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Proteomics International presents a novel blood test for diagnosing Endometriosis at international conference

- **New blood test for endometriosis offers improved early screening for the disease, correctly identifying up to 90 per cent of patients with the condition in a study of over 900 participants**
- **Biomarkers identified that change concentration as severity of endometriosis increases**
- **Research also suggests the current gold standard for diagnosis—an invasive surgical procedure—may be misdiagnosing some patients**
- **A simple blood test could provide early screening to rule in or rule out the need for invasive surgery in women presenting with symptoms of endometriosis**
- **Results presented at the international conference for the Society for Reproductive Investigation at their 70th Annual Scientific Meeting held in Brisbane**
- **Endometriosis affects one in nine women and currently diagnosis typically takes an average of 7.5 years**

Proteomics International Laboratories Ltd (Proteomics International; the Company; ASX: PIQ) is pleased to announce its latest results for its potential new world-first blood test for diagnosing endometriosis. The results indicate strong diagnostic performance of the test and were presented on Friday at the 70th Annual Meeting of the international Society for Reproductive Investigation (SRI), held in Brisbane, March 21 – 25, 2023.

The simple test uses biomarkers—protein 'fingerprints' in the blood—to screen for the painful condition. Research presented at the conference shows current versions of Proteomics International's test can identify endometriosis, with the Company's preferred prototype correctly identifying up to 90 per cent of patients when comparing moderate or severe endometriosis to symptomatic controls (no endometriosis) in a 901 person study.

Proteomics International Managing Director Dr Richard Lipscombe said the results were exciting but also thought provoking. "*We have a potential screening test for endometriosis—a simple blood test to help determine who should have an invasive laparoscopy and who should not. But the study also suggests the current gold standard for diagnosis—an invasive surgical procedure—may be misdiagnosing some patients, particularly in the early stages of endometriosis.*"

Endometriosis is a common and painful disease that affects one in nine women and girls, often starting in teenagers. It occurs when tissue similar to the lining of the uterus grows in other parts of the body where it does not belong. At the moment, there is no simple way to test for the condition, which can cause pain and infertility, and costs Australia $9.7 billion each year[1].

The current gold standard for detection is an invasive laparoscopy followed by histopathology, a surgical

[1] www.endometriosisaustralia.org

procedure where a camera is inserted into the pelvis through a small cut in the abdominal wall and then a biopsy is taken for analysis. On average, it takes women 7.5 years to be diagnosed[2].

Proteomics International's test works by measuring the concentration of biomarkers in the blood that are associated with endometriosis. The Company has identified a panel of 14 biomarkers that change concentration as the severity of endometriosis increases and has built a series of statistical models using different combinations of these biomarkers to diagnose disease. Analysis shows these biomarkers all relate to biological pathways that could be linked to the unwanted tissue growth that occurs in endometriosis.

Dr Lipscombe said the models are all showing the same thing. "*Our simple blood tests can detect endometriosis, but the data also indicates some people may currently be misdiagnosed in the early stages of the disease*," he said. "*We think this is possible because during a laparoscopy it can be difficult for surgeons to definitively detect and confirm the small lesions that occur when uterine tissue starts growing in the wrong place*."

Dr Lipscombe said we need to study more people in the early stages of endometriosis or with symptoms and to look more closely at their existing diagnosis. "*These results are an exciting development in our work to better understand this complex disease and improve the diagnosis of it."*

Proteomics International's preferred diagnostic model targets a potential early screening test to rule in or rule out the need for invasive surgery by distinguishing symptomatic controls from moderate and severe endometriosis, and achieved sensitivity (Sn) of 90%, specificity (Sp) of 90%, and an AUC (area under the ROC curve) of 0.84. The test employs the 'traffic light' scoring system successfully developed for the Company's world-first test for predicting the onset of diabetic kidney disease, PromarkerD, which is currently rolling out in the USA.

The endometriosis diagnostic test is being developed in collaboration with the Royal Women's Hospital and the University of Melbourne [ASX: 4 August 2021, 1 August 2022].

The Royal Women's Hospital Director of Research, Professor Peter Rogers said that a non-invasive test for endometriosis could save women years of suffering. *"Endometriosis symptoms often start when women are teenagers,"* he said. "*But because it's so hard to diagnose, girls can struggle with unexplained pain throughout their lives. We're hoping to prevent this with a simple, accessible blood test that can be ordered by a family GP*."

Proteomics International has filed patents in all major jurisdictions for a method to measure a panel of protein biomarkers to determine whether a subject has endometriosis.

Society for Reproductive Investigation, 70th Annual Scientific Meeting oral presentation (O-115); [copy attached; summary below]

Titled: *A Novel Plasma Protein Biomarker Test for Diagnosing Endometriosis*

Kirsten Peters[1], Elizna Schoeman[1], Christina Andronis[1], Scott Bringans[1], Tammy Casey[1], Lianzhi Chen[1], Deem Ismail[1], Jun Ito[1], Mufaidha Raju[1], Pearl Tan[1], Richard Lipscombe[1], Peter Rogers[2,3], Sarah Holdsworth-Carson[2,3,4].
[1] Proteomics International, Perth, Australia; [2]University of Melbourne, Melbourne, Australia; [3]Royal Women's Hospital, Melbourne, Australia; [4]Epworth HealthCare, Melbourne, Australia

Summary of Study

Method: To test the performance of the biomarkers, Proteomics International's scientists compared 901 samples across three groups: women who had been diagnosed with endometriosis through a laparoscopy (N=494), and two control groups: healthy individuals (N=153) and, patients with pelvic pain but surgically-diagnosed absence of endometriosis (symptomatic controls) (N=254).

In grading the severity of endometriosis by laparoscopy the surgeons used the US rASRM (revised American Society for Reproductive Medicine) score, whereby a score of 1-5 was classified minimal, 6-15 mild, 16-40

[2] www.endometriosis-uk.org

moderate and >40 severe endometriosis. The clinical samples were collected over several years (2012-2019) from patients who attended a Royal Women's Hospital Pelvic Pain Clinic**.**

Plasma protein biomarkers were measured by mass spectrometry and statistical analysis performed using multivariate logistic regression, followed by use of the 'traffic light" system to optimise test performance.

Results: The results demonstrated multiple versions of the test were effective in discriminating endometriosis and stages of disease severity:

- For symptomatic controls (no endometriosis) vs moderate + severe endometriosis: AUC = 0.84; optimised Sn = 90%, Sp = 90%. The model can separate most individuals, but there are still some patients that would be misclassified. Comment: an important 'real world' comparison.
- For healthy controls vs severe endometriosis: AUC = 0.97; optimised Sn = 89%, Sp =95%. The model achieved excellent separation of controls from severe endometriosis. Comment: indicates biomarkers are discriminating between endometriosis and non-endometriosis. In addition, the biomarker scores also suggested many "symptomatic controls" may actually have endometriosis, whilst some patients classified by laparoscopy as "minimal endometriosis" may not have endometriosis.

In interpreting these results, it is important to recognise that endometriosis is a highly complex condition with a broad spectrum of clinical indications. Consequently, endometriosis is not necessarily a simple positive versus negative test, and further work may be required to detect these subtle variations.

Conclusions:

- A series of models were developed where biomarkers added significant value to clinical factors for diagnosing endometriosis from both symptomatic and healthy controls.
- A panel of 14 protein biomarkers was defined for diagnosing endometriosis, all with biological functions relevant to disease pathophysiology.
- The data suggests that some individuals may be being misdiagnosed in the early stages of the disease.

Next steps:

- Confirm the clinical performance and clinical utility of the test in independent patient cohorts;
- Accelerate pathways to commercialisation of the biomarker panel as a new diagnostic screening test for endometriosis. Proteomics International believes a validated test will garner significant interest, both commercially and in the clinic.

Glossary

Sensitivity (Sn) (true positive rate)	The ability of a test to correctly identify those with the disease. E.g. sensitivity of 90% means that for every 100 people with endometriosis, the test correctly diagnosed 90 with the condition.
Specificity (Sp) (true negative rate)	The ability of the test to correctly identify those without the disease. E.g. specificity of 85% means that for every 100 people with symptoms but no endometriosis, a test correctly identifies 85 as not having the condition.
AUC	"Area Under the ROC Curve". A receiver operating characteristic curve, or ROC curve, is a graphical plot that illustrates the performance of a classifier system.
Interpreting AUC values	Conventionally the clinical significance of AUC is: > 0.7 acceptable discrimination > 0.8 excellent discrimination > 0.9 outstanding discrimination

For comparison, the statistical performance of the Prostate-Specific Antigen (PSA) diagnostic test (blood test measuring the concentration of the PSA protein) for the diagnosis of prostate cancer is[3]:

- Prostate cancer versus no cancer: AUC 0.68, P = <0.001
- PSA cut-off threshold 3ng/ml: Sensitivity 32%, Specificity 87%

Authorised by the Board of Proteomics International Laboratories Ltd (ASX: PIQ).

ENDS

About the Promarker™ Platform

Proteomics International's diagnostics development is made possible by the Company's proprietary biomarker discovery platform called Promarker, which searches for protein 'fingerprints' in a sample. This disruptive technology can identify proteins that distinguish between people who have a disease and people who do not, using only a simple blood test. It is a powerful alternative to genetic testing. The technology is so versatile it can be used to identify fingerprints from any biological source, from wheat seeds to human serum. The Promarker platform was previously used to develop PromarkerD, a world-first predictive test for diabetic kidney disease, that is currently being commercialised. Other tests in development include for asthma & COPD, oesophageal cancer, diabetic retinopathy and oxidative stress.

About Proteomics International Laboratories (PILL) (www.proteomicsinternational.com)

Proteomics International (Perth, Western Australia) is a wholly owned subsidiary and trading name of PILL (ASX: PIQ), a medical technology company at the forefront of predictive diagnostics and bio-analytical services. The Company specialises in the area of proteomics – the industrial scale study of the structure and function of proteins. Proteomics International's mission is to improve the quality of lives by the creation and application of innovative tools that enable the improved treatment of disease.

For further information please contact:

Dr Richard Lipscombe
Managing Director
Proteomics International Laboratories Ltd
T: +61 8 9389 1992
E: enquiries@proteomicsinternational.com

Dirk van Dissel
Investor Relations & Corporate Advisor
Candour Advisory
T: +61 408 326 367
E: dirk@candouradvisory.com.au

Kyle Moss
Corporate Advisor
Euroz Hartleys
T: +61 8 9488 1400
E: kmoss@eurozhartleys.com

3 pubmed.ncbi.nlm.nih.gov/15998892/

A Novel Plasma Protein Biomarker Test for Diagnosing Endometriosis

Kirsten Peters[1], Elizna Schoeman[1], Christina Andronis[1], Scott Bringans[1], Tammy Casey[1], Lianzhi Chen[1], Deem Ismail[1], Jun Ito[1], Mufaidha Raju[1], Pearl Tan[1], Richard Lipscombe[1], Peter Rogers[2,3], Sarah Holdsworth-Carson[2,3,4]

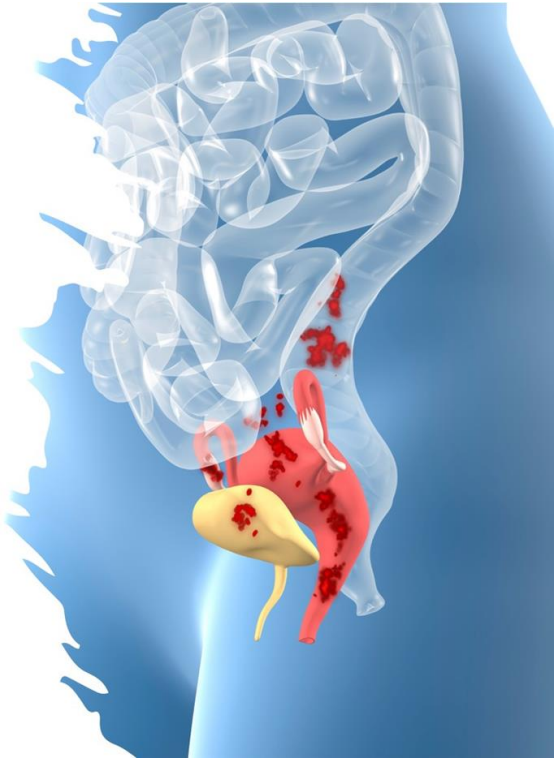
[1] Proteomics International, Perth, Australia; [2] University of Melbourne, Melbourne, Australia; [3] Royal Women's Hospital, Melbourne, Australia; [4] Epworth HealthCare, Melbourne, Australia

March 24th, 2023

Presented at the 70th Annual Scientific Meeting of the Society for Reproductive Investigation (SRI), Brisbane, Australia

Introduction & Aim

- Endometriosis (endometrial-like tissue outside the uterus) is a common inflammatory gynecological condition, affecting approximately 10% of women globally.
- It is a major cause of female infertility and individuals can suffer from intense and chronic pain which substantially impacts quality of life.
- Diagnosis is often delayed (average 7.5 yrs), usually by invasive laparoscopy with histological verification, however, it is costly and requires a skilled physician.
- Recent guidelines from ESHRE[1] suggest that imaging may offer an accurate alternative to laparoscopy, at least for ovarian and some cases of deep endometriosis.



A non-invasive diagnostic test allowing earlier diagnosis is needed.

Aim: to validate a panel of protein biomarkers for use in endometriosis diagnosis.

[1] Becker, et al. European Society of Human Reproduction and Embryology (ESHRE) guideline: Endometriosis. Hum. Reprod. Open **2022**, 26.

Participants

- Plasma samples (n=901) were analysed across three clinical groups:
  - **Endometriosis** cases confirmed with laparoscopy/histopathology (n=494),
  - **Symptomatic Controls** with surgically confirmed absence of endometriosis (n=254)
  - **Healthy Controls** (n=153) (general pop, excl any with Endo-associated symptoms).

Endo and Pelvic Pain Clinic
Royal Women's Hospital,
Melbourne, Australia
2012-2019

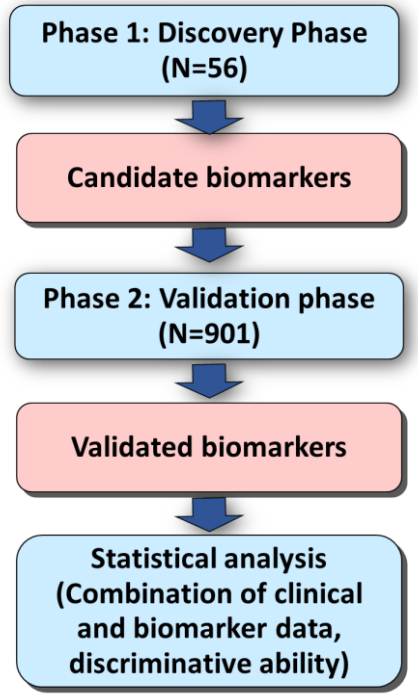
Endo severity rASRM:
- minimal (n=254)
- mild (n=75)
- moderate (n=67)
- severe (n=97)
- missing (n=1)

	Endometriosis (N=494)	**Symptomatic Controls** (N=254)	**Healthy Controls** (N=153)
Age (years)	30 ± 7	31 ± 8	28 ± 9
BMI (kg/m^2)	25 ± 5	27 ± 6	25 ± 6
Smoking status (% current or past)	40	44	30
Age at Menarche (years)	13 ± 2	13 ± 2	13 ± 2
Family history of Endometriosis (%)	28	23	3
Pain (% menstrual/pelvic/intercourse)	93/81/76	93/83/77	0
Cycle Length (% 14-20/21-27/28/29+/other* days)	32/8/30/21/9	46/9/21/14/10	0/19/50/31/0
Gravidity (% 0/1/2/3+)	68/16/10/6	51/14/10/25	73/9/8/10
Live births (% 0/1/2/3+)	80/10/8/2	63/15/11/11	81/5/12/2
Ethnicity (% AS/SMR/AFR/EUR/Other/Unknown)	11/0.6/0.6/76/5/7	4/0.8/0.8/82/6/6	17/5/1/62/6/9
Exogenous hormone medication (% oral/IUD/Depo inj)	21/7/2	29/17/2	24/7/1

*Cycle Length other=unknown, unsure or not cycling; AS=South or East Asian, SMR=South American, AFR=African, EUR=European.

Proteomics & Statistical Analysis

- A proteomics mass spectrometry platform was used to identify potential plasma protein biomarkers for endometriosis in a small discovery cohort followed by validation in a larger cohort (Figure 1).
- A total of 51 candidate protein biomarkers were assessed.
- Clinical characteristics and biomarker concentrations were compared between endometriosis cases, symptomatic controls and healthy controls using t-tests or chi-squared tests.
- Multivariate logistic regression was used to develop models for diagnosing endometriosis 1) clinical model, 2) clinical+biomarker model.
- Model performance was assessed by AUC-ROC curves (area under the receiver operating characteristic curve) and sensitivity (Sn) and specificity (Sp) determined.
- Dot plots were also used to visualize the model predictions.

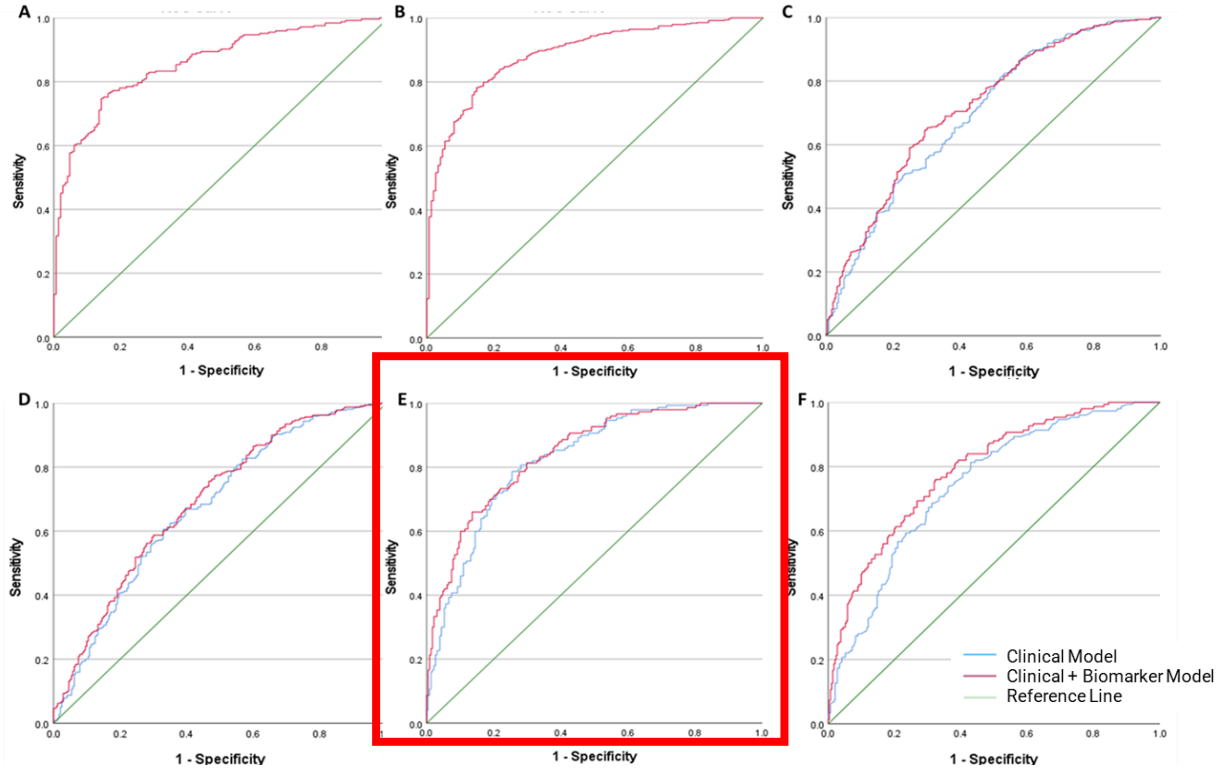


Proteomics workflow for discovery and validation of endometriosis biomarkers

Models for Endometriosis

- A series of models for endometriosis were developed.
- Models to diagnose symptomatic controls or Endo from healthy controls had excellent AUCs (Fig A/B).
- Key clinical factors associated with Endo - age, BMI, cycle length, number of times pregnant, hormone use and ethnicity.
- After adjustment for clinical factors, several protein biomarkers were found to be independently associated with Endo and significantly improved model performance (*P*<0.05) (Fig C-F).
- The best performing model was for diagnosing moderate/severe Endo from symptomatic controls (AUC=0.84) (Fig E).

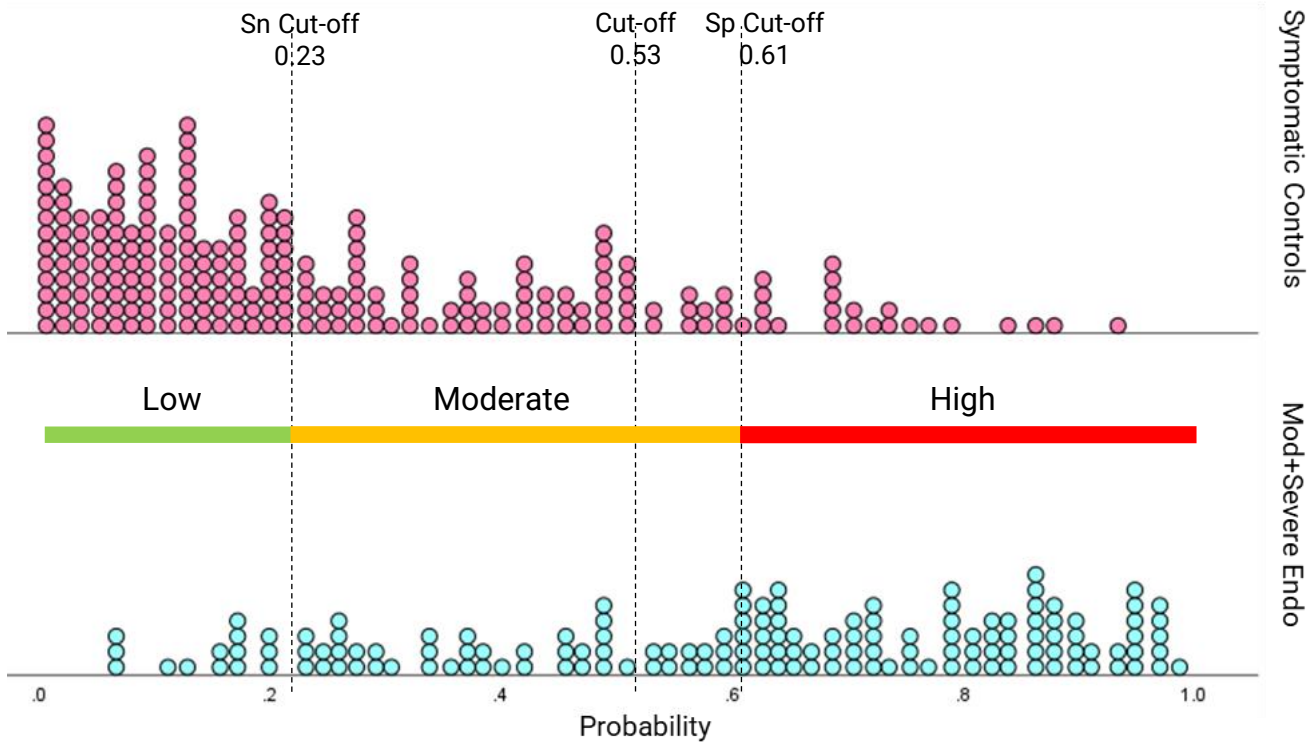
Biomarkers added significant value to clinical factors for diagnosing endometriosis



A. Healthy vs Symptomatic Controls – AUC=0.86 *biomarker only*
B. Healthy vs Endometriosis – AUC=0.89 *biomarker only*
C. Symptomatic Controls vs Endometriosis – AUC=0.72, ΔAUC p=0.018
D. Symptomatic Controls vs Min+Mild Endo – AUC=0.69, ΔAUC p>0.05
E. Symptomatic Controls vs Mod+Severe Endo– AUC=0.84, ΔAUC p=0.028
F. Min+Mild Endo vs Mod+Severe Endo – AUC=0.79, ΔAUC p=0.003

"Area Under the ROC Curve". A receiver operating characteristic curve, or ROC curve, is a graphical plot that illustrates the performance of a classifier system.
AUC>0.70 acceptable, >0.80 excellent, >0.90 outstanding model performance to separate the clinical groups. Minimal/Mild/Moderate/Severe Endo as defined by rASRM stages.

Symptomatic Controls vs Moderate/Severe Endo



- Dot plot of the best performing model 'Symptomatic Controls vs Moderate+Severe Endo'

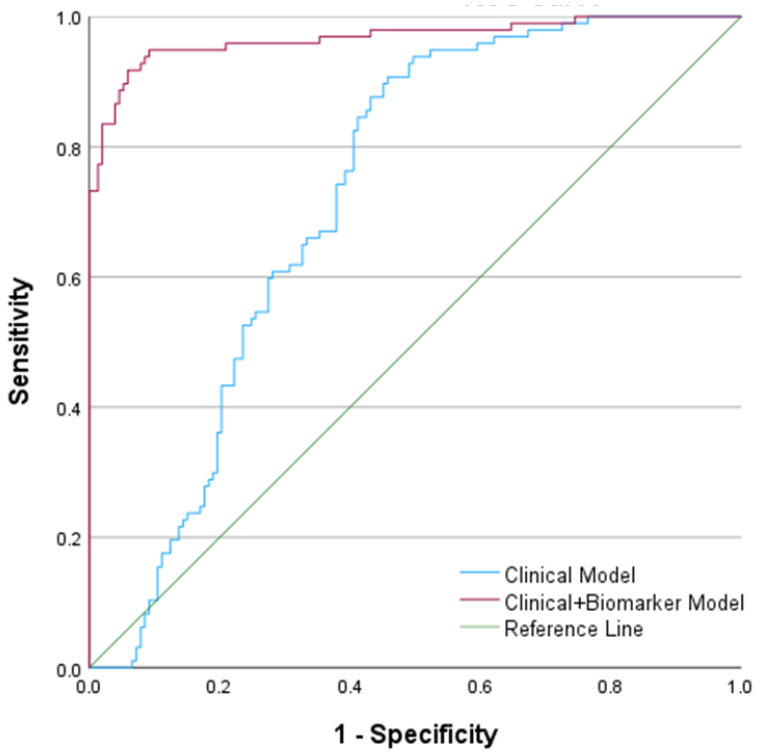
- AUC=0.84 (excellent discrimination)
- Cut-off ≥0.53 → Sn 66%, Sp 86%
- Model can separate most individuals, but there are still some individuals that would be misclassified.

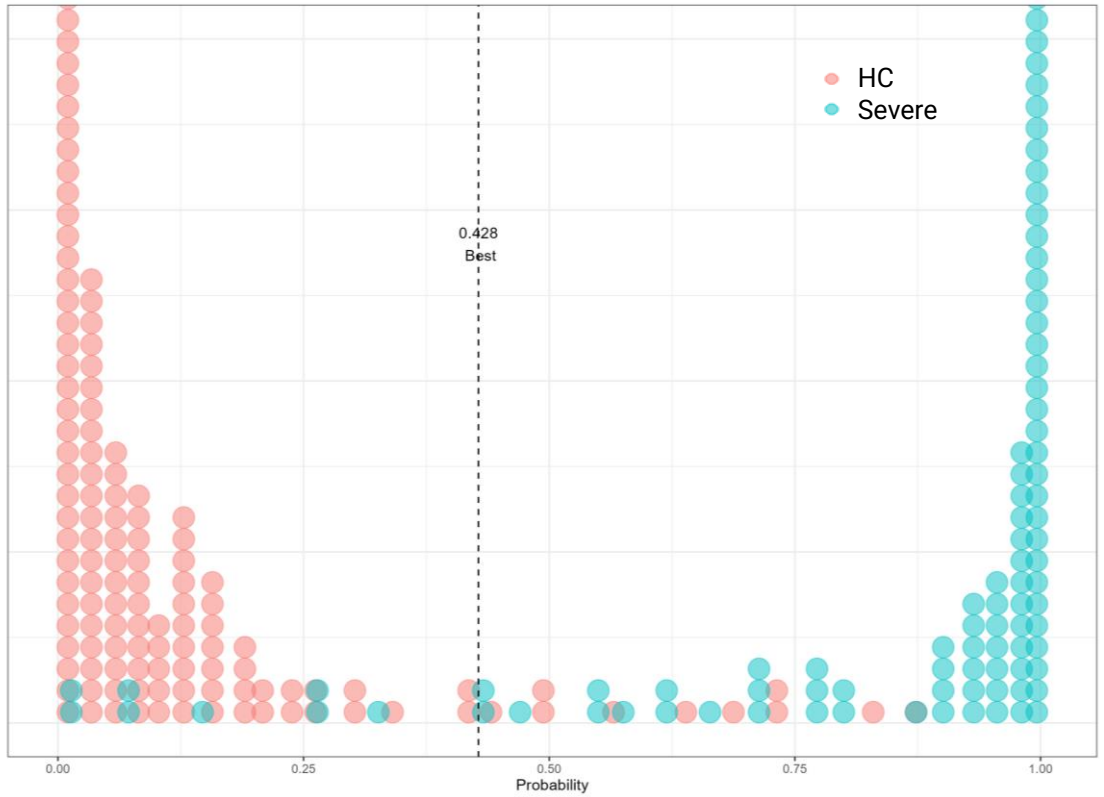
- Two cut-offs 'Traffic-Light' risk classification:
  - low, moderate or high risk
  - Sn cut-off ≥0.23 → **Sn 90%,** Sp 58%
  - Sp cut-off ≥0.61 → Sn 57%, **Sp 90%**

An example of how the test might work in the clinic

"Area Under the ROC Curve". A receiver operating characteristic curve, or ROC curve, is a graphical plot that illustrates the performance of a classifier system. Sn = Sensitivity, Sp = Specificity, AUC>0.70 acceptable, >0.80 excellent, >0.90 outstanding model performance to separate the clinical groups. Minimal/Mild/Moderate/Severe Endo as defined by rASRM stages.

Healthy Controls vs Severe Endo





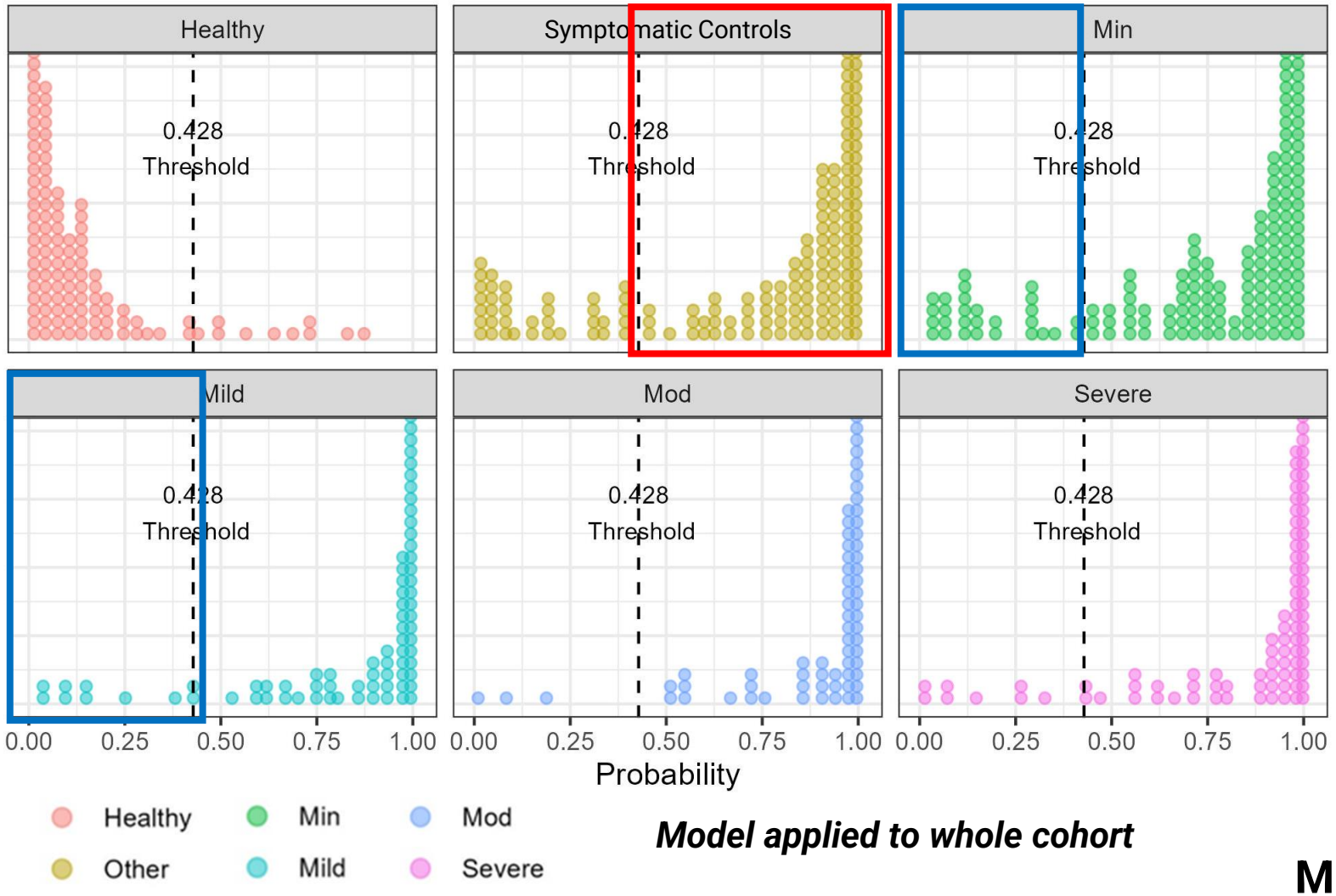
- Outstanding separation of Healthy Controls from Severe Endo.
  - Clinical AUC=0.72
  - **Clinical + Biomarker AUC=0.97 → Sn 89%, Sp 95%**

ΔAUC p<0.0001

Given the high Sn/Sp, no traffic-light approach was considered for this model

"Area Under the ROC Curve". A receiver operating characteristic curve, or ROC curve, is a graphical plot that illustrates the performance of a classifier system. Sn = Sensitivity, Sp = Specificity, AUC>0.70 acceptable, >0.80 excellent, >0.90 outstanding model performance to separate the clinical groups. Minimal/Mild/Moderate/Severe Endo as defined by rASRM stages.

Healthy Controls vs Severe Endo



Model applied to whole cohort

- Excellent separation of Healthy Controls from Severe Endo.
  - AUC=0.97 → Sn 89%, Sp 95%
- There are Symptomatic Controls who map similarly to the Endo cases across all stages from Minimal to Severe (probabilities >0.428 threshold – red box).
- There are also Minimal/Mild Endo cases who map like the Healthy Controls. This is also seen in the Mod/Severe Endo cases (probabilities <0.428 threshold – blue box).

Are these people misdiagnosed?

Multiple models show the same effect

Minimal/Mild/Moderate/Severe Endo as defined by rASRM stages. Sn=Sensitivity, Sp=Specificity.

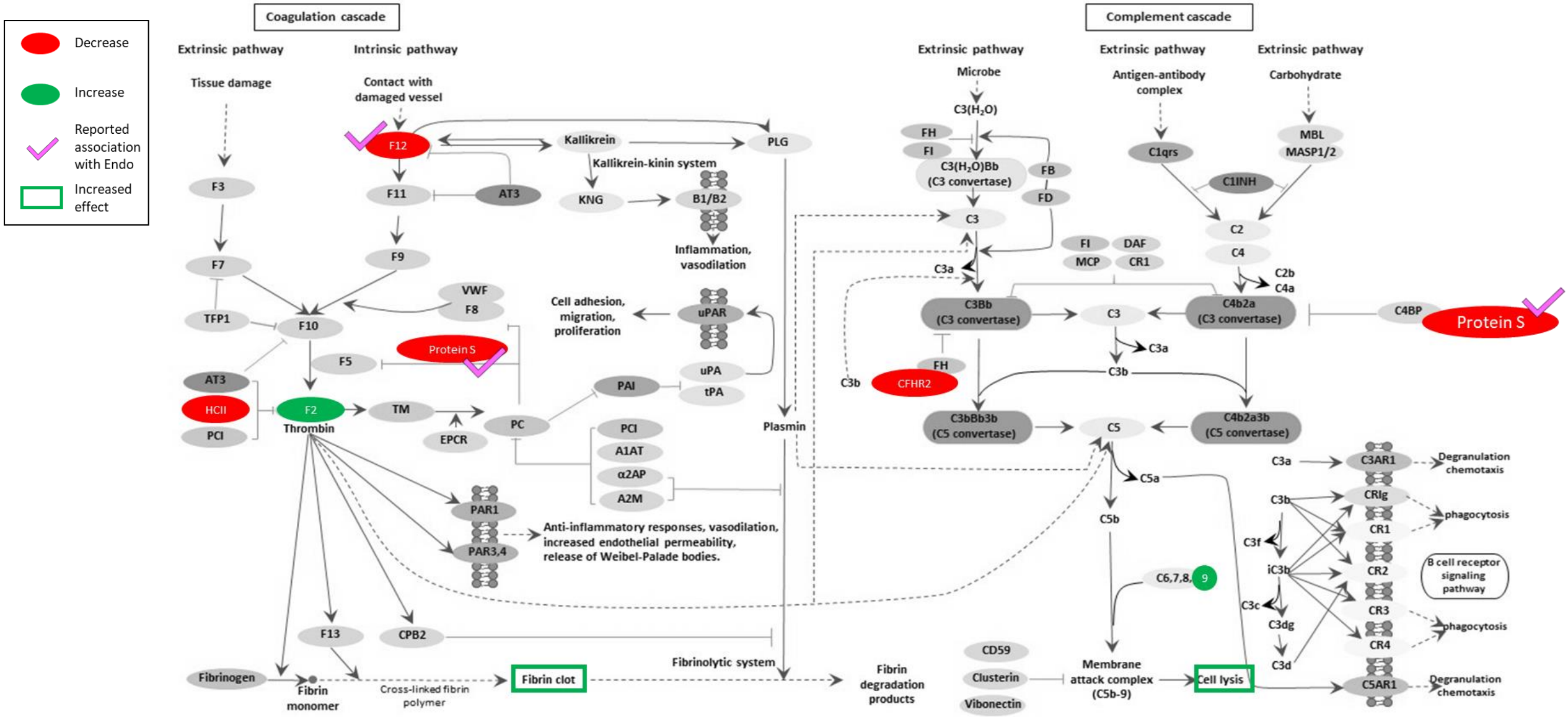
Protein Pathways/Processes

There were 14 key protein biomarkers identified in the modelling that fit into a number of different physiological pathways, and some are in more than one pathway:

- ↑ Coagulation and complement cascades (6 biomarkers),
- ↑ ER stress and UPR signaling pathways (2 biomarkers),
- ↑ Angiogenesis (1 biomarker),
- ↑ Proinflammatory signal (1 biomarker),
- ↑ Abnormal lipid metabolism (1 biomarker),
- ↑ Oxidative stress protection (1 biomarker),
- ↓ Lubrication and tissue homeostasis (1 biomarker),
- ↑ Neuropathic pain signals (1 biomarker)

Coagulation & Complement Cascades (6 biomarkers)

Proteomics International



Conclusions

- This study identified a panel of 14 plasma biomarkers that were associated with endometriosis, all with biological functions relevant to disease pathophysiology.
- A series of models were developed where biomarkers added significant value to clinical factors for diagnosing endometriosis from both symptomatic and healthy controls.
- These models have the potential to be used as a simple diagnostic blood test for endometriosis to help determine who should have an invasive laparoscopy and who should not.
- The data also suggests that some individuals may be misdiagnosed in the early stages of the disease.
    - Confounders – batch effect, age of samples, collection of samples, pain markers, micro-lesions?
- Next steps:
    - Confirm the clinical performance and clinical utility of the models in independent patient cohorts;
    - Explore additional clinical classifications of endometriosis - superficial peritoneal, ovarian and deep endometriosis subtypes.

Contact

Dr Kirsten Peters

Head of Clinical Studies/Senior Biostatistician

T: +61 8 9389 1992

E: kirsten@proteomics.com.au

Dr Richard Lipscombe

Managing Director

T: +61 8 9389 1992

E: richard@proteomics.com.au

www.proteomics.com.au

ASX:PIQ

Proteomics International LABORATORIES LTD