Pathophysiology of endometriosis

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Disclosure: shareholder EndoSAT
Pathophysiology for surgeons

• What is endometriosis?

• Different presentations: 1 disease?

• Progressive and recurrent?

• What do we know?

• What is surgically useful?
The Endometrium

- Glands & stroma
  - during menstrual cycle = dating
  - Pregnancy -> decidualisation
- Functionalis and basalis
  - Different hormonal control
- Junctional zone & spiral arteries
The Endometriual function

- The most regenerative tissue
- Hormonal sensitivity
- Peristalsis
- Pregnancy
  - Invasion
  - Immunology
Endometriosis: Facts 1
different presentations – prevalences - histology

100% Retrograde menstruation

80% Subtle with remodeling

15% typical

10% cystic

1% deep

adeno myosis, mullerianosis, stromatosis, pockets,
## Symptoms vary with lesion

<table>
<thead>
<tr>
<th></th>
<th>Prevalence</th>
<th>Pain</th>
<th>Infertility</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subtle</strong></td>
<td>80%</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td><strong>Typical</strong></td>
<td>25%</td>
<td>in 50% +</td>
<td>?</td>
</tr>
<tr>
<td><strong>Cystic</strong></td>
<td>10%</td>
<td>in 80% +++</td>
<td>++++</td>
</tr>
<tr>
<td><strong>Deep</strong></td>
<td>2-3%</td>
<td>in 95% ++++</td>
<td>???</td>
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<tr>
<td><strong>Adenomyosis</strong></td>
<td></td>
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<tr>
<td><strong>Peritoneal pockets – Müllerianosis - Choristoma</strong></td>
<td></td>
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<tr>
<td><strong>Stromatosis</strong></td>
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</table>
**Pathophysiology: Sampson Theory**

**Sampson: retrograde menstruation**

- Viable cells in menstruation
- Retrograde menstruation
- Viable cells in PF
- Implantation potential
  - In humans, in primates, in nude mice, in vitro

**Metaplasia**
Prevalence of subtle lesions

Koninckx et al 1991

n= 1297, 918, 267
Microscopical endometriosis

• In normal peritoneum
  • 10-15%
  

• In lymph nodes
  • 15% in deep endo

• In bowel deep endo
Bowel resection: 10-20% positive margins


- 27% Roman Horace fertil Steril 2016

Bowel occult microscopic endometriosis in resection margins in deep colorectal endometriosis specimens has no impact on short-term postoperative outcomes

Horace Roman, MD PhD$^{1,2}$, Clotilde Hennetier, MD$^1$, Basma Darwish, MD$^1$, Alexandra Badescu, MD$^{1,3}$, Marie Csanyi, MD$^4$.

Moutaz Aziz, MD$^4$, Jean-Jacques Tuech, MD PhD$^5$, Carole Abo, MD$^1$
SHOULD WE REVISE THE DEFINITION OF ENDOMETRIOSIS?

ENDOMETRIOSIS SURGERY AND ERADICATION OF ALL ENDOMETRIAL CELLS AND STROMA

CONCLUSION

A tentative conclusion might be that the occurrence of small, macroscopically invisible, nests of endometrial glands and stroma is much more frequent than we thought on the peritoneum, in pelvic lymph nodes, and inside the bowel walls, at least in women with endometriosis. It is also strongly suggested that most of these endometrial cell nests do not constitute a clinical pathology or develop into more severe lesions, nor do they cause pain or infertility.

Microscopic endometriosis: impact on our understanding of the disease and its surgery
Philippe R. Koninckx, M.D., Ph.D.
Jacques Donnez, M.D., Ph.D.
Ivo Brosens, M.D., Ph.D.
Fertil steril, 2016, 105,305-6
SHOULD WE REVISE THE DEFINITION OF ENDOMETRIOSIS?

Clinical evidence strongly suggests that the mere presence of endometrial glands and stroma outside the uterus should no longer be considered to be a clinical pathology by definition. Unfortunately, we cannot distinguish between glands and stroma that have no clinical importance and may disappear spontaneously and those that will develop into endometriosis causing pain and infertility. Hopefully, immunohistochemistry and/or molecular biology one day will allow us to define specific activities or processes causing the endometriosis pathology and therefore to distinguish between normal and pathologic “endometrial-like tissue outside the uterus.”

ENDOMETRIOSIS SURGERY AND ERADICATION OF ALL ENDO METRIAL CELLS AND STROMA
What is endometriosis?

Glands and stroma outside the uterine cavity

- Always pathology?
  - No
  - We need another definition
Subtle lesions? Microscopic?

- White & red vesicles, flame like
  - More tetrograde menstruation?
  - Progressive: the large majority not
- Microscopic: no known associated pathology
  - Lymph nodes
  - Bowel resection
  - Pain & infertility
- For the surgeon:
  - not too aggressive
  - No wide perineum ablation
Pathophysiology: Theories

Sampson

- Viable cells in menstruation
- Retrograde menstruation
- Viable cells in PF

We see

We Imagine

Implantation potential
In humans, in primates, in nude mice, in vitro

Abdominal wall growth stops fibrosis
Evidence for progression?

- Subtle to typical?
  
  No evidence in the human in primates

- Typical to severe cystic deep?
  
  Circomstantial evidence of the contrary

  historical data in Leuven:
  typical in 1980; deep in the 90’s in primates: no evolution
A recurrent disease?

- Subtle: 100%
- Typical: 20%
- Cystic: 5%
- Deep: 1%
Prevalences and age

Fig 2 Prevalence (red) of subtle, typical and deep endometriosis in women with infertility (n=1297), pain (n=918) and infertility and pain (n=267). Subtle endometriosis decreases with age whereas typical cystic and deep endometriosis increase with age. The total prevalence (yellow) remains unchanged. (from 45)
**Clonality in endometriosis**

Genetic damage to single progenitor cell

- **Cystic ovarian endometriosis**  Yes
  - Jimbo et al (1997) *Am J Pathol* 150, 1173; 21 samples from 11 endometriomas; Marker = X-linked *HUMARA* gene
  - 21/21 samples monoclonal
  - Tamura et al (1998) *Lab Invest* 78, 213; 25 epithelial cells from 25 archival endometriomas; Controls = 25 matched ovarian stroma tissue; Marker = X-linked *PGK* gene
  - 10/25 samples informative (all 10 monoclonal)

- **Deep endometriosis**
Germline predisposition

• Familial clustering

• Twin studies: MZ >> DZ

• Heredity
  symptom onset age in non-twin sisters
  6-9x increased prevalence in 1st degree relatives

  15% prevalence in 1st degree relatives (using MRI)
Facts?

- Progression or end stage?
- Cystic and deep are clonal
- Wise to consider separately
- Associated with (typical)
  - Pain and infertility
  - Low grade inflammation in peritoneal fluid
  - Hereditary, age
  - ........food, ......fat
  - Cancer, nerve cells in endometrium
  - For surgeons: association is not causal
Pathophysiology of endometriosis

The theories
Sampson
Angiogenic-lymphogenic spread
Endometriotic disease theory

The modulators
peritoneal fluid
immunology
genetics
Evidence for progression?

• Subtle to typical?

• Typical to severe?

No evidence in the human in primates

Why progression in some women only?

Endometriosis is a genetic disease

• Hereditary
• Deep and cystic are clonal in origin

Total body radiation, Dioxin
The Endometriotic Disease Theory

Genetic mutation cause a cell to become tumorous

Köninckx P.R., Kennedy S., Barlow D.,
Gyn Obstet Invest 1999,47,1-10
The Endometriotic Disease Theory
Koninckx P.R., Kennedy S., Barlow D., Gyn Obstet Invest 1999, 47, 1-10

Endometriosis

Subtle lesions

Retrograde menstruation,
Remodeling,

Endometriotic disease

Deep

Genetic mutation favorised by heredity immunology volume environment

Cystic Ovarian Adhesions

Typical
Conclusion I

which cell?

endometriosis versus endometrium?
stem cells?
basalis or functionalis?
“cancer like” invasion & metastasis?

Environment

blood
peritoneal fluid
JZ ‘blocks’ invasion

altered immunology

endometrium
peritoneal cavity
systemic
Haematologic and lymphogenic spread

- Haematologic
  - Lung endometriosis
- Lymphatic
  - Deep endo
  - Umbilical?
Peritoneal fluid

• An ovarian exudate
  • Volume // with ovarian activity

Peritoneal fluid

- An ovarian exudate
  - Volume
    // with ovarian activity
  - steroid hormone concentrations always higher than in plasma
  - protein concentrations: 60% of plasma lower for larger molecules


LUF as a cofactor for Endometriosis

• Since
  • steroid hormone concentrations are low  Koninckx PR, De Moor P, and Brosens IA. (1980) Diagnosis of the luteinized unruptured follicle syndrome by steroid hormone assays on peritoneal fluid. Br. J. Obstet. Gynaecol. 87, 929-934

Peritoneal fluid in Endometriosis

- Low grade inflammation with more and activated macrophages

- Increase in chemotactic activity by 20kD protein
  

- Decreased by medical therapy
  

- In vitro secretion of a monocyte chemoattractant
  

- RANTES
  
Peritoneal fluid in Endometriosis

- More and activated Macrophages = secretion products
  - Bax+ macrophages  

- angiogenic activity
  - in vivo  
  - TGF β  
  - VEGF  

- Cytokines  
Peritoneal fluid in Endometriosis

• Others
  • The IGF system
  • Platelet activating factor and altered fibrinolytic system
  • Prostaglandins
• decreased NK cell activity
  • inhibition of activity
  • increased shedding of ICAM-1 by endometrial cells
  • high local concentrations of glycodelins


(Somigliana, Vigano, et al. 1996)
Decreased NK cell activity

• Decreased activity
  • In plasma & peritoneal fluid
  • More in more severe endo
  • Local shielding and glycodelins?
• The chicken or the egg
  • 4 mths after excision of deep endo
    CA125 decreased
    NK and endometrium resistance unchanged


potent suppressive activity in mixed lymphocyte cultures

- requires 18 h of contact
- min - max dose : 5-50µgr/ml
Angiogenetic factors

- Bioassay
- VEGF
- TGF

Like most benign tumors

Chicken allantoic membrane
Oosterlynck, Waer, Koninckx 1994

Koinckx P.R., Kennedy S., Barlow D., Gyn Obstet Invest 1999,47,1-10
Endometriosis as a benign tumour

A benign tumour means genetic predisposition and an insult

age radiation dioxin

progression

environment, immunology
A benign tumor

- loss in E-cadherin receptors in some foci of endometriosis  

- suppressing metalloproteinase secretion in vitro with progesterone or with a natural inhibitor, inhibits endometriosis formation  
Pollution and Endometriosis

- Dioxins and PCB’s
- Is Endometriosis increasing in the human?
- Is endometriosis linked to pollution?
  - Animal models
  - Human
Endometriosis in Rhesus Monkeys chronically exposed to dioxin

Endometriosis in Rhesus Monkeys

THE SHORTEST TIME BETWEEN IRRADIATION AND ENDOMETRIOSIS IS 6 YEARS
Dioxin Concentrations in women with endometriosis

Mayani A, Barel S, Soback S, Almagor M. Human Repr 1997, 12, 373-375

% of women with detectable dioxin in blood

- No: 1/35
- I+II: 3/24
- III+IV: 5/20

Dioxin concentration (part per trillion)

Stage of Endometriosis

- No
- I+II
- III+IV
Plasma Dioxin concentrations & risk


• Rhesus monkeys 25ppt 4 years (Bowman, 1989)
  • T1/2 : 180-780 days
  • adipose tissue concentration : 250-810 ppt
• Humans (Mocarelli, 1991)
  • T1/2 : 7 years
  • Seweso subjects : 2.000-35.000 ppt
• Area under time-concentration curve for 14 years
  • rhesus monkeys 388-1.400 (*1000)
  • humans Seweso 5.500-112.000 (*1000)
Dioxin and Radiation

- Dioxin binds to the DNA through specific receptor
  - Pseudo steroid
  - Direct DNA effect
  - Transmissible effect e.g., sperm up to third generation

- Radiation has a direct DNA effect
Genetic predisposition

- Loss of heterozygosity
- Germ cell predisposition
Loss of Heterozygocity

Germline Mutation = Heredity

First Hit

Second Hit

Where should we look for a first hit?

in the endometrium of women with & without endometriosis

Somatic Mutation
Endometrium in Endometriosis


Genetics of endometriosis

Which Gene?

Oxegene project

Stephen Kennedy

University of Oxford

Specific genes
Non hypothesis driven
Linkage analysis
Molecular genetic evidence

Genomewide Linkage Study in 1,176 Affected Sister Pair Families Identifies a Significant Susceptibility Locus for Endometriosis on Chromosome 10q26

Susan A. Treloar,1,4 Jacqueline Wicks,1,4 Dale R. Nyholt,1,4 Grant W. Montgomery,1,4 Melanie Bahlo,1,2 Vicki Smith,6 Gary Dawson,6 Ian J. Mackay,6 Daniel E. Weeks,7 Simon T. Bennett,6 Alisoun Carey,6 Kelly R. Ewen-White,3 David L. Duffy,1,4 Daniel T. O’Connor,5 David H. Barlow,8 Nicholas G. Martin,1,4 and Stephen H. Kennedy8

1Cooperative Research Centre for Discovery of Genes for Common Human Diseases, 2Walter and Eliza Hall Institute, and 3Australian Genome Research Facility, Melbourne; 4Queensland Institute of Medical Research and 5Queensland Endometriosis Research Institute, Brisbane, Australia; 6Oxagen, Abingdon, United Kingdom; 7Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh; and 8Nuffield Department of Obstetrics and Gynaecology, University of Oxford, Oxford, United Kingdom
Linkage analysis 2006

- Extremely expensive
- Probably 2 chromosomes identified
- Highly secretive for patent reasons
- Clinically irrelevant for the next 10 years
- Potentially very important
For the surgeon: Cancer Stem cells – progression - endometriosis ??
Ultramicro-trauma in the endometrial-myometrial junctional zone and pale cell migration in adenomyosis

Fertility and Sterility® Vol. 104, No. 6, December 2015

Mohamed G. Ibrahim, M.Sc., a Vito Chiantera, M.D., a Sergio Frangini, M.D., a Shadi Younes, M.Sc., a Christhardt Köhler, M.D., b Eliane T. Taube, M.D., c Johanna Plendl, M.D., d and Sylvia Mechsner, M.D. a

Conclusion(s): The myofiber disarray in the inner myometrium, and the nuclear membrane irregularities in adenomyosis, are evidence for ultramicro-trauma in adenomyosis. The migrating nonleukocytic pale cells may be involved.

Van Gieson staining: Collagen fibers stain red; cytoplasm stains brown. (A) The inner myometrium in a nonadenomyosis patient. The smooth muscle fibers are parallel to the basal endometrial glands. (B) The endometrial-myometrial interface in adenomyosis: The basal endometrium dips down (circle) into the inner myometrium, disrupting the regular interface (magnification outside of inset: ×100). (C) The inner myometrium in adenomyosis. The smooth muscle fibers are arranged in diverse directions. Magnifications are ×200, unless otherwise noted.

Immune-expression of (A, B) CD45 and (D, E) CD68 in the basal endometrium in adenomyosis. The glandular epithelial cells could not show any positive staining. (C and F) Positive controls: (C) CD45 positive immune cells in spleen and (F) CD68 positive macrophages among intestinal crypts. (A and D) Dapi stain. (G and H) E-cadherin immune-expression in the basal glands at the EMIZ in adenomyosis. (G) magnification: x200; (H) x400. (I) Diagrammatic illustration of the pale cells’ role in the common pathogenesis of endometriosis and adenomyosis. Because they are located eccentrically in the basal endometrial glands, the pale cells can migrate into the myometrium (lower section), where they develop into adenomyotic lesions. Those in close contact with the glandular lumen (concentric position) can migrate through the uterine cavity into the peritoneal cavity, where they develop into peritoneal endometriosis.
Epidemiology of Subtle, typical, cystic and deep endometriosis: a systematic review

Philippe R. Koninckx *,§
Anastasia Ussia §,©
Jörg Keckstein §
Arnaud Wattiez #
Leila Adamyan &

Acknowledged: Jacquez Donnez, Camran Nezhat, Charles Koh, Antonio Setubal

Deep Endometriosis surgeons > 20 Years

Gynaecological surgery, in press
Conclusions

• Typical, cystic, and deep: different endpoints

• Impression that deep is progressing
Endometriosis 2015

Tissue
proliferative
Invasive
Immunologic

Genomic
incident

Peritoneal
cavity

3 or more diseases
rarely  proliferative
Non recurrent

Definition has to be changed
No animal model - as for placentation

Koninckx PR  Ussia A
Moscow 2015
For the surgeon?

- excise
For the surgeon?

- If no adhesions: Cystic corpus luteum
- Excise / coagulate small
- Pathology
  - Glands and stroma are always found
For the surgeon?

- Every day more arguments not to be too aggressive
  - Leave a rim of fibrosis
  - Small resections

- Some are different
For the surgeon: the literature

- **Research: Significance**
  - Increases with N
  - Does not permit a conclusion of the population

- **Clinically useful**
  - Outcome
  - Accidents
  - Sensitivity/specificity for population

Length of women and men

170          180  cm

170            180  cm

Increases with N

Does not permit a conclusion of the population