Bleeding of the endometrium
Endometritis or functional?

Claire Bourgain
Forpath masterclass
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We know about

- Endometrial epithelium
- Endometrial stroma
  Proliferation, secretion, breakdown...
- Endometrial blood vessels

But what do we know about

- Endometrial leukocytes?
- Endometrial inflammation?
Normal endometrial leukocytes
Immune Cells in the Female Reproductive Tract

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Question

• In a normal condition, the endometrium contains scarce leukocytes?

• In the luteal phase, endometrial leukocytes may represent
  – Up to 25% of the total cell population
  – >40% of the total cell population?
Question

- In a normal condition, endometrial leukocytes are cycle-dependent and express hormone receptors.

- The basal and functional layer of the endometrium contain similar proportions of leukocytes.

- The largest population of endometrial inflammatory cells in the luteal phase are:
  - Regulatory T-cells
  - Macrophages
  - Plasma cells
  - NK-cells
Endometrial complexity

- Dynamic
- Layered
- Heterogenous
Distribution of endometrial leukocytes

• Basal aggregates
  – CD19 B cell core, T cells, macrophage halo
  – Proliferative phase 300-400 cells
  – Secretory phase 3000-4000 cells

• Scattered stromal cells
  – T cells (T8>T4)
  – B cells in very low number
  – CD56+CD16- NK cells
    • Low in proliferative phase
    • Increase to 30-40% of stromal cells in the luteal phase
  – Macrophages
  – DC cells
CD56+ cells

Blois et al. (2011); Lee et al. (2011)
Endometrial CD56+ cells

• uNK cells
• Specific phenotype CD56+CD16-
• Different from PB NK cells
• Low cytolytic activity
• High cytokine production
• 70% of lymphocytes at the feto-maternal interface
Schumacher et al, Front Immunol, 2014
NK and DC cell modulation

Blois et al, 2011
Menstrual cycle distribution of uterine natural killer cells is altered in menorrhagia.

*Article · Sep 2013 · Fertility and Sterility*
Endometrial leukocytes

• Innate part of endometrial tissue
• Drivers of important physiological and pathological functions
• Subject to cyclic variation
• Topographical differences
• Emerging data
• Guidelines for assessment are lacking!
Endometritis
Endometritis

Pregnancy-related
- Postpartum
- Post Cesarean section
- Puerperal sepsis
  - Enterococcus
  - Streptococcus
  - chlamydia

Unrelated to pregnancy
- Ascending infection
- IUD
- PID
  - Acute endometritis
  - Chronic endometritis
Endometritis

• Infectious
  – Acute or chronic
  – TBC, CMV

• Other
  – Sarcoidosis
  – Arteritis
  – Xanthogranulomatous
  – Mechanical
  – Carcinoma-associated
  – Immune-related?
Endometritis diagnosis

• One plasma cell is sufficient
• Requests presence of lymphocytes and plasma cells
• Requests presence of lymphocytes, plasma cells and neutrophils

• What is normal?
• What is pathological?
Acute endometritis

- Uncommon
  - Post-partum
  - Post-abortion
  - Chlamydia or Neisseria infection

- Excess of neutrophils
- Micro-abcesses
- Glandular destruction
- Outside menstrual period
Menstrual breakdown

- Necrotic predecidua
- Stromal neutrophils
- Stromal NK cells
- Glandular apoptosis

Report endometrium with menstrual breakdown
Criteria acute endometritis

• ≥5 neutrophils/ X400 HPF
• ≥ 1 plasma cell/ X120 LPF
• Intra-epithelial neutrophils only
Acute and chronic endometritis

• Spectrum towards chronic endometritis

• Classical features of acute endometritis
  • $\geq 2$ plasma cells/2HPF
  • Stromal changes
Post-menopauze-Exclude carcinoma
Chronic Endometritis

• Long-standing excess of inflammatory cells

Elusive diagnosis

• Which cells?
  – Plasma cells
    – One plasma cell?
    – Two plasma cells?
    – Plasma cells in at least 2 HPF?
  – 3 or more plasma cells?
Chronic endometritis

• Old diagnostic criteria
• < current knowledge on normal endometrial immune cell population
• < current knowledge on functional disturbances (infertility, RPL, endometriosis)
• Plasma cells?
• Need for updated guidelines
  – Immunohistochemistry?
Chronic endometritis

Intrinsic endometrial pathology

• Difficult to date endometrium
• Spindled stromal cells, edema, cellular density
• Presence of plasma cells in an appropriate context ≥ 2-3/2HPF
  – CD 138
  – Increased B-cells in an lymphocytic infiltrate
• Beware: Plasma cells may be seen in any breakdown endometrium
Limitations of the criteria used to diagnose histologic endometritis in epidemiologic pelvic inflammatory disease research

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Chronic endometritis-problems

- Plasma cell misidentification
  - 25% of endometria with HE plasma cells are CD138 negative
  - In 30% of women without PID risk, plasma cells can be identified by IHC
- Plasma cells are not specific

- NO active search for plasma cells in an otherwise normal looking endometrium
Chronic endometritis conditions

• AUB 3-10%

• NO active search for plasma cells in an otherwise normal looking endometrium
• Lymphocytic infiltrate ≠ endometritis
Chronic endometritis

• Response to injury
  – Infection
  – Stagnation
  – (pre-)neoplastic
  – Idiopathic

• Association with
  – Myoma
  – Polyp
    • Micropolyp>macropolyp
Xanthogranulomatous endometritis
Actinomyces endometritis
Endometritis with retained POC
Endometrial leukocytes in fertility
CD56+ cells and reproduction

• uNK increased
  – in recurrent miscarriage
  – in recurrent implantation failure after IVF

• uNK count test in RM or RIF?

• Which therapy?
  – Prednisolone?

• NO standardized test to define ‘high’ uNK!

• Exponential change in late luteal phase
Endometrial CD56+ cells

Standardisation of uterine natural killer (uNK) cell measurements in the endometrium of women with recurrent reproductive failure

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Method

• Variability of cell counts

• Neutral buffered formalin, 24-48h
• 3800 stromal cells, closest to luminal edge
• Image J program
• No glandular or luminal epithelium
• % uNKcells/total stromal cells
Tissue collection
- Timing of sample: LH+7
- Mode of biopsy: pipelle sampler

Tissue fixation and processing
- Fixation: 10% neutral buffered formalin 24-48 hours, room temperature
- Processing: routine processing into paraffin wax, NOT xylene free

Tissue immunostaining and assessment
- Section thickness: 3μm, coated slides, ensure water bath is clean, stain within 1 month of cutting
- CD56 antibody: Leica Biosystems (NCL-CD56-504)
- Secondary/tertiary reagents: If hand staining then recommend use of Vectastain Elite ABC kit
- Detection system: DAB, light haematoxylin counterstain
- Controls: negative (at minimum omission of primary antibody, preferably non-immune serum); known positive tissue with every staining run

Quantification
- Capture of images: 10 x400 images; start with luminal edge in view and image every 2nd field of view; if necessary can count 4 images deep from luminal edge.
- Counting: One operator/centre. Use Image J cell counter plug in to count total number of stromal cells and immunopositive uNK cells. Ensure that whole cells are being counted and not just fragments of DAB staining i.e. must see associated nucleus
- Number of cells counted: count at least 3800 stromal cells
- Calculation of results: express as % of total stromal cells
Characterization of Uterine NK Cells in Women with Infertility or Recurrent Pregnancy Loss and Associated Endometriosis

Article · May 2014 · American Journal Of Reproductive Immunology
%CD56+ stroma cells

- uRPL
- UI
- Endometriosis
- Fertile

(b) Images: RPL, UI, Fertile
The graph illustrates the percentage of NKp46+ stroma cells across different conditions: uRPL, UI, Endometriosis, and Fertile. The y-axis represents the percentage of NKp46+ cells, ranging from 0 to 5.

- **uRPL** shows a higher percentage compared to the other groups.
- **UI** and **Endometriosis** have a moderate percentage.
- **Fertile** has the lowest percentage.

The p-value for the difference between uRPL and the other groups is 0.03*.

**Additional images**:
- **Goat IgG** shows sparse staining.
- **RPL** shows moderate staining with focal areas highlighted.
- **UI** shows more intense staining with defined areas highlighted.
- **Fertile** shows minimal staining with a few highlighted areas.
First do no harm: uterine natural killer (NK) cells in assisted reproduction

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ABSTRACT: Natural killer (NK) cells are a type of lymphocyte circulating in peripheral blood named because of their effector functions in killing target cells. Immune cells that share similar phenotypic characteristics but are poor killers populate the uterine lining at implantation and during early pregnancy when the placenta is established. The functions of these uterine NK (uNK) cells are essentially unknown but available data point to a role in regulating placenation in concert with other elements of the decidua and invading trophoblast cells. Despite the lack of scientific rationale and advice from clinical governing bodies, such as the Human Fertilisation and Embryology Authority, an increasing range of tests and therapies are still offered to women undergoing IVF or attending recurrent miscarriage clinics based on the myth that uterine NK cells need suppressing to prevent damage to the embryo. New treatments can be introduced at whim with subsequent demands for expensive trials to prove/disprove their efficacy. The evidence that targeting uNK or peripheral blood NK cells assists women with recurrent pregnancy failure is lacking. Healthcare professionals and patients should very carefully evaluate the practice of immunomodulation to enhance pregnancy outcome. A discussion on how to move towards stricter regulation of immunotherapy in non-hospital settings is now needed because it is clear that the potential risks and costs of these therapies outweigh any benefits.

Key words: uterine natural killer cells / assisted reproduction / miscarriage / immunotherapy / embryo
Chronic endometritis and fertility

- General infertile patients 4%
- IVF patients 15%
- RIF patients 14-42%
- ≥ 3 RPL patients 27-58%
- Endometriosis 52%
Indication for endometrial biopsy

- Rule out anatomic lesions
- Bleeding causes inflammation
- Inflammation causes bleeding
- Fertility assessment
Take home message

• Rule out myoma, polyp, hyperplasia, malignancy
• Criteria for acute and chronic endometritis
• Any breakdown can be associated with plasma cells
• Bleeding is associated with altered immune cell content
• Fertility assessment
  – Timing of the cycle is mandatory
  – Rule out POC retention
  – Endometriosis, RPL and RIF are associated with higher endometritis incidence- emerging evidence of an immune factor
  – Criteria for normal immune cell assessment are currently lacking
THANK YOU