Mφs within the follicular immune microenvironment. This insight offers a new perspective research on POR and underscores the potential of Mφs modulation as a future therapeutic strategy in future interventions.

T Cell Dynamics and Follicular Health

In ART patients undergoing ovarian stimulation, total T cell and CD8 T cell counts gradually declined with decreasing ovarian function and increasing age, while CD4 T cells progressively increased. CD4 T cells count was identified as a key independent factor influencing POR.

Compared with studies on Mφs, studies on T cells in ovarian FF and their effects on oocytes are limited. Some findings align with ours findings: for example, the proportion of T lymphocytes in the FF is significantly greater in patients with idiopathic infertility than in controls,[37] suggesting a role for T cells in folliculogenesis and oocyte maturation. Changes in T cell subtypes and cytokine profiles in the FF are linked to a diminished ovarian reserve, with patients with DOR showing higher CD8 T cell counts and an elevated CD8/CD4 ratio than controls.[30] CD8 T cells inhibit GCs proliferation and promote GCs apoptosis via the intrinsic apoptotic pathway,[68] possibly explaining why CD8 T cells are most abundant in POR patients with worst prognosis. In patients with PCOS who have ovulatory dysfunction, T cell imbalances, including a reduced percentage of CD8 T cells, lower CD69 and IFN-γ levels, and increased PD-1 expression,[26] can also observed in the FF. These findings suggest T cell dysfunction may play a role in PCOS pathogenesis.

These findings indicate that variations in T cell subset, through cytokines secretion and GCs interactions, may influence ovarian function, impairing follicle development and the response to COH. Studies have shown that in patients undergoing IVF and receiving COH, the CD8 T cell count in the FF changes, while the total T cell and CD4 T cell counts remain stable.[69] Since the CD4 T cell count was identified as an independent factor for POR in our study and Th/Tc ratios in peripheral blood have been linked to IVF outcomes,[21] CD4 T cells may serve as a peripheral marker for predicting prognosis. Further research into the role of T cells in the ovarian microenvironment could help identify potential therapeutic strategies for POR, including adjusting T cell ratios to improve ovarian function.

The Critical Role of DCs in the FF Warrants Further Attention

As key immune system components, DCs continuously survey peripheral tissues, capture and process antigens, migrate to lymphoid organs, and present antigens to T cells.[70–72] Classical DCs are divided into cDC1 and cDC2. Ultimately, cDC1 efficiently cross-present antigens to CD8 T cell, while cDC2 more efficiently stimulate CD4 T cell.[73,74] DCs are multifunctional, maintaining immune tolerance and inducing inflammation.[75] In the ovary, DCs support cumulus expansion, ovulation, luteinization, gonadotropin and progesterone production, and lymphangiogenesis, with their depletion potentially leading to ovulation issues, follicle rupture failure, and inadequate luteal formation.[76–78] In patients with ovulatory dysfunction, DCs from the peripheral blood show upregulation of proinflammatory genes and increased oxidative stress, which disrupt intercellular communication.[79] Therefore, dysregulated ovarian DCs could lead to ovarian dysfunction and infertility.[80]

In the ovarian microenvironment, the precise roles of the cDC1 and cDC2 subtypes remain unclear, and no prior studies have examined changes in cDC1 and cDC2 cells specifically in patients with POR. Our study is the first to identify these alterations, demonstrating that cDC1 and cDC2 counts were significantly elevated in the low prognosis group, particularly among the DOR group, with a substantial increase in the number of cDC2. Logistic regression analysis revealed that an elevated cDC2 count markedly increased the likelihood of POR, and an AUC greater than 0.7 suggests the potential of cDC2 as a diagnostic biomarker for POR. Further investigation is needed to assess whether DC count in the peripheral blood of patients with POR exhibit similar changes to those observed in the FF. Should these findings be consistent, cDC2 in the FF or peripheral blood could serve as a valuable prognostic indicator for infertile patients undergoing ART and may emerge as a therapeutic target to improve clinical outcomes.

Potential Clinical Implications

In cancer therapy, immune cell therapy represents an emerging treatment strategy, and for POR—an area lacking effective interventions—immune cell therapy may hold significant promise. With regard to Mφs, our findings indicate reduced Mφs infiltration in the follicular microenvironment of POR patients, alongside increased polarization towards M1 and M2 phenotypes. Potential strategies include enhancing Mφs recruitment to the ovary, preventing depletion of ovarian resident Mφs, reprogramming Mφs to augment their phagocytic capacity, or inhibiting Mφs polarization. Chemokine-related pathways such as CSF1/CSF1R[81] and CCL2/CCR2[82] have been shown to play crucial roles in Mφs recruitment, and blocking these pathways remains one of the most extensively studied methods for Mφs depletion. Phagocytic capacity could also be enhanced by either promoting “eat-me” signals through the use of opsonizing

antibodies or by attenuating "don't-eat-me" signals.[83,84] Moreover, studies on TGF-β and CD40 demonstrate that the direction of Mφs polarization is controllable.[85,86]

Regarding T cells, T cell therapies in oncology, such as the introduction of chimeric antigen receptors (CARs) into large numbers of peripheral autologous T cells, have achieved remarkable success in hematological malignancies.[87] For POR, the observed decrease in the CD4/CD8 ratio suggests that increasing this ratio may enhance ovarian responsiveness to COH. Specific approaches could be utilized, such as selecting certain T cell subsets for genetic modification and preparing them at defined CD4 ratios to yield consistent CAR-T cell products for clinical applications.[88] This could ensure reproducible in vivo efficacy and facilitate the identification of factors linked to either therapeutic success or toxicity. Additionally, CD4 T cells can be expanded ex vivo; for instance, a novel cryo-thermal therapy has been reported to induce CD4 T cell proliferation.[89]

In the case of DCs, we observed significant increases in cDC1 and cDC2 levels. DCs are primary inducers of immune responses, and targeting these cells may provide a novel approach to improving immune responses, particularly when T cell targeting alone is insufficient.[90] Since the presence of DCs within tumors is often associated with favorable prognoses, DC-targeted therapies have typically focused on enhancing DC function, increasing their numbers, or circumventing the tumor microenvironment to promote systemic de novo antitumor immunity.[91] In contrast, in POR, reducing the levels of cDC1 and cDC2 within the ovarian FF may improve ovarian function, providing a novel research direction. Further exploration of the roles and functions of cDCs in FF may reveal new opportunities for treating POR. Additionally, if elevated cDC2 levels are further validated in the peripheral blood of POR patients, cDC2 may emerge as a novel biomarker for predicting POR.

Limitation

The limitations of this study included a relatively small sample size, which may have reduced the statistical power and generalizability of the findings, and a single-centre design that could have introduced selection bias, limiting the applicability of the results to broader populations. The lack of longitudinal data restricted insights into the dynamic changes in immune cell populations over time and treatment progression, and the study's focus on a limited range of immune cell types (Mφs, T cells, DCs, and neutrophils) did not allow it to fully capture changes in complete ovarian immune microenvironment, potentially omitting alterations in influential cell types such as B cells and natural killer cells. Additionally, the use of FF from patients treated with ovulation-inducing drugs may have affected immune cell distribution and activity, thus, the result may not entirely reflect changes in immune profiles in a natural state. The selection of immune molecular markers also presented limitations, as it may have led to relatively coarse classification of various immune cell subtypes, potentially overlooking functionally distinct cell populations. Finally, while the study revealed an association between immune cell proportions and ovarian responsiveness, further investigation is needed to elucidate the molecular mechanisms underlying these findings and to confirm the potential clinical applications of immune modulation in improving ovarian function.

Conclusion

Overall, the FF contains many immune cells, and the proportions of these immune cells are closely related to the number of retrieved oocytes and the ovarian reserve. We observed a decrease in total Mφs infiltration in the follicular microenvironment of patients with POR, accompanied by increased polarization towards M1 and M2 phenotypes. T cell infiltration was also elevated, with a reduction in the CD4/CD8 ratio. Levels of cDC1 and cDC2 were significantly increased. Additionally, we identified that the total number of Mφs, CD4 T cell count, and cDC2 cell count are key factors influencing POR, with cDC2 showing potential as a biomarker for predicting POR. The immune balance in the FF is likely a critical determinant of follicle growth and development, suggesting that modulating the immune environment in patients with poor prognosis could represent a viable therapeutic strategy. Nevertheless, the potential efficacy of this approach in overcoming ovarian hyporesponsiveness remains largely unexplored and warrants further investigation.

Data Sharing Statement

No data regarding any of the subjects in the study have not been previously published unless specified. Data will be made available to the editors of the journal for review or query upon request.

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Disclosure

The authors declare that they have no competing interests.

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