Why did all biomarker identification studies fail in endometriosis?

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Diagnosis of endometriosis

• Current gold standard:
  – Laparoscopic visualization of endometriotic lesions, followed by histological confirmation

• Advantage
  – Can remove the lesion after seeing it
Downside

- Invasive
- Carries risks of morbidity and, very rarely, mortality
- Expensive
- The accuracy depends on the experience and skill levels of the surgeon
- Not suitable for screening purpose
- Incidence estimate difficult
- Makes the monitoring of treatment difficult
- Problem in determining recurrence

\[\text{Dx delay}\]
The need for non-invasive biomarkers

• Non-invasive
• Typically much less expensive than laparoscopy
• Shorten or eliminate the diagnostic delay
Applications of biomarkers

• Diagnosis
• Prognosis
  – Fertility outcome
  – Recurrence risk
• Screening
• Patient stratification
  – Precision medicine
• Monitoring the response to treatment
• Course of the disease
Types of biomarkers

• **Sources**
  – Peripheral blood
    • Serum, plasma
  – Urine
  – Saliva
  – Endometrial fluid
  – Menstrual debris
  – Peritoneal fluid

• **Purpose**
  – Diagnosis
  – Prognosis

• **Variety**
  – miRNA
  – DNA
  – RNA
  – Cytokines
  – Chemokines
  – Hormones
  – ...

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Current status

• A hot topic for extensive reviews
  – May et al. HRU 2010
  – May et al. HRU 2011
  – Nisenblat et al. Cochrane Database Syst Rev 2016a
  – Nisenblat et al. Cochrane Database Syst Rev 2016b
  – Agrawal et al. Mol Sci 2018

• Consensus: Not a single biomarker has been clinically validated
What went wrong?
Failure is the mother of success.

-- A Chinese Proverb
Failure is infinitely more educational than success.
Challenges

• Heterogeneity
  – Different subtypes of endometriosis
  – Age
    • Premenarcheal
    • Cyclic
    • Postmenopausal
  – Variation in symptomology
    • Pain
      – Different kind of pain
    • Infertility
    • Both

• Co-morbidities
  – Adenomyosis
  – Uterine fibroids
  – Autoimmune diseases
  – Other chronic diseases

Ahn et al. F&S 2017
What have been done wrong?
A revisit of 70 studies on biomarkers

- These studies were evaluated by Nisenblat et al. 2016
- Published between 1986-2014
- Evaluated diagnostic performance of 47 blood biomarkers
- Total number of cases: 5,356
- Total number of controls: 3,470
- Highest sensitivity reported: 0.81
- Highest specificity reported: 0.87
Number of published biomarker studies 1986-2014
Cumulative # of published biomarker studies 1986-2014

Annual growth rate 13.1%
Distribution of #cases per study

Median = 56.5
Distribution of #controls per study

Median = 38
Clinical suspicion

Gate criteria

Index test

Laparoscopy

Endometriosis

No endometriosis

N=36 (51.4%)

Two-gate design

Clinical suspicion

Gate 1 criteria

Study group

Of cases

Index test

Sensitivity

N=34 (48.6%)

Gate 2 criteria

Study group

Of controls

Index test

Specificity
Indications for surgery

- Ovarian cysts
- Pain
- Infertility
- Unreported
- All the above
Combidities

- Unreported
- W/ fibroids
- W/ adenomyosis
- Exclusive
Index test vs. biomarkers

- Several (separately tested)
- Several (tested in combination)
- Mixed
- 1 biomarker
Biomarker novelty

- New test
- Replication (same)
- Replication (different)
- Unreported
Other features

- 23 of 70 (32.9%) studies did not give any information on age for cases
- 22 of 70 (31.4%) did not give any info on age for controls.
- 67/70=95.7% used rASRM classification system, only 4.3% unreported
- 62/70 (88.6%) did not report whether the operator(s) was/were blinded or not
- 43/70=61.4% specified the threshold. 38.6% did not.
- 64/70=91.4% did not validate their results in an independent sample
- 65 of 70 (92.9%) studies did not retest
- 62/70=88.6% of studies had missing data that could affect the interpretation of the results.
- 64/70=91.4% of studies did not provide diagnostic criteria
An analysis of 41 studies published in 2015-2018

• These studies were published after the Ephect guidelines in 2014
  – 2015: 9
  – 2016: 15
  – 2017: 10
  – 2018 (up to now): 7

• Studies on biomarker for recurrence were excluded

• Total number of cases: 2,572
• Total number of controls: 1,839
Other features

- 4 of 41 (9.8%) studies did not give any information on age for cases or controls
- 16 of 41 (39.0%) used single-gate (cohort) design, and the rest used two-gate (case-control) design
- 6/41=14.6% did not do histological confirmation, and 2 (5%) unreported
- 36/41=(12.2%) did not report whether the operator(s) was/were blinded or not
- Only 1/41=2.4% specified the threshold. 97.6% did not.
- 64/70=91.4% did not validate their results in an independent sample
- 39 of 41 (95.1%) studies did not retest
- 11/41=26.8% of studies did not report if there are missing data
Geographic distribution

Asia
Australia
Europe
North America
South America
Co-occurrence with adenomyosis

Unreported

Included

Excluded

Co-occurance with adenomyosis
Co-occurrence with uterine fibroids

- Unreported
- Excluded
- Included

Co-occurrence with uterine fibroids
Co-occurrence with other comorbidities

- Unreported
- Excluded
- Included
# of cases per study

![Histogram showing the distribution of cases per study with a median of 41.](image)

- Median: 41
# of controls per study

Frequency

0 50 100 150 200 250 300 350
0 5 10 15 20
median=30
Summary

• Typically small or moderate sample sizes
  – Not able to cover all stages
  – Impossible to cover all combinations of important factors: age, subtype, menstrual phase, comorbidity, symptomology
• Lacks detail
• Susceptible to biases
• No validation/retest
• No proof that the marker is lesion-specific
What should we consider?

• Where does the biomarker come from?
  – Any in vivo data?
  – Is the change
    • Subtype-dependent
    • progression-dependent?
    • Symptomology dependent?
  – Specific to endometriosis?

• How can we eliminate the “Humburg effect”?

• Can we have preclinical evidence first?
Basic requirements

• Large sample sizes
  – to account for variations in age, menstrual phase, rASRM stage, subtypes, symptomology and severity, and common co-morbidity

• Endometriosis-specific
  – Once all visible lesions are removed, there is a change; Retest
  – Correlation with lesional histology
    • Stage of progression
  – Support from in vivo data
Basic requirements

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• Validation

• Harmonization all protocols for phenotyping, biobanking, and experimentation
Conclusions

• The biomarker studies published during the 1986-2018 period overall have low quality
• Most of them can easily succumb to various biases
• Most of them are difficult to be generalized to more general population/subtypes
• The statistical power is typically low
• To maximize our chance in identifying biomarkers in the future, there are certain requirements need to be fulfilled
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