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

Dx delay

- 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

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
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  - Fassbender et al. Biomed Res Int 2015
  - Liu et al. Cochrane Database Syst Rev 2015
  - Gupta et al. Cochrane Database Syst Rev 2016
  - 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

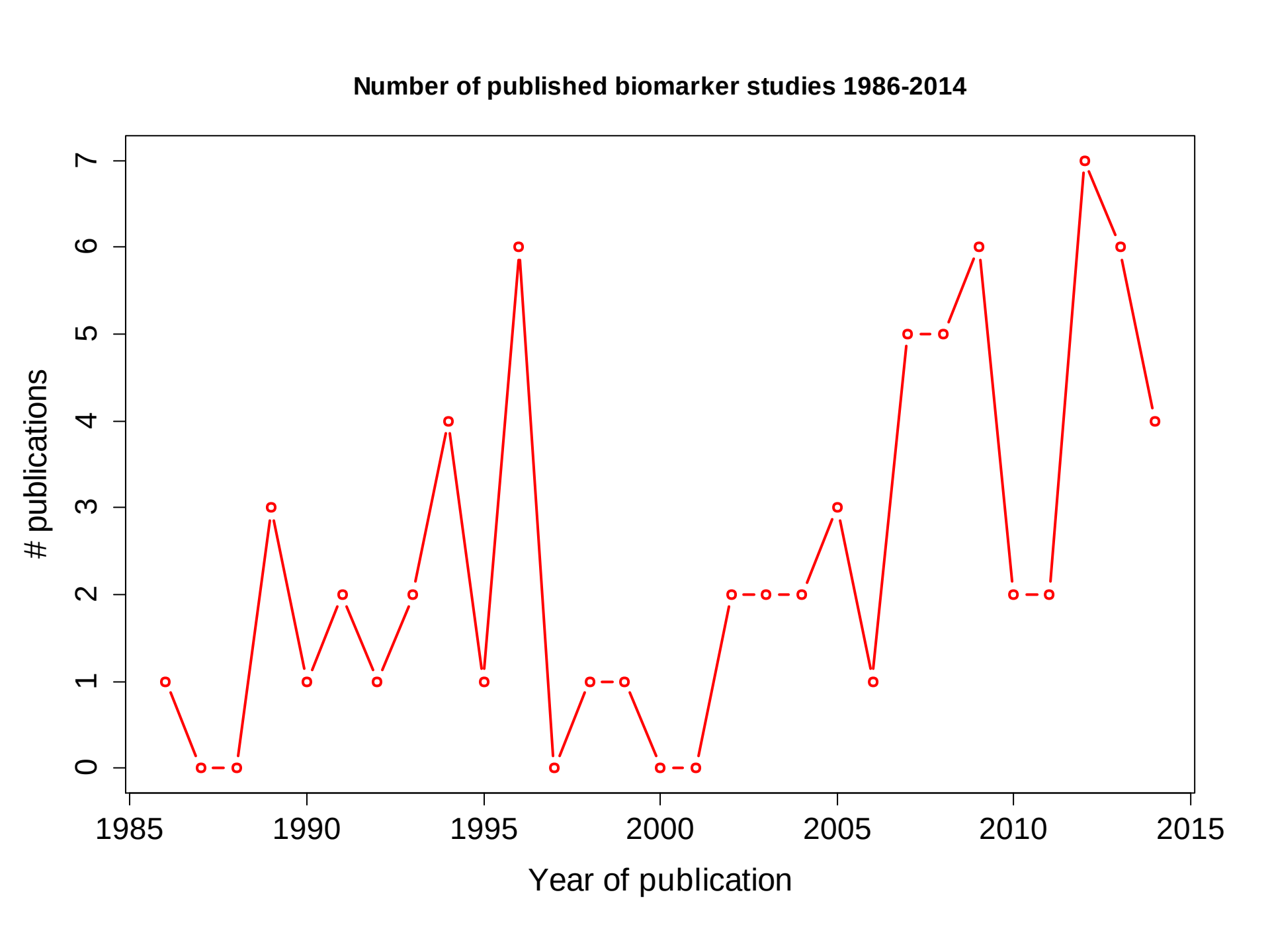
- Heterogeneity
  - Different subtypes of endometriosis
  - Age
    - Premenarcheal
    - Cyclic
    - Postmenopausal
  - Variation in symptomology
    - Pain
      - Different kind of pain
    - Infertility
    - Both
- Co-morbidities
  - Adenomyosis
  - Uterine fibroids
  - Autoimmune diseases
  - Other chronic diseases

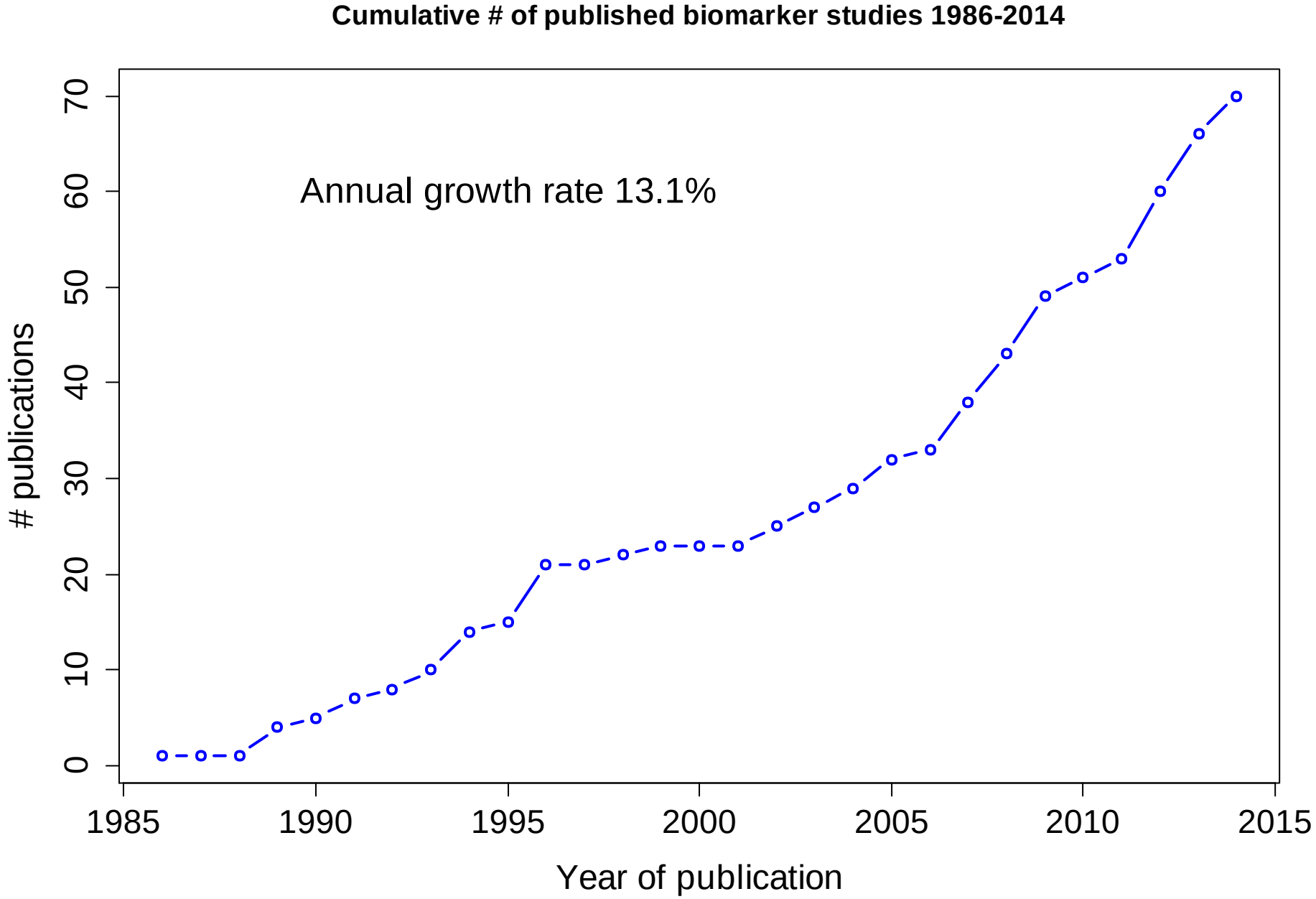
Ahn et al. F&S 2017

What have been done wrong?

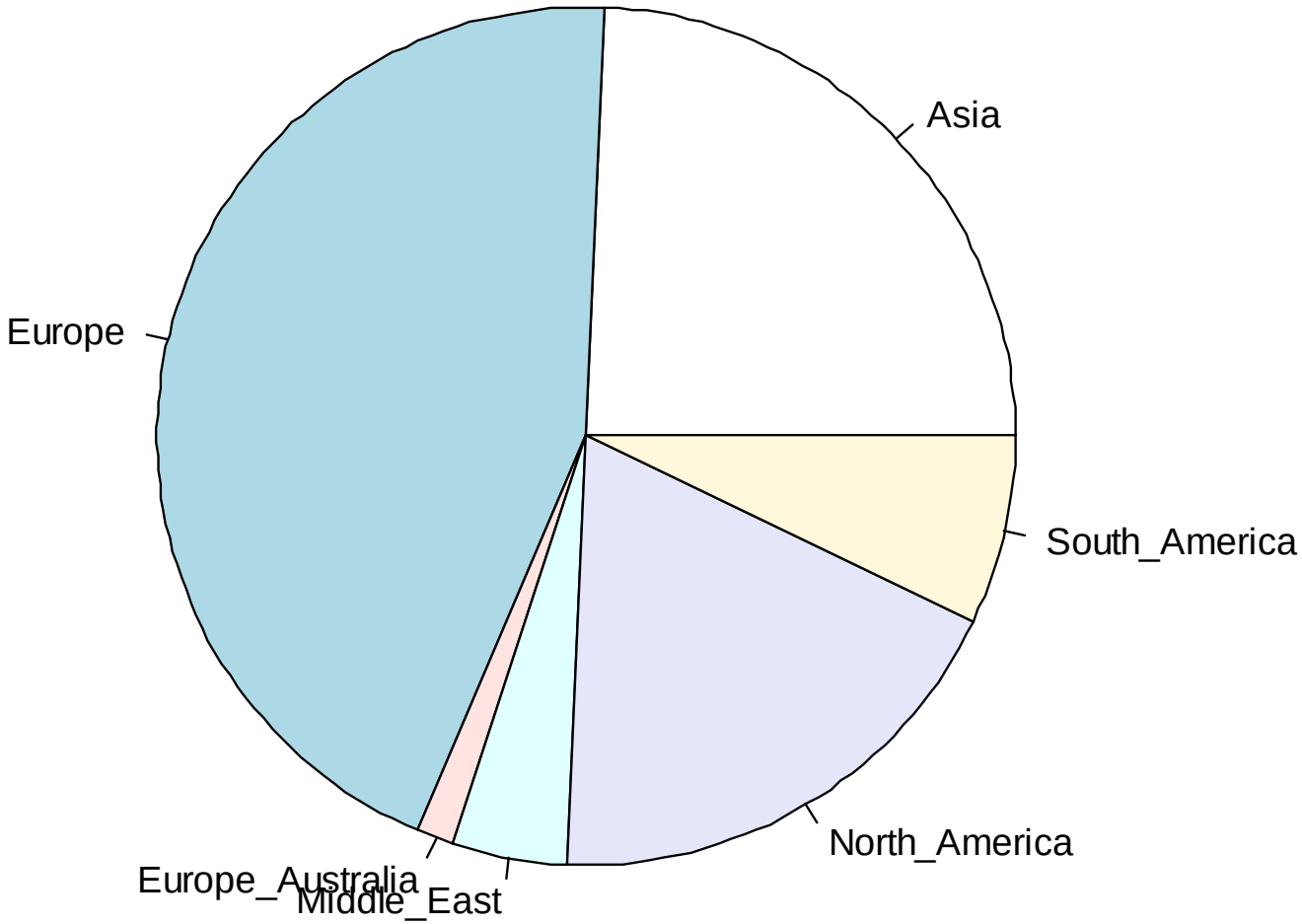
A revisit of 70 studies on biomarkers

- 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

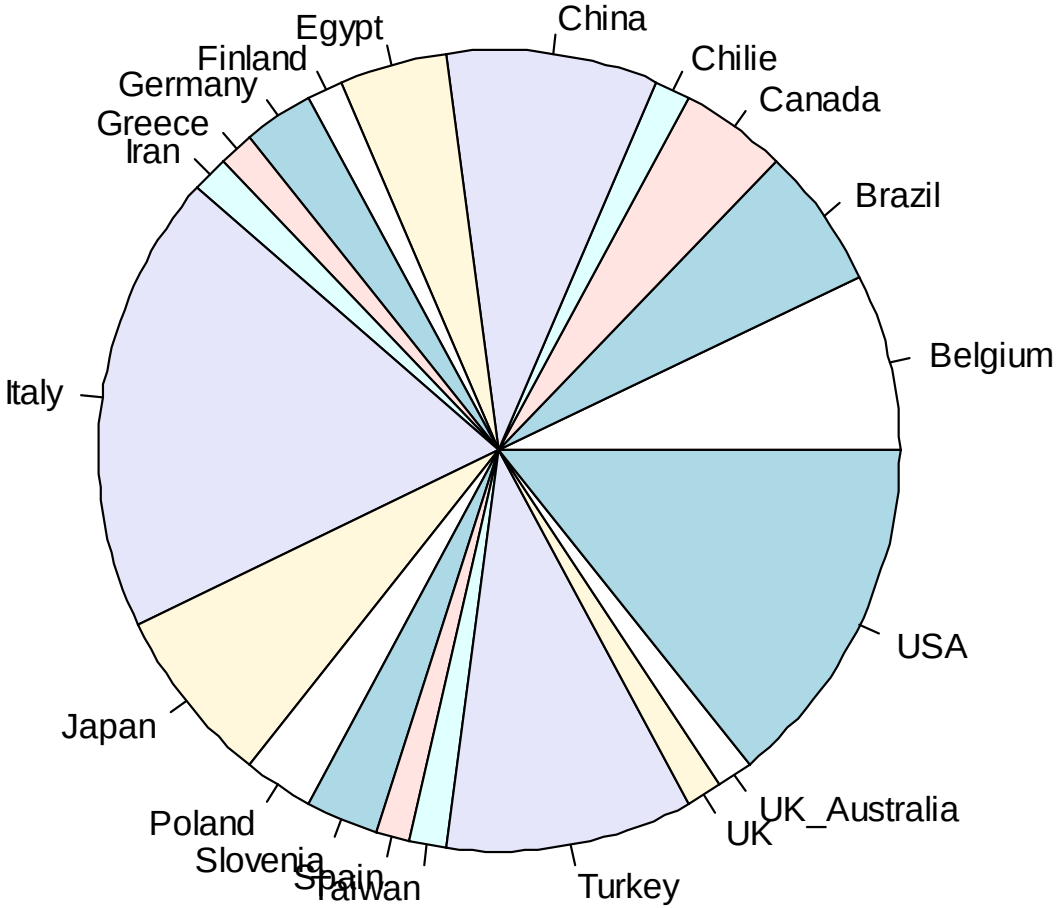

Number of published biomarker studies 1986-2014
publications
0
1
2
3
4
5
6
7
1985
1990
1995
2000
2005
2010
2015
Year of publication

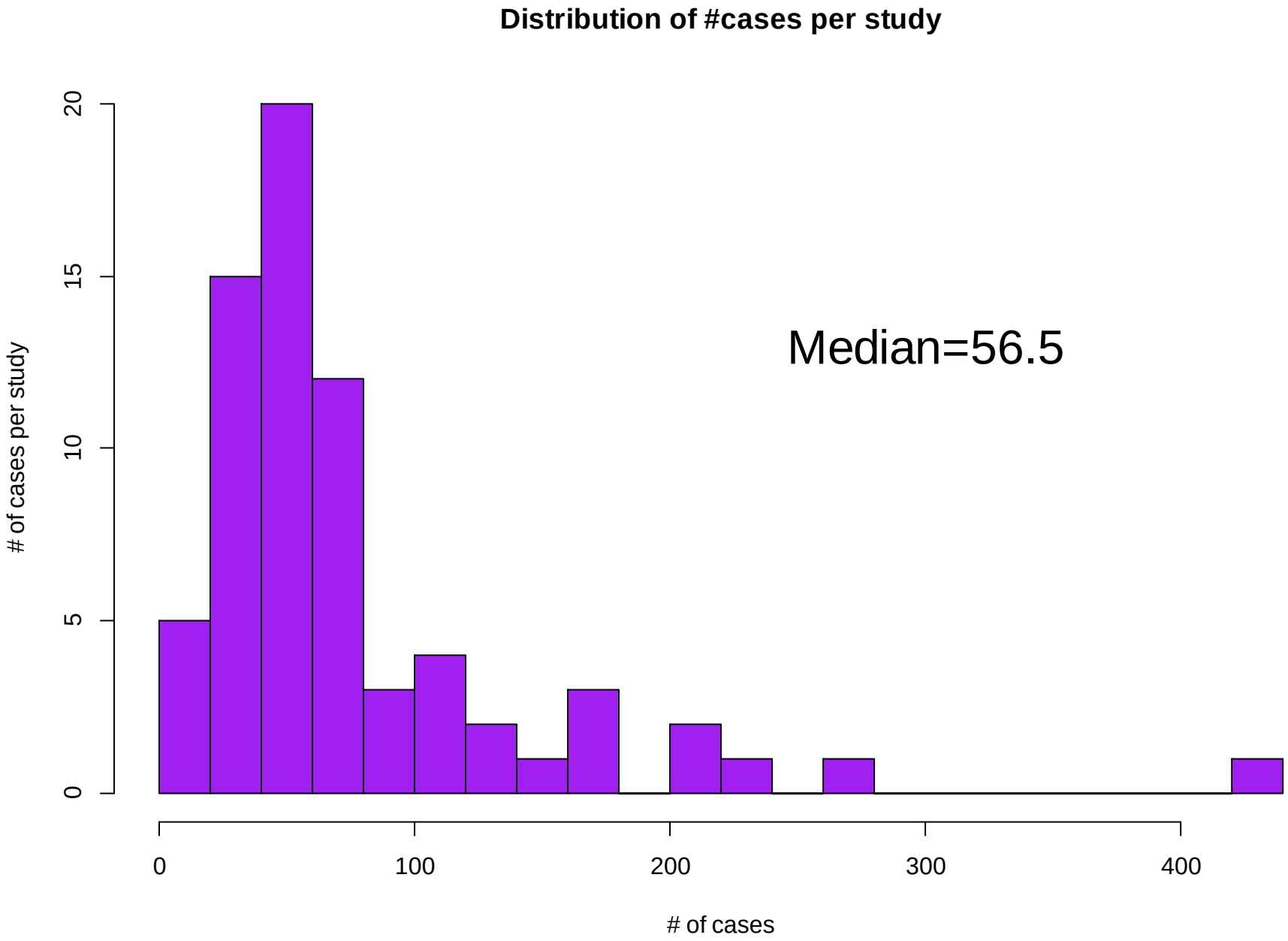

Cumulative # of published biomarker studies 1986-2014
Annual growth rate 13.1%
publications
0
10
20
30
40
50
60
70
1985
1990
1995
2000
2005
2010
2015
Year of publication

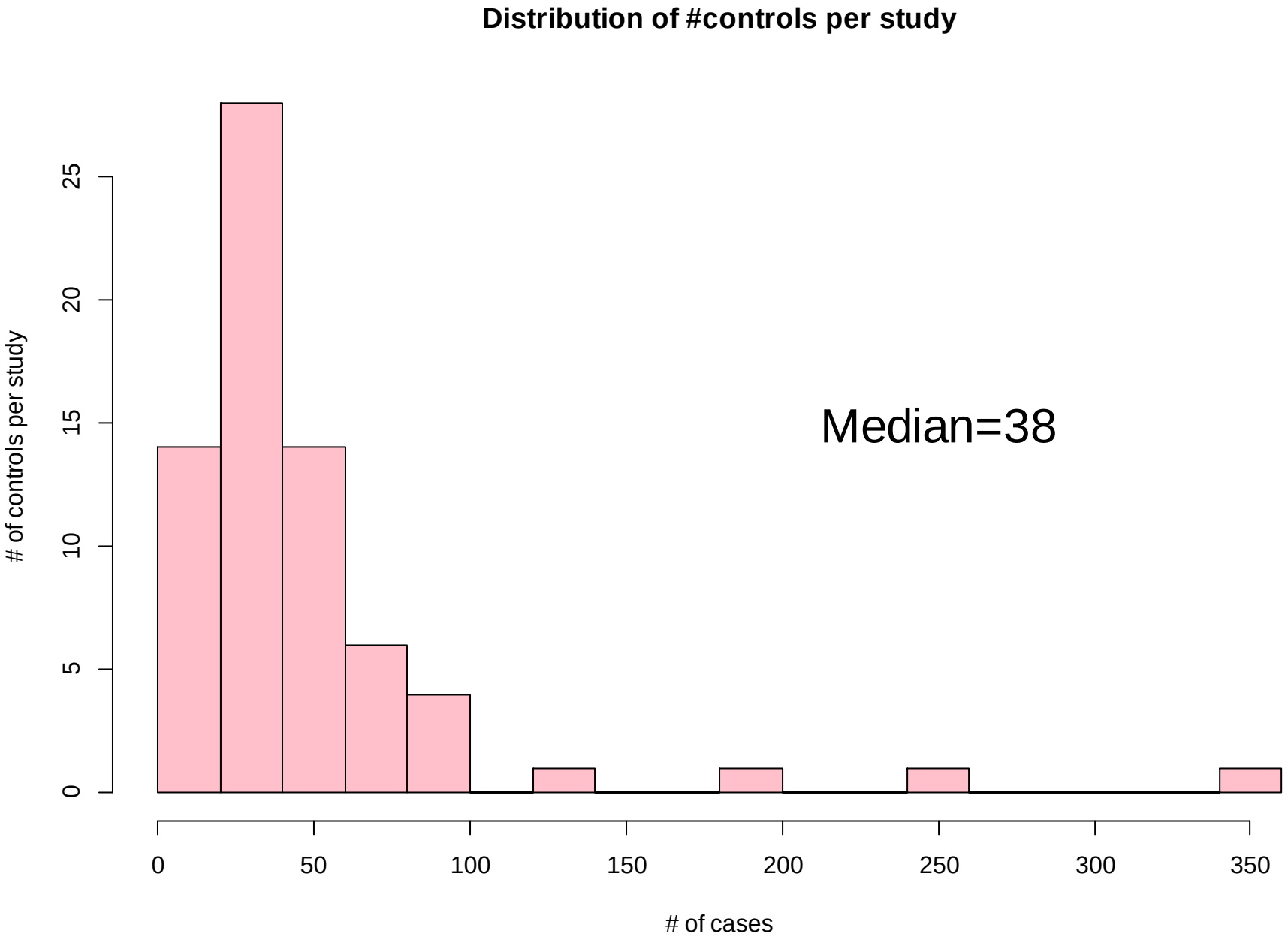
Number of publications by continent



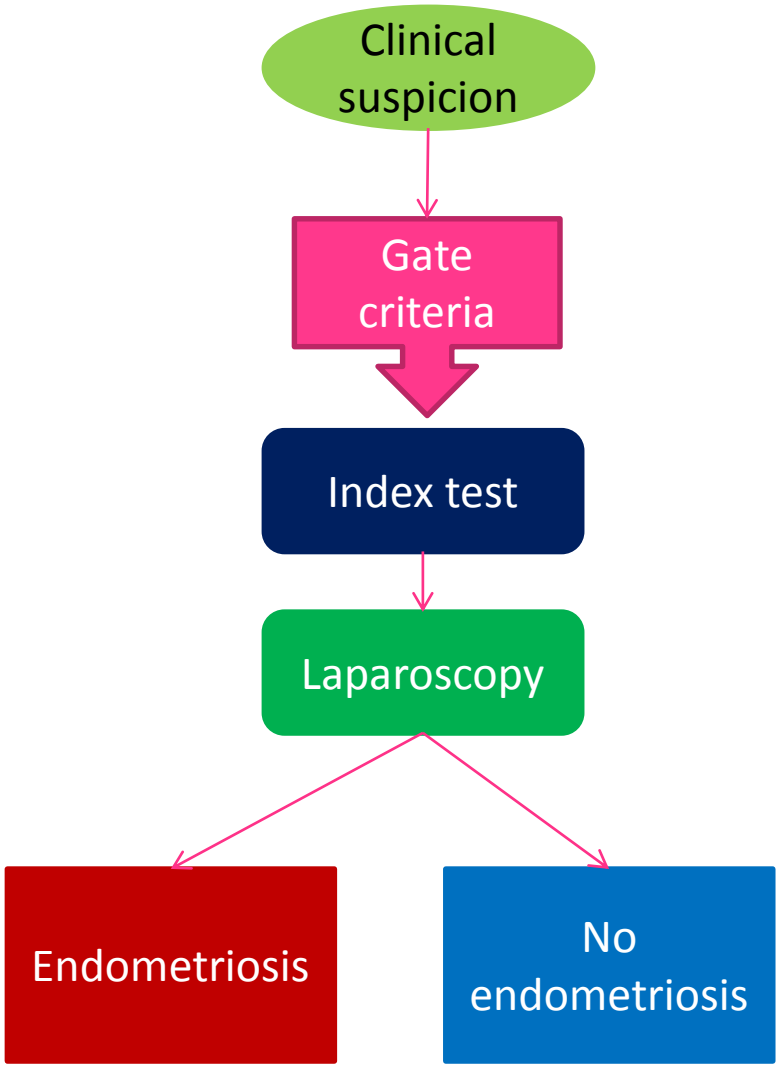
Number of publications by country




Distribution of #cases per study
# of cases per study
0
5
10
15
20
Median=56.5
0
100
200
300
400
# of cases

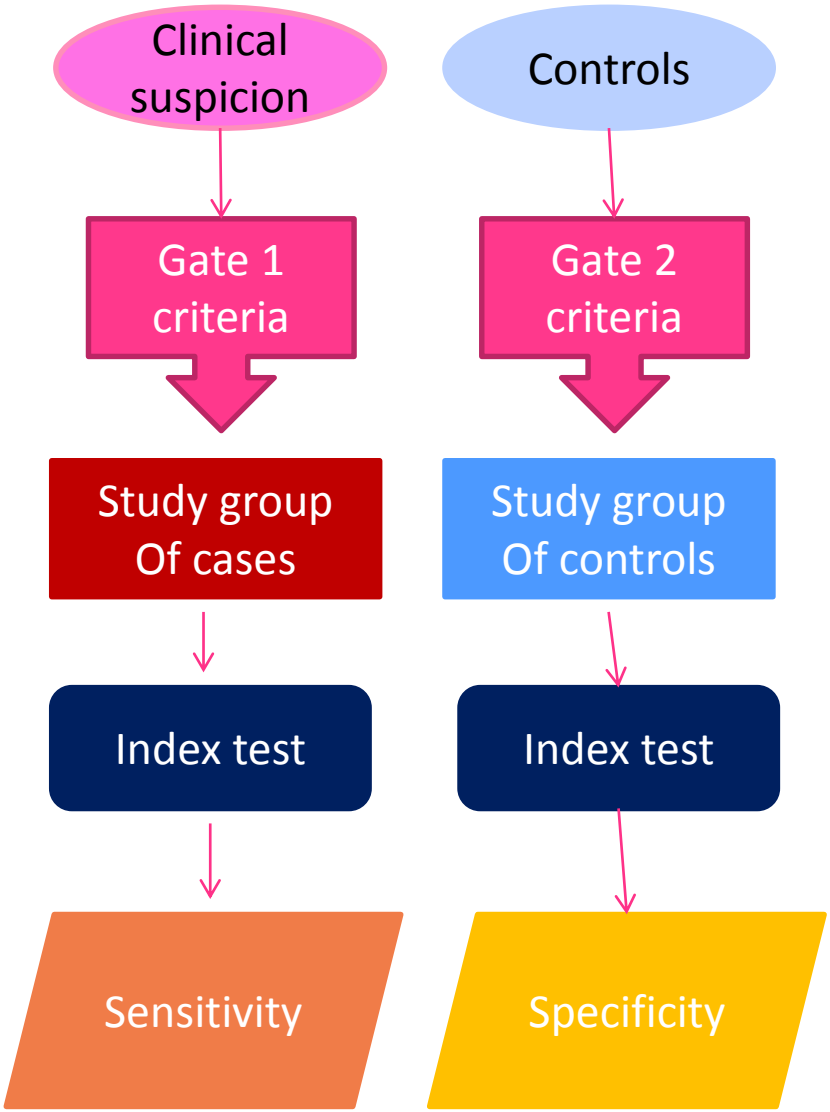

Distribution of #controls per study
# of controls per study
0
5
10
15
20
25
Median=38
0
50
100
150
200
250
300
350
# of cases

Single-gate design



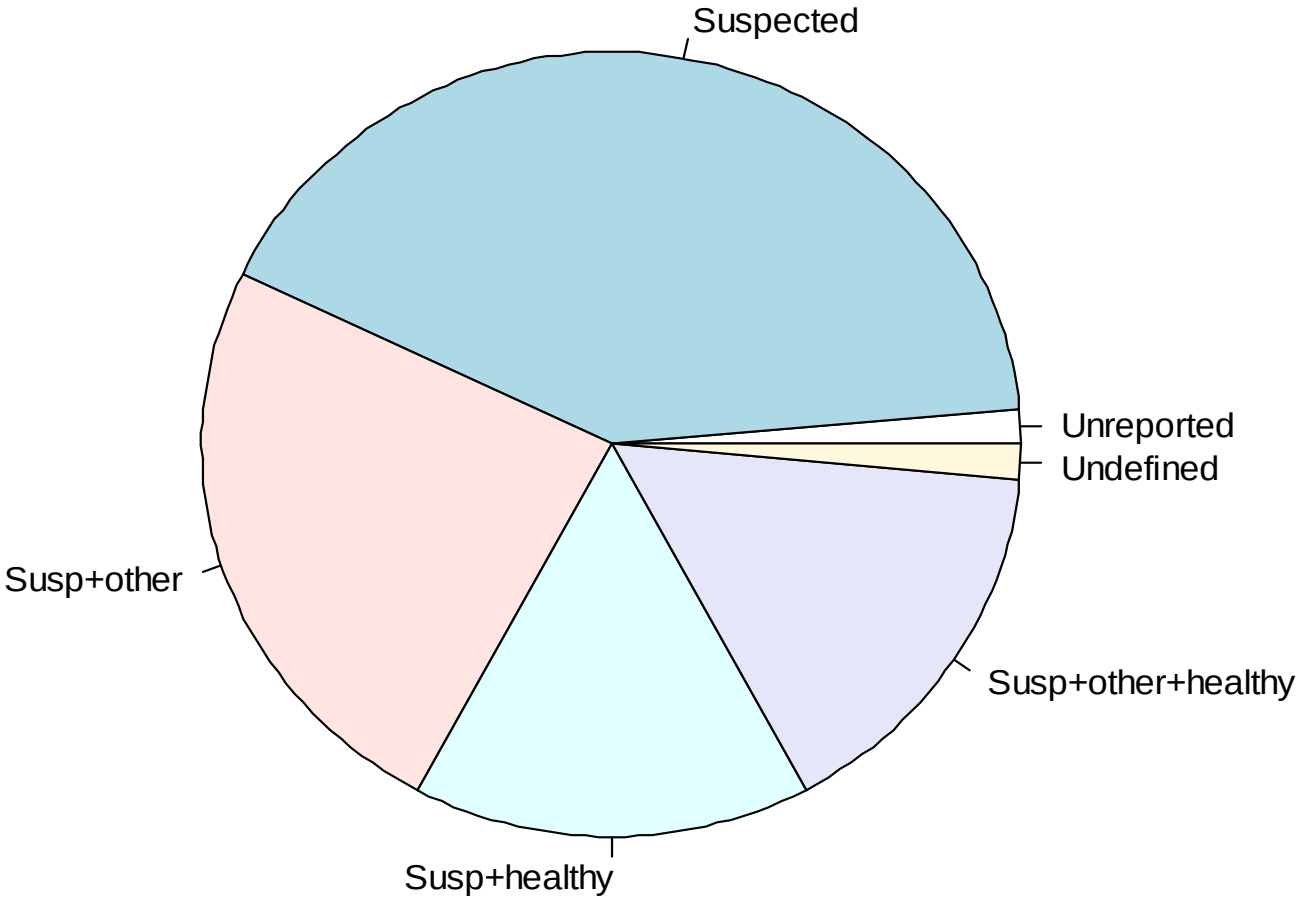
N=36 (51.4%)

Two-gate design

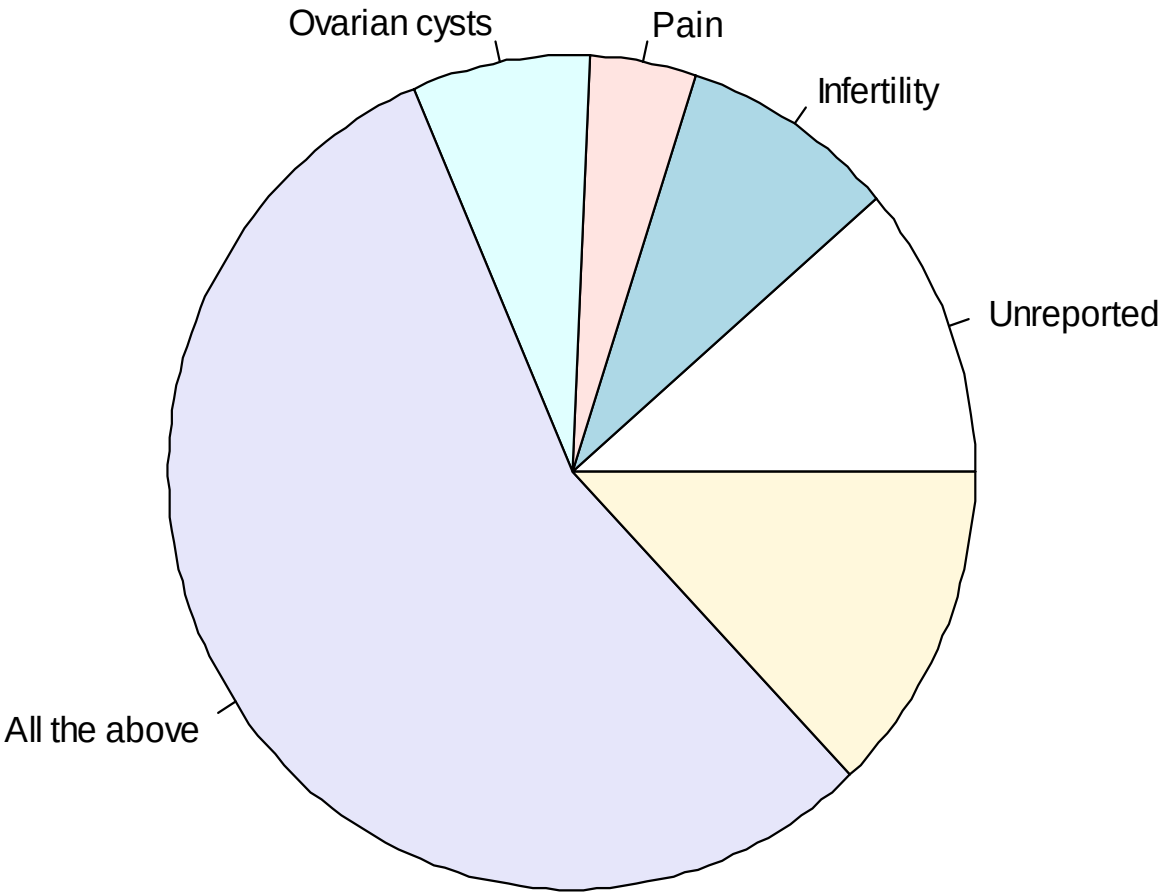


N=34 (48.6%)

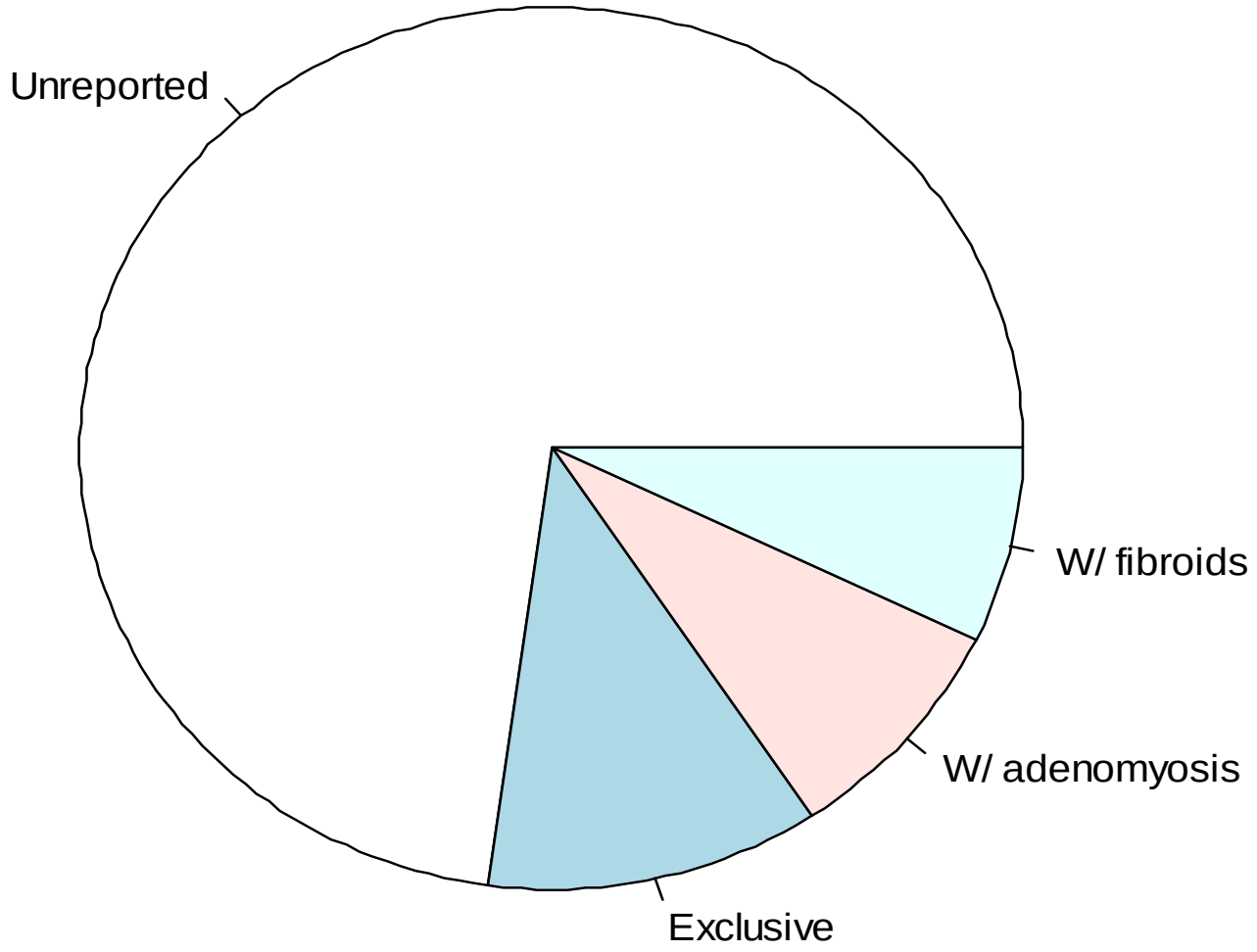
Population sampled



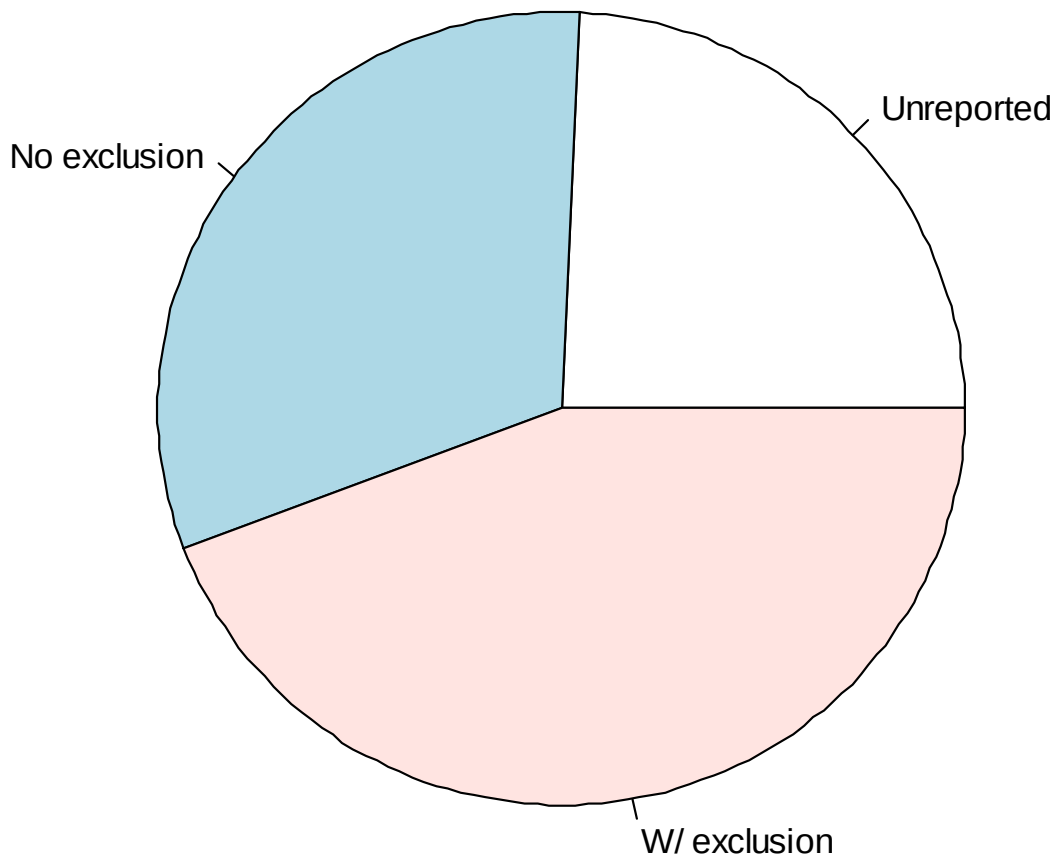
Indications for surgery

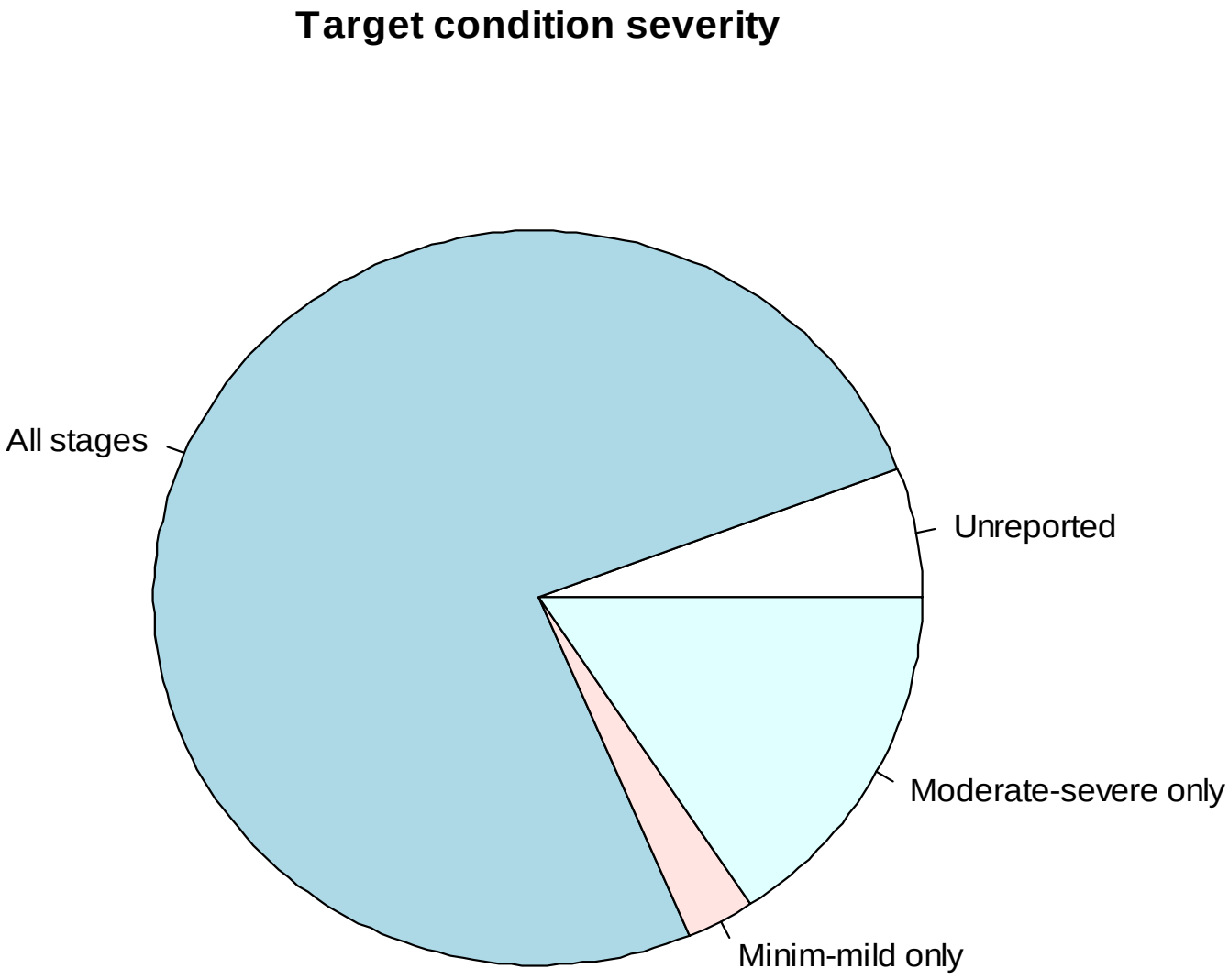


Combidities

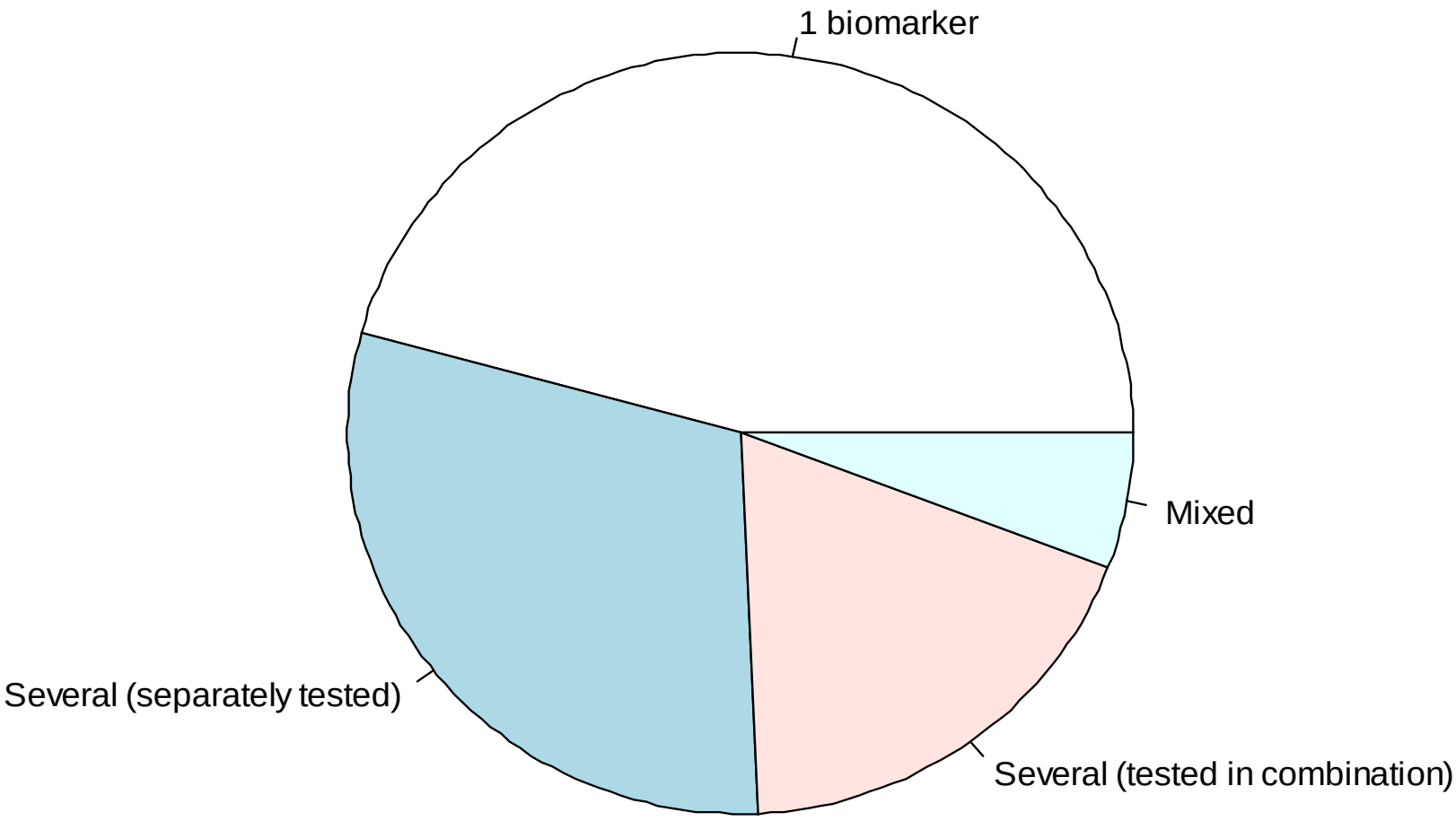


Exclusions

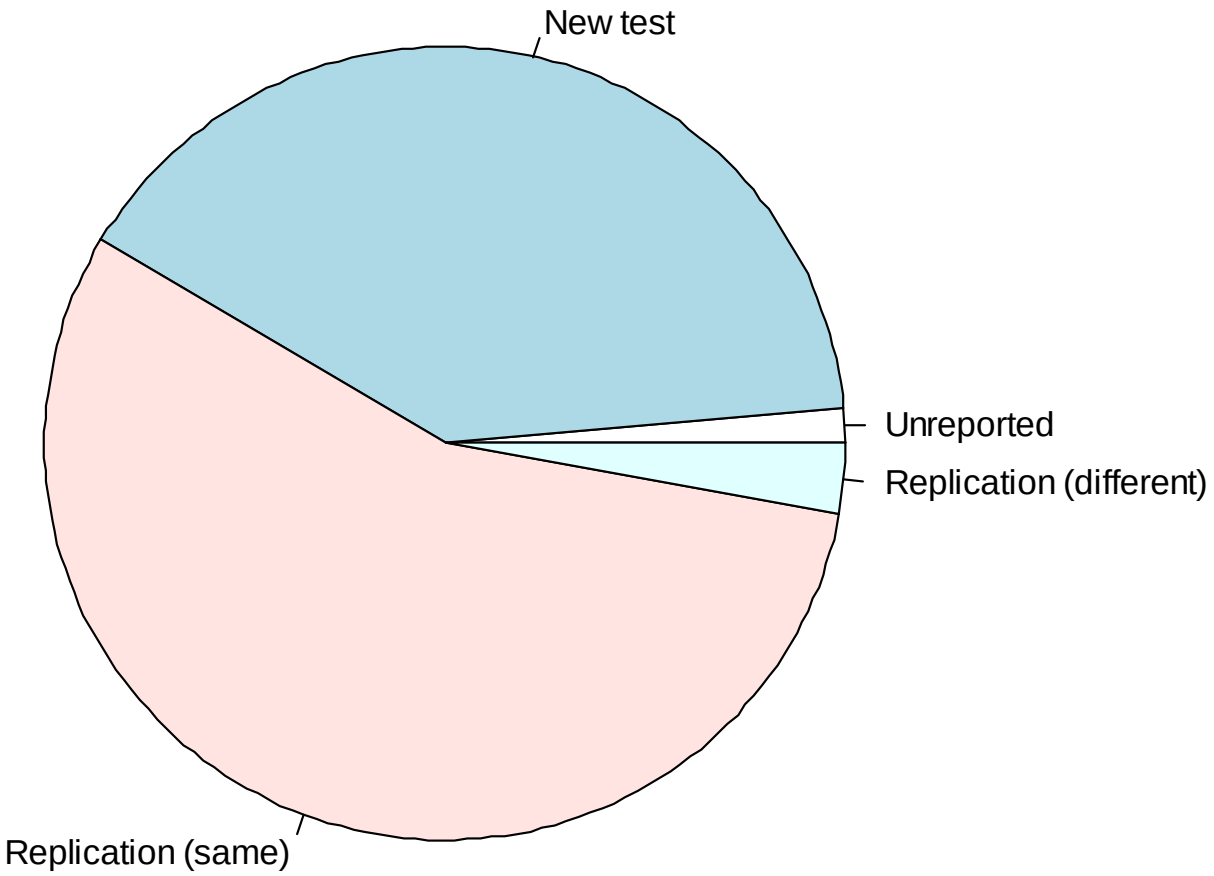



Target condition severity
All stages
Unreported
Moderate-severe only
Minim-mild only

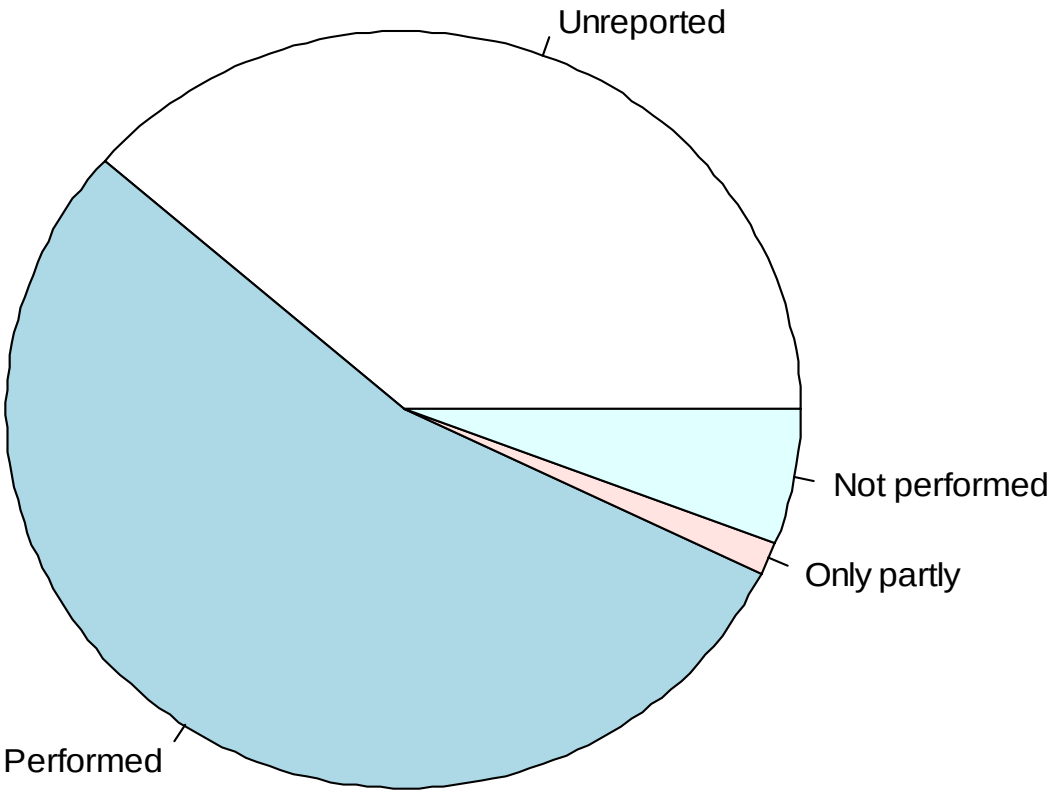
Index test vs. biomarkers



Biomarker novelty



Reference histology



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

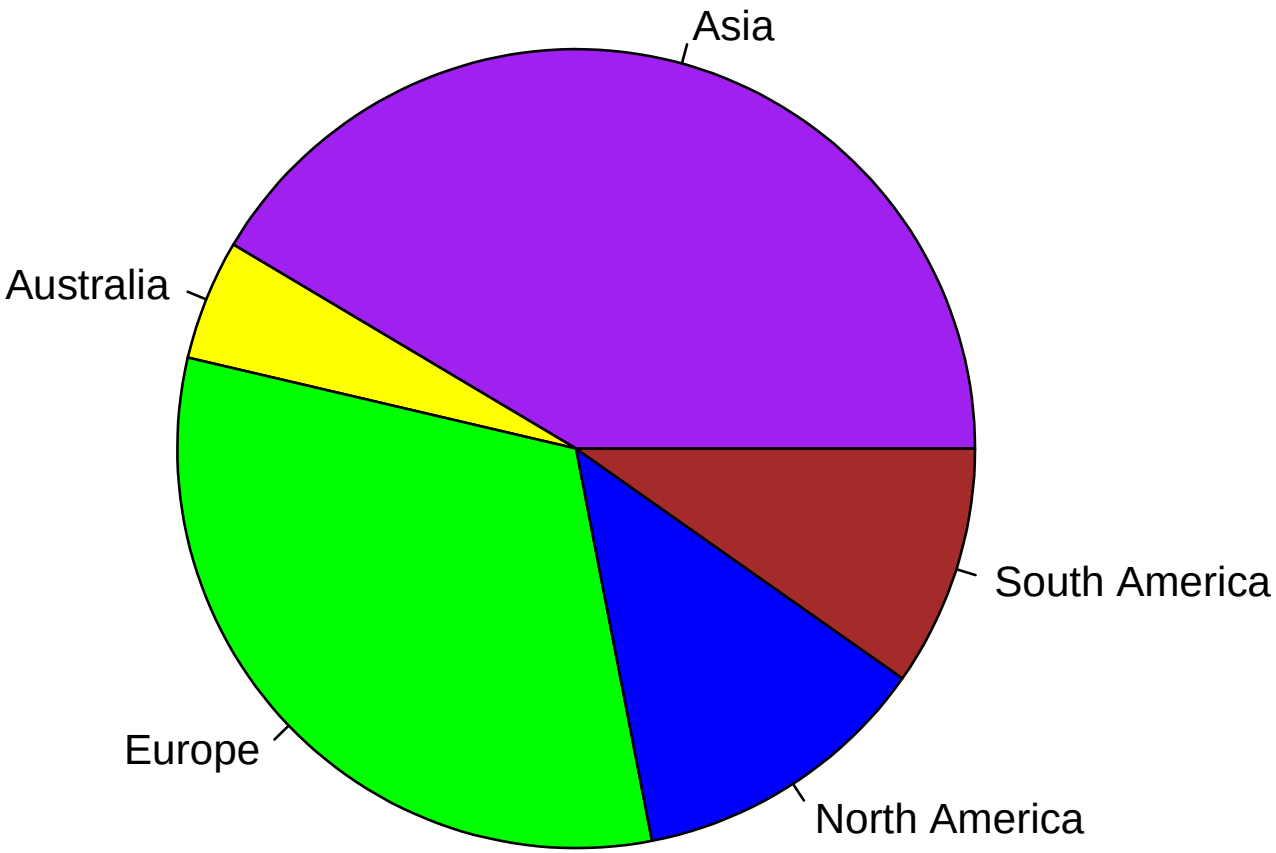
An analysis of 41 studies published in 2015-2018

- 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

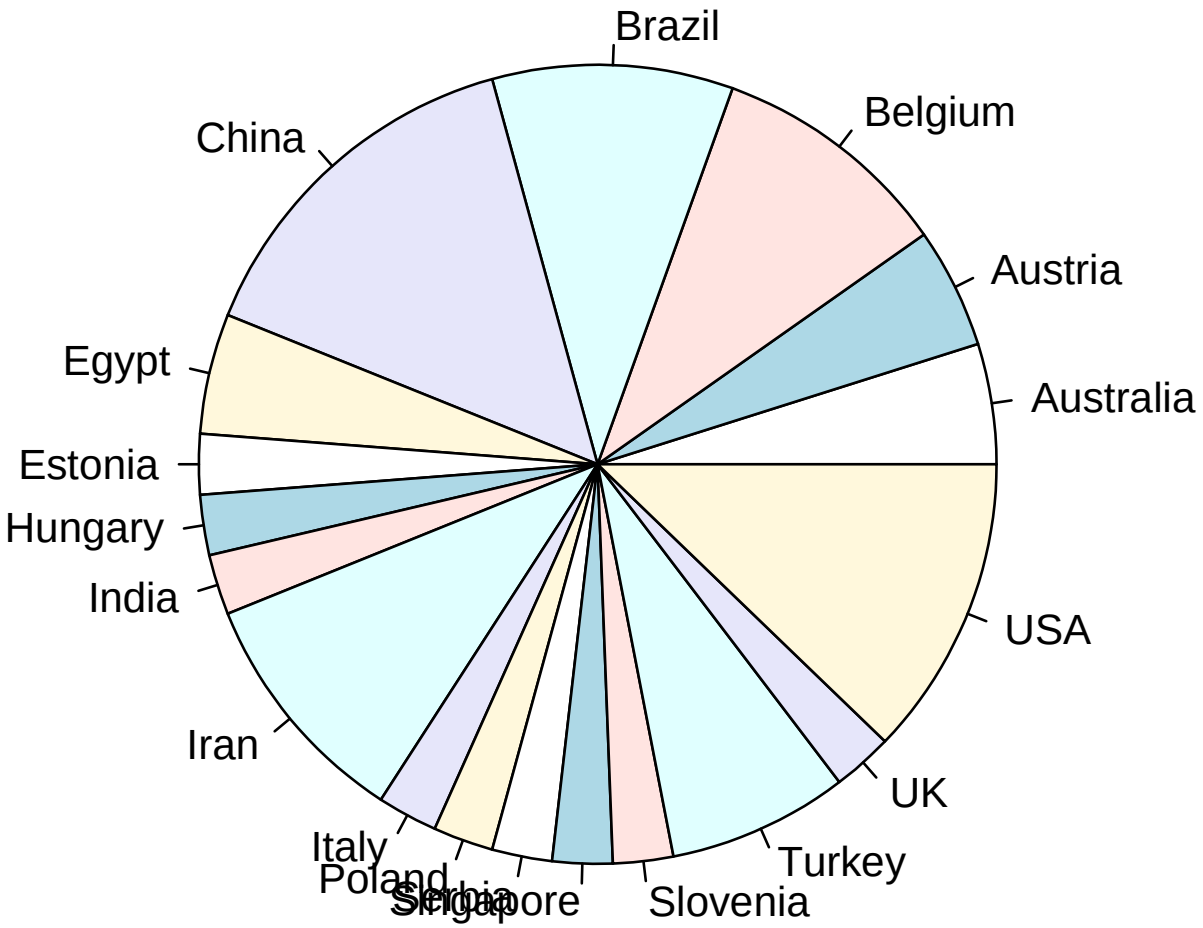
Other features

- 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

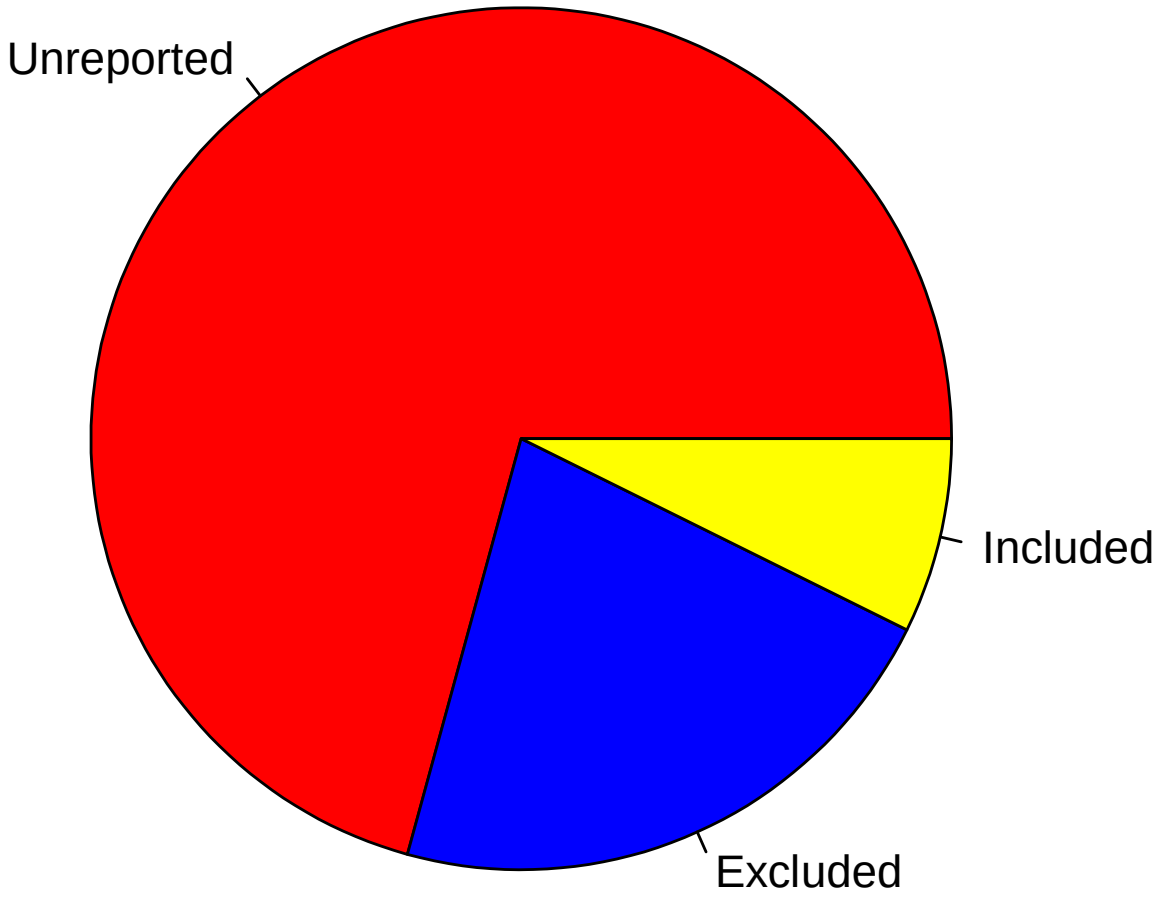
Geographic distribution



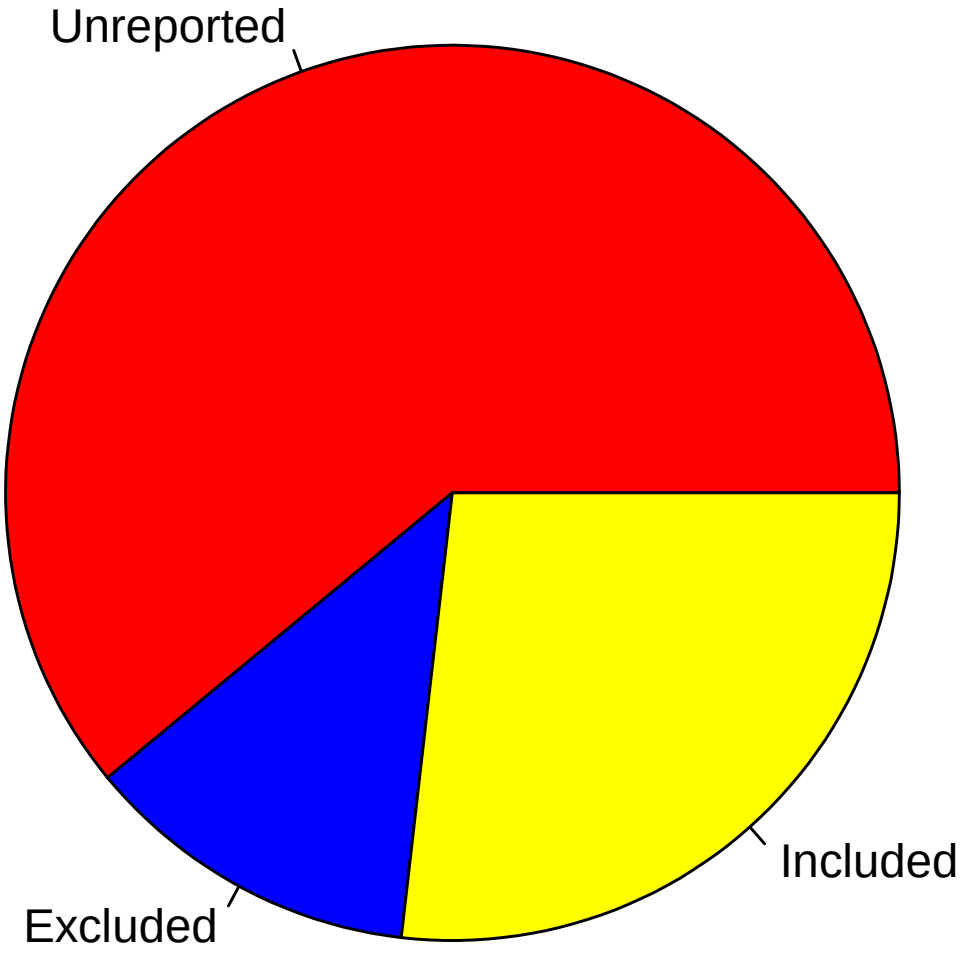
Number of studies by country



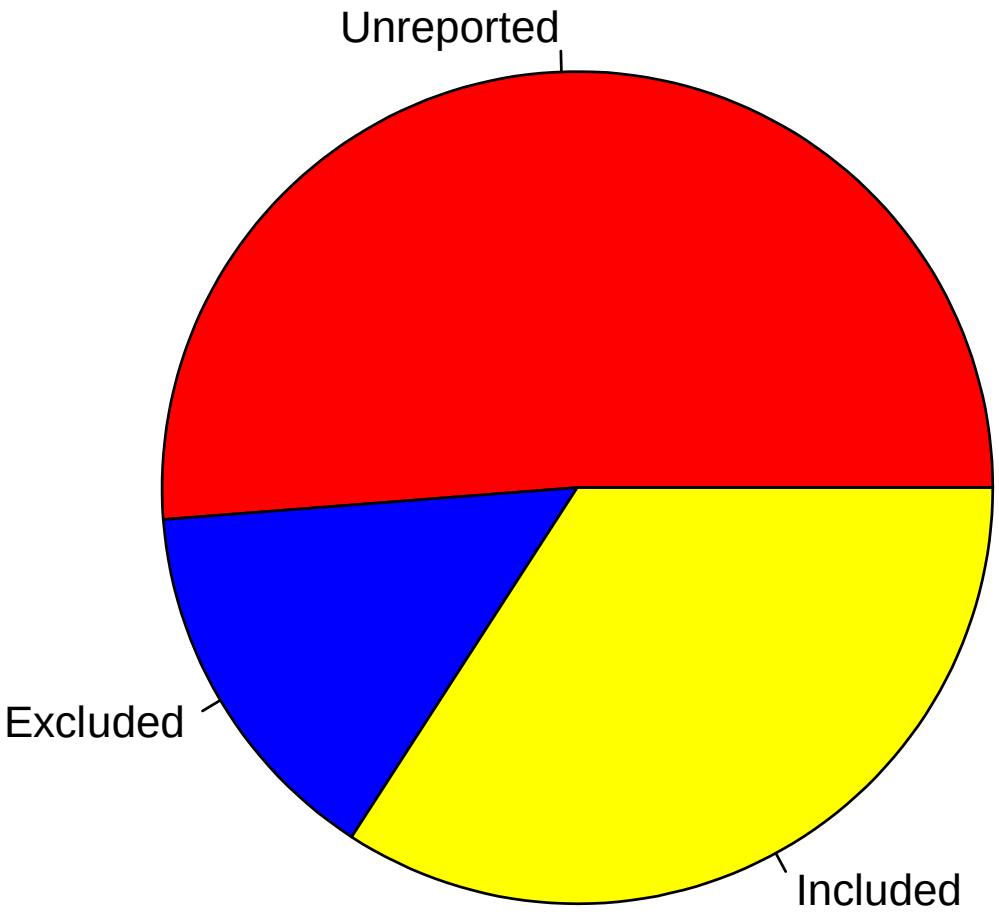
Co-occurrance with adenomyosis

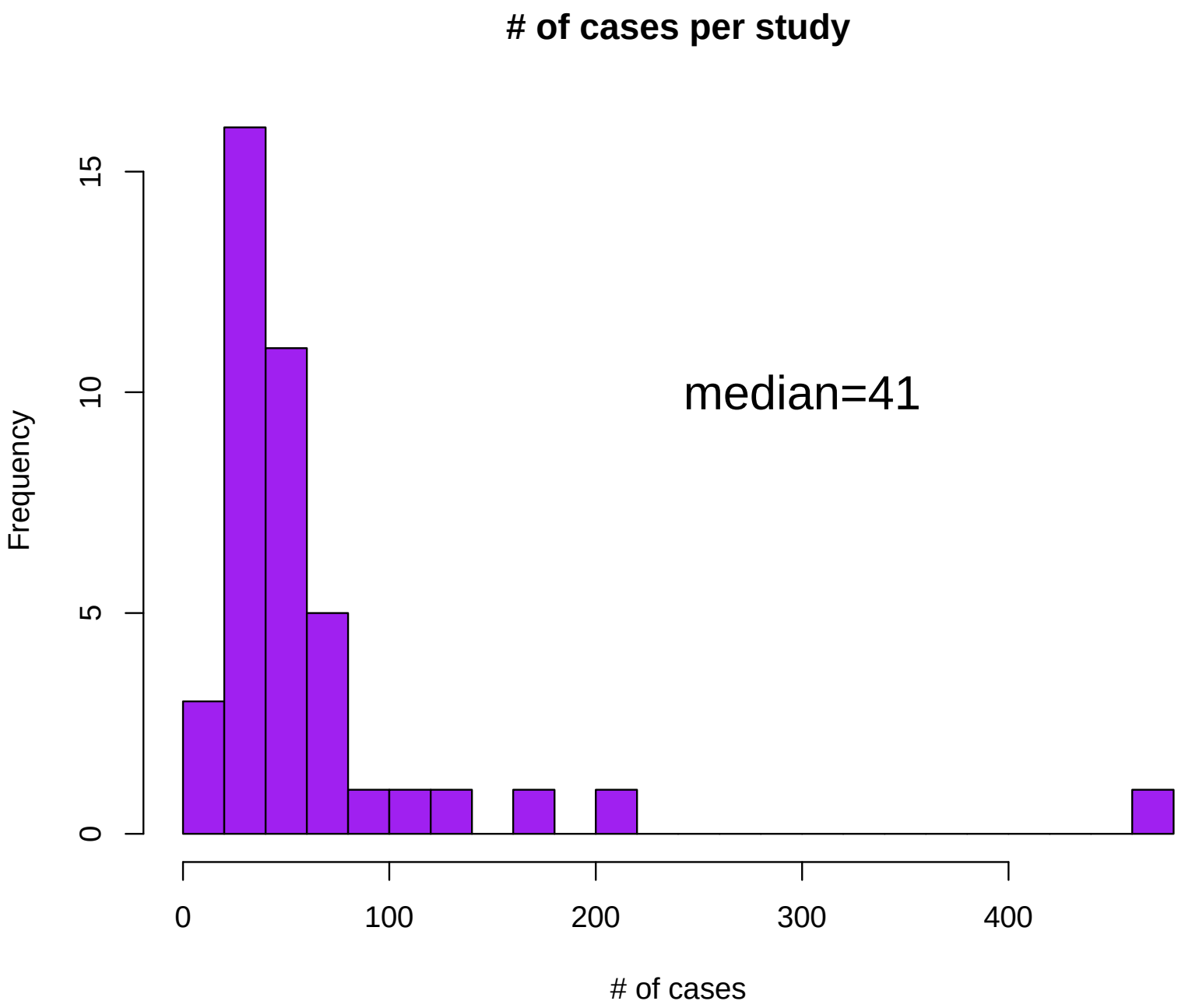


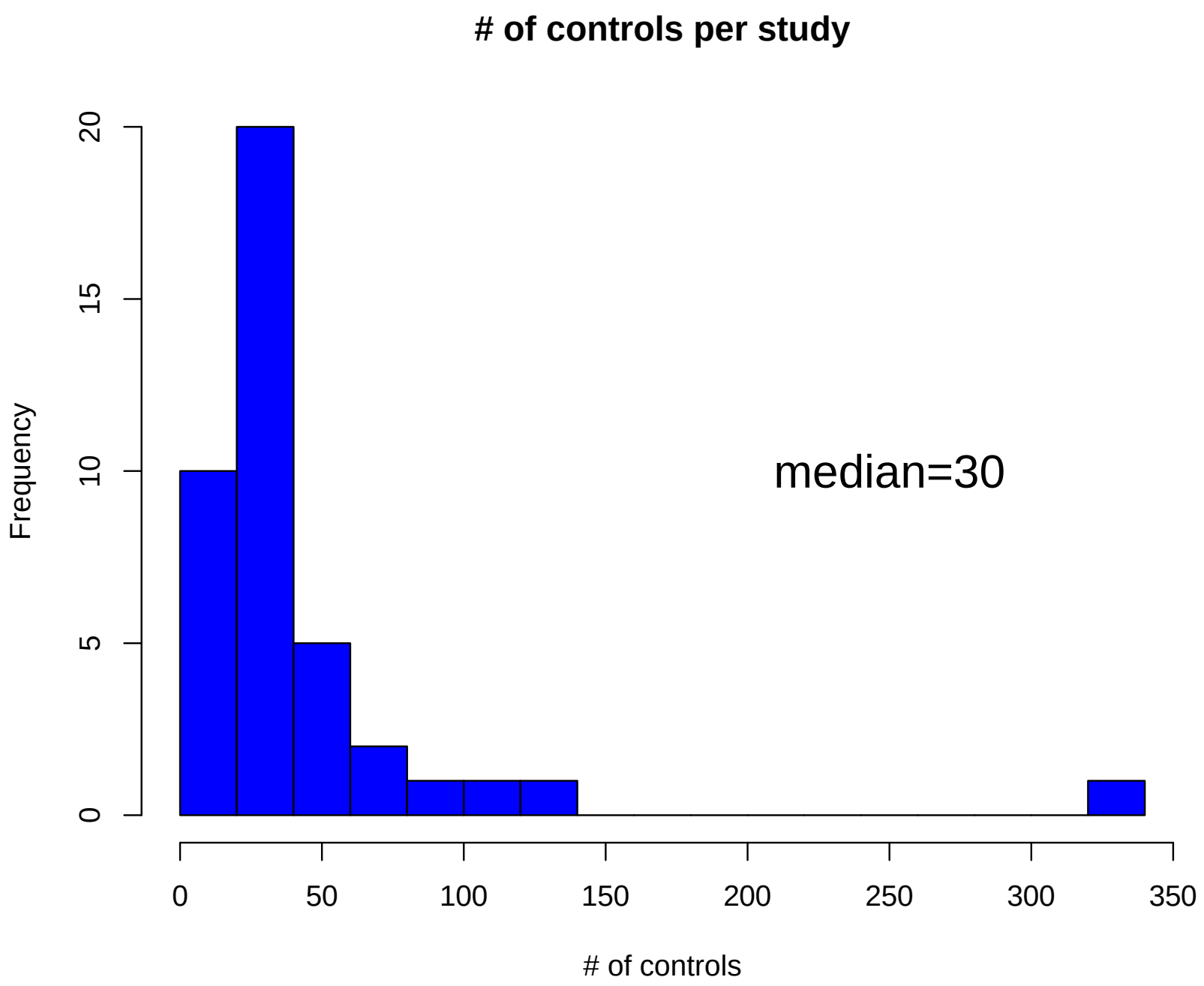
Co-occurrance with uterine fibroids



Co-occurrance with other comorbidities




# of cases per study
median=41
Frequency
0
5
10
15
0
100
200
300
400
# of cases


# of controls per study
Frequency
0
5
10
15
20
median=30
0
50
100
150
200
250
300
350
# of controls

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, co-morbidity, 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

- Large sample sizes
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  - Once all visible lesions are removed, there is a change; Retest
  - Correlation with lesional histology
    - Stage of progression
  - Support from in vivo data
- 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

Acknowledgment

- Vicki Nisenblat
- Fengyi Xiao

7th Asian Conference on Endometriosis

September 14-16, 2018
Taipei, Taiwan