B-cell lymphoma 6 protein (BCL6) as a potential biomarker for endometriosis: can it be used to predict endometriosis' presence and/or severity?

STATEMENT OF COMPLIANCE

This document is a protocol for a clinical research study. The study will be conducted in compliance with all stipulations of this protocol, the conditions of ethics committee approval, the NHMRC National Statement on Ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

Contents

Deleted: 9

Deleted: 11

Deleted: 13

PROTOCOL SYNOPSIS

Title	B-cell lymphoma 6 protein (BCL6) as a potential biomarker for endometriosis: can it be used to predict endometriosis' presence and/or severity?
Objectives	**Primary:** To evaluate BCL6 expression in luteal phase endometrial biopsy in women with and without endometriosis at laparoscopy. **Secondary:** 1) To assess whether or not there is any correlation between BCL6 levels and the severity of endometriosis at laparoscopy, based on r-ASRM classification 2) To assess whether including the results of patients' long-form Endometriosis Health Profile Questionnaire (EHP-30) in the statistical analysis (in combination with their BCL6 result) improves the likelihood of correctly predicting the presence, absence, and/or severity of endometriosis at laparoscopy
Study Design	Case-control study, based on prospectively-collected data.
Planned Sample Size	184 participants
Selection Criteria	**Inclusion criteria:** - Women aged between 18 – 50yo - Women who have a regular menstrual cycle - Women who are already planning to undertake laparoscopy, for any indication - English speaking - Capacity and willingness to give written informed consent - Willingness to comply with the study - Willingness to have their planned procedure undertaken in the luteal phase of their menstrual cycle

	Exclusion criteria: - Women who have an absolute contra-indication to endometrial biopsy, such as viable (ongoing) and wanted intra-uterine pregnancy - Women with a non-viable or unwanted pregnancy - Women who have a relative contra-indication to endometrial biopsy, such as: cervical or uterine infection - Women in whom undertaking an (otherwise unnecessary) endometrial biopsy may worsen a pre-existing condition (e.g. Asherman's syndrome, intra-uterine adhesions)
Study Procedures	(1) Participants to complete an online questionnaire pre-operatively (long-form EHP-30 questionnaire) (2) Endometrial biopsy to be taken intra-operatively, and sent for analysis of BCL6 levels (3) Endometriosis to be staged intra-operatively using the r-ASRM classification system

Statistical Procedures **Sample Size Calculation** **Analysis Plan**	Participants' EHP-30 answers will be uploaded automatically from 'RedCap' to Excel, for analysis using SPSS statistical software. Participants' r-ASRM score will also be entered into Excel. This will be ordinal data; either: no endometriosis, or stage I / II / III / IV endmetriosis. Participants' BCL6 results will be continuous data, ranging from 0 to 4. r-ASRM score versus BCL6 will be presented as a box plot. Our sample size of 184 patients is based on the following assumptions: (1) For approximately one-third of our patients, endometriosis is *not* suspected pre-operatively; 10% of these patients will be found to have endometriosis at laparoscopy (2) For approximately two thirds of our patients, endometriosis is expected to be found at laparoscopy; approximately 50% of these patients will be found to have endometriosis at laparoscopy Therefore, approximately 37% of our patients will have endometriosis. Assuming BCL6 test's sensitivity and specificity for endometriosis are ≥ 85% (from Evans-Hoeker et al[1]), a sample size of 184 will provide precision of +/- 10% for 95% confidence intervals, and 80% power to detect a difference of 0.374 in average BLC6 levels between controls and cases. Receiver-operator curve analysis will be performed to assess BCL6 as a biomarker for endometriosis, and to find the optimal cut-off score for BCL6 that differentiates between cases and controls. Students t test will be used to compare mean BLC6 levels between cases and controls. Logistic regression analysis and multi-variable modelling will enable us to ascertain whether or not participants' EHP-30 answers improve the predictive value of BCL6: this results will be presented as a nomogram or classification tree analysis. P values of <0.05 will be considered significant
Duration of the study	Nine months

GLOSSARY OF ABBREVIATIONS

ABBREVIATION	TERM
AGES	Australasian Gynaecological Endoscopy and Surgery society
ASRM	American Society of Reproductive Medicine
BCL6	B-cell lymphoma 6 protein
CARE	Centre for Advanced Reproductive Endosurgery
EHP-30	Endometriosis Health Profile Questionnaire
HREC	Human Research Ethics Committee
r-ASRM	Revised American Society for Reproductive Medicine
SAE	Serious adverse event
STAT3	Signal Transducer and Activator of Transcription 3
SUSAR	Suspected Unexpected Serious Adverse Reaction

1. STUDY MANAGEMENT

1.1 Principal Investigator

Dr Alison Bryant-Smith

MBBS/BA MPH MSurgEd MRCOG FRANZCOG

AGES Accredited Training Program trainee

Centre for Advanced Reproductive Endosurgery (CARE)

408 / 69 Christie St, St Leonards, 2065

Email: al.bryantsmith@gmail.com

Phone: +61 (0) 455 022 020

1.2 Associate Investigators

A/Prof Alan Lam

MBBS (Hons) FRCOG FRANZCOG

Director, CARE

408 / 69 Christie St, St Leonards, 2065

Phone: +61 (02) 9966 9121

Dr Jessica Lowe

MBBS (Hons) MPHTM FRANZCOG

Consultant gynaecologist, CARE, Concord Hospital

408 / 69 Christie St, St Leonards, 2065

Phone: +61 (02) 9966 9121

Adj. Prof. Annabelle Farnsworth

MBBS (Hons) FRCPA FIAC

DipCytopath(RCPA) RANZCOG (Hon)

Medical Director, DHM Pathology

95 Epping Road, Macquarie Park, 2113

Phone: +61 (02) 9855 5180

Dr Sophie Corbett Burns

MBBCh BAO FRCPA

Consultant histopathologist, Douglass Hanly Moir

95 Epping Road, Macquarie Park, 2113

Phone: +61 (02) 9855 5180

Dr Alison Gee

MBBA MM(RH&HG) FRANZCOG CREI

Consultant fertility specialist, Genea

Level 3 / 321 Kent St, Sydney, 2000

Phone: +61 (02) 9221 9398

A/Prof George Condous

MBBS MD(Lon) FRCOG FRANZCOG

Consultant gynaecologist, Sydney University

Suite 2 / 8 Northcote St, St Leonards, 2065

Phone: +61 (02) 1300 851 968

1.3 Statistician

Dr Rachel O'Connell

BMath, MMedStat, PhD

Statistician, NHMRC Clinical Trials Centre, and the Kolling Institute for Medical Research

Royal North Shore Hospital

Reserve Rd, St Leonards, 2065

+61 (02) 9926 4731

1.4 Sponsors

Site	Sponsor
Royal North Shore Hospital	Northern Sydney Local Health District
Royal Prince Alfred Hospital	Sydney Local Health District
Concord Repatriation General Hospital	Sydney Local Health District
Nepean Hospital	Nepean Blue Mountains Local Health District
The Mater Hospital	St Vincent's Hospital
North Shore Private	North Shore Private
Strathfield Private	Strathfield Private

1.5 Funding and resources

Pathology Company 'Douglass Hanly Moir' has agreed to perform the BCL6 tests on endometrial biopsy samples free of charge. In exchange for funding, fertility company 'Genea' will be acknowledged in publications. No study data or personal information will be sent to either of these companies.

2 INTRODUCTION AND BACKGROUND

2.1 Background Information

Endometriosis is the presence of endometrial glands and stroma in sites other than the uterine cavity. The exact pathogenesis of endometriosis remains controversial, but the most widely held theory is that of 'retrograde menstruation': when endometrial cells flow backwards through the Fallopian tubes and into the peritoneal cavity during menstruation. Hence, endometriotic lesions typically implant on and grow between pelvic organs, such as the ovaries, Fallopian tubes, and the lining of the abdominal and pelvic cavities[2].

Thought to affect approximately 10% of reproductive-age women, endometriosis can lead to many and varied symptoms, including severe dysmenorrhoea (period pain), dyspareunia (pain during penetrative intercourse), chronic pelvic pain, and infertility[2].

The diagnosis of endometriosis may be suspected based on a patient's history and physical examination. Imaging modalities (such as ultrasound and magnetic resonance imaging) can strengthen the suspicion of severe endometriosis (e.g. endometriotic ovarian cysts and deeply

infiltrating nodules) with a high degree of sensitivity. However, superficial peritoneal endometriosis cannot be identified by any imaging modality at present.

The only way to definitively confirm the presence or absence of endometriosis is to undertake laparoscopy (keyhole surgery). Undertaking laparoscopy, and excising suspected endometriotic lesions for histological analysis, remains the gold standard for diagnosing and managing endometriosis.

Because there is no reliable non-invasive way to diagnose endometriosis (esp. superficial endometriosis), there is often a significant delay in diagnosis. In addition: in approximately 55% of patients who undertake laparoscopy for pelvic pain (to investigate possible endometriosis), no endometriosis is found[3].

There are no serum markers (blood tests) which have been found to diagnose endometriosis with adequate sensitivity and specificity[4]. Hence, further research is needed to establish relatively non-invasive methods of diagnosing endometriosis. This would allow clinicians to: avoid unnecessary laparoscopic surgery if endometriosis is thought to be unlikely, based on a biomarker result; and to have a lower threshold to undertake laparoscopy if the biomarker result is suggestive of endometriosis.

Regarding the authors' credentials: A/Prof Alan Lam (AL) and Dr Alison Bryant-Smith (ABS) have significant experience in laparoscopic surgery for endometriosis. A/Prof Lam is one of Australia's most highly regarded laparoscopic gynaecologists, and has previously been the President of the Australasian Gynaecological Endoscopy and Surgery (AGES) society. He has also served on the board of the World Endometriosis Society, and the International Society of Gynecological Endoscopy. Dr Bryant-Smith is a consultant gynaecologist with a special interest in endometriosis, who is currently undertaking a two-year AGES accredited training program (Fellowship) in advanced laparoscopy, with A/Prof Lam as her mentor.

Dr Jessica Lowe is a consultant gynaecologist, who has sub-specialised in advanced laparoscopic gynaecology. She has completed various research projects in the past, including the 'PARROT trial', which was a multi-centre, pragmatic, stepped-wedge cluster-randomised controlled trial.

Adj. Prof Annabella Farnsworth (AF) is a specialist gynaecological histopathologist, and is well known both in Australia and internationally for her contributions to gynaecological pathology. Dr Sophie Corbett-Burns (SCB) is a consultant histopathologist, with a special interest in gynaepathology.

Dr Alison Gee is a consultant obstetrician/gynaecologist, who specialises in infertility and reproductive medicine. She is an accredited fertility sub-specialist, with a special interest in endometriosis-related infertility.

A/Prof George Condous is a consultant gynaecologist, who has specialised in the diagnosis and management of endometriosis. He has published over 100 papers in international peer-reviewed journals, and is highly regarded internationally for both his clinical and research acumen.

2.2 Research Question

To what extent are B-cell lymphoma 6 protein (BCL6) levels on endometrial biopsy (taken during the luteal phase) predictive of endometriosis at laparoscopy?

2.3 Rationale for Current Study

As noted above, there is a dire need for a relatively non-invasive diagnostic tool for endometriosis. This would save many women from having an unnecessary laparoscopy: as mentioned above, 55% of women who have laparoscopy to investigate pelvic pain (and a clinical suspicion of endometriosis) do not actually have any endometriosis found at subsequent laparoscopy[4]. In addition, having a reliable non-invasive marker of endometriosis has been predicted to be a paradigm shift for the diagnosis and treatment of infertile women[1].

In addition, a reliable biomarker would guide the management of some women towards an earlier laparoscopy. For example: *in vitro* fertilisation is half as likely to be successful in women with endometriosis[5]. Diagnosing endometriosis non-invasively and undertaking earlier laparoscopic excision, would improve such women's fecundity, and may negate the need for them to undertake *in vitro* fertilisation at all[5].

Previous research investigated the possibility of using nerve fibres in endometrial biopsies as a biomarker for endometriosis. There were some promising studies initially[6–8]; however, these promising results could not replicated by several subsequent research groups[9–11].

A promising biomarker that has been explored recently is B-cell lymphoma 6 protein (BCL6): in 2017 Yoo and colleagues[12] concluded that BCL6 is one of several biomarkers that are over-expressed in the endometrium of women with endometriosis, and postulated that it may be involved in endometriosis' pathogenesis. Lessey et al[13] outline how endometriosis leads to sustained activation of a transcription factor called Signal Transducer and Activator of Transcription 3 (STAT3): STAT3 phosphorylation then stimulates BCL6 expression, thereby explaining a possible mechanism for why BCL6 is abnormally high in the endometrium of women with endometriosis. Evans-Hoeker et al[1] undertook a prospective case-control study, and determined that the BCL6 level at which endometriosis was highly likely was 1.4: BCL6 protein expression was significantly higher in the luteal/secretory phase of the menstrual cycle in women with endometriosis, compared to controls

(women without endometriosis). A cut-off of 1.4 provides a positive likelihood ratio of 15.4, and a negative likelihood ratio of 0.04 for endometriosis[1].

Hence, we are proposing to undertake a prospective study, in which patients who are already undergoing laparoscopic surgery (for any indication) have concurrent endometrial biopsy collected, using dilatation and curettage method. The endometrial biopsy sample will be sent for laboratory analysis, to determine the participant's BCL6 level. The patient's endometriosis (or lack thereof) will be surgically staged according to the revised American Society of Reproductive Medicine (r-ASRM) classification system, which is the standard scoring system for endometriosis[14] (please see Appendix 13.1). Statistical analysis will allow conclusions to be made, regarding to what extent BCL6 on endometrial biopsy are predictive of the presence/absence and stage of endometriosis. If so, this will further develop the possible use of BCL6 as a relatively non-invasive biomarker to improve patient counselling and management, potentially negating the need to stage their endometriosis laparoscopically.

3 STUDY OBJECTIVES

Our null hypothesis is that there is no correlation between BCL6 levels on luteal phase endometrial biopsy between control (no-endometriosis) and women with endometriosis.

3.1 Primary Objective

To evaluate BCL6 expression in luteal phase endometrial biopsy in women with and without endometriosis at laparoscopy.

3.2 Secondary Objectives

1) To assess whether or not there is any correlation between BCL6 levels and the severity of endometriosis at laparoscopy, based on r-ASRM classification

2) To assess whether including the results of patients' long-form Endometriosis Health Profile Questionnaire (EHP-30) in the statistical analysis (in combination with their BCL6 result) improves the likelihood of correctly predicting the presence, absence, and/or severity of endometriosis at laparoscopy

4 STUDY DESIGN

This study will be designed and carried out in compliance with the National Health and Medical Research Council's 'National Statement on Ethical Conduct in Human Research'[15].

4.1 Type of Study

Case-control study, derived from prospectively-collected samples.

4.2 Study Design

This will be a case-control study, derived from prospectively-collected samples.

Women who fulfil the selection criteria will be invited to participate in the study. Many of these women will already be planned to undertake a concurrent endometrial biopsy, at the time of their laparoscopy. If not: we will talk them through a consent form for endometrial biopsy, using the standard dilatation and curettage method. If they agree to participate, their procedure will be booked to occur during the luteal phase of their menstrual cycle (i.e. the two weeks leading up to an expected period).

A urinary pregnancy test will be completed immediately pre-operatively (i.e. on the day of the planned operation), to exclude any pregnant women (as per the exclusion criteria outlined below).

Patients will be asked to complete an online version of the long-form EHP-30 questionnaire pre-operatively[16]. The EHP-30 is a reliable, validated instrument to measure the health-related quality of life of women with endometriosis. It asks participants (over the course of 30 questions) to recall how often in the last four weeks they have experienced various symptoms of endometriosis. The 'long-form' version includes six additional sections (using 23 questions) covering areas such as: work; relationships with children; sexual relationship(s); and feelings about the medical profession, treatment, and infertility.

At their planned procedure, endometrial biopsy will be taken, using the standard dilatation and curettage method. As a guide, approximately 0.5cm3 of tissue will be biopsied, weighing approximately 2 grams. The endometrial biopsy sample will be sent to the laboratory for analysis of the BCL6 level. Laparoscopy will be undertaken (as planned), and the patient's endometriosis (if present) will be scored. Clinicians will use the best-known, and most widely used, classification system for endometriosis: the revised American Society for Reproductive Medicine (r-ASRM) classification system (please see Appendix 13.1). This involves documenting and scoring patients' endometriotic implants (seen at laparoscopy) based on their location and size, the location and thickness of any adhesions (internal scarring), plus the extent to which the 'Pouch of Douglas' (the area between the uterus and bowel) is involved. Patients will then end up being classified as having either: no endometriosis; stage I (minimal); stage II (mild); stage III (moderate); or stage IV (severe) endometriosis[14].

Later statistical analysis will aim to determine to what extent patients' BCL6 levels (on luteal phase endometrial biopsy) is predictive of the presence and severity of endometriosis at laparoscopy. Additional analysis will include patients' EHP-30 results as a predictive variable, to assess if the combination of BCL6 level plus EHP-30 answers improves the predictive value of these two screening tools for endometriosis.

4.3 Number of Participants

184. We will need to recruit 68 patients who are found to have endometriosis at laparoscopy, and 116 who are not.

4.4 Study sites

Patients who are already planning to undertake laparoscopic surgery (for any indication) with any of the investigators will be invited to participate. AL and ABS consult with patients in St Leonards, and operate at The Mater hospital (North Sydney), North Shore Private Hospital (St Leonards), and the Royal North Shore Hospital (St Leonards). Dr Lowe operates at Strathfield Private Hospital (Strathfield) and Concord Hospital (Concord). A/Prof Condous operates at Nepean Hospital (Kingswood). The surgical processes and procedures at each of these hospitals are very similar, with minute differences only in regards to the surgical equipment utilised.

The BCL6 laboratory analysis will be performed at the Douglass Hanly Moir laboratory in Macquarie Park.

4.5 Expected Duration of Study

It is expected that it will take approximately nine months to undertake this study, with recruitment beginning as soon as ethical approval and funding have been received. We expect that it will take approximately six months from the commencement of recruitment through to performing the operations on all participants. We expect statistical analysis and writing of any related reports and journal articles to take an additional three months.

4.6 Outcome Measures

Participants will be asked to fill out an online version of the long form of the EHP-30 questionnaire, which is a reliable, validated instrument to measure the health-related quality of life of women with endometriosis[16].

Participants' endometrial biopsy samples will be sent to the Douglass Hanly Moir laboratory for BCL6 analysis.

Clinicians will use the r-ASRM classification system to score participants' endometriosis intra-operatively (please see Appendix 13.1). This involves documenting and scoring patients': endometriotic implants (that are seen at laparoscopic surgery) based on their location and size, the location and thickness of any adhesions (bands of scarring between internal organs); plus the extent to which the 'Pouch of Douglas' (the area between the uterus and bowel) is involved. Patients will then end up being classified as having either: no endometriosis; stage I (minimal); stage II (mild); stage III (moderate); or stage IV (severe) endometriosis.

5 STUDY TREATMENTS

Some participants in this study would already be planning to undertake endometrial biopsy, in addition to planned laparoscopy (for example, to investigate heavy menstrual bleeding). Others may originally only be planning to undertake laparoscopy, and will (if they participate) undergo an endometrial biopsy (as part of this study), over and above the planned laparoscopy.

While the patient is anaesthetised, endometrial biopsy will be performed using a 'dilatation and curettage' technique. This involves:

- Gently dilating the cervical canal up to 5mm diameter, using 'cervical dilators'
- Using a 'curette' to take a sample of the lining of the uterus ('endometrium')

This procedure is outlined in the attached 'Patient information and consent' form, as are the potential complications, including:

- Uterine perforation (approximately 1 in 300 women)
- Formation of intra-uterine adhesions (scar tissue) (approximately 1 in 60 women)
- Cervical injury (approximately 1 in 25 women)
- Infection (approximately 1 in 20 women)
- Blood loss > 100mLs (approximately 1 in 10 women)[17–20]

(Please see Appendix 13.2 for this document.)

The potential implications of the above possible complications and their management include:

(1) Uterine perforation: collection of blood tests for full blood count; injury to bladder or bowel (1 in 15 patients who have a uterine perforation); intra-abdominal haemorrhage (1 in 6 patients who have a uterine perforation); need for laparotomy (very rare)[21]

(2) Intra-uterine adhesions: lighter menstruation (4 in 5 women with intra-uterine adhesions); sub-fertility (1 in 8 women with intra-uterine adhesions); cyclic pelvic pain (1 in 30 women with intra-uterine adhesions); recurrent pregnancy loss (1 in 8 women with intra-uterine adhesions). Management of the above issues involves hysteroscopy (a procedure performed under general anaesthetic to visualise the lining of the uterus with a small video camera), and resection of the intra-uterine adhesions (separating the intra-uterine scarring, using 'diathermy' electrosurgical devices).[22]

(3) Cervical injury: repair of any cervical tear is performed immediately, using several absorbable stitches.

(4) Infection: mild infections (the majority) are treated with tablet antibiotics; severe infections (very rare) require admission to hospital and intravenous antibiotics.

(5) Blood loss > 100mLs: observation; collection of blood tests for full blood count; administration of medications such as syntocinon and/or tranexamic acid; in the cases of very rare major haemorrhage (> 1000mLs), laparotomy (open surgery) may be needed

The above potential complications of dilatation and curettage can be compared with the potential risks of laparoscopic surgery, which include the following:

- Conversion to an open operation ('laparotomy') (approximately 1 in 70 women)
- Injury to urinary tract (bladder or ureters) (approximately 1 in 100 women)
- Injury to major (big) blood vessel (approximately 1 in 200 women)
- Injury to minor (small) blood vessel (approximately 1 in 350 women)
- Later development of hernia through one of the scars (approximately 1 in 500 women)
- Injury to gastrointestinal tract (stomach or bowels) (approximately 1 in 1000 women)[19,23,24]

After the endometrial biopsy tissue has been sent to Douglass Hanly Moir (DHM) pathology laboratories, they will be analysed to assess the BCL6 level. DHM then routinely stores all pathology slides and paraffin block for ten years. After this point, samples are incinerated.

6 PARTICIPANT ENROLLMENT AND RANDOMISATION

6.1 Recruitment

Patients are referred to our gynaecology clinics by other clinicians (e.g. general practitioners, other gynaecologists, fertility specialists) for a range of gynaecological complaints and conditions, including: pelvic pain, pelvic organ prolapse, leiomyomata, endometriosis, ovarian cysts, infertility, heavy menstrual bleeding, and urinary incontinence. All patients who fulfil the inclusion criteria (as outlined below) will be invited to participate.

6.2 Eligibility Criteria

Inclusion criteria will be:

- Women aged between 18 – 50yo
- Women who have a regular menstrual cycle (i.e. menstrual cycle length 21 - 35 days inclusive)
- Women who are already planning to undertake laparoscopy, for any indication
- English speaking
- Capacity and willingness to give written informed consent
- Willingness to comply with the study
- Willingness to have their planned procedure undertaken in the luteal phase of their menstrual cycle

Exclusion criteria will include:

- Women aged < 18yo or > 50yo
- Women with an irregular menstrual cycle (i.e. menstrual cycle length < 21 days or > 35 days)
- Women who have an absolute contra-indication to endometrial biopsy, such as viable (ongoing) intra-uterine pregnancy
- Women who have a relative contra-indication to endometrial biopsy, such as: active cervical or uterine infection
- Women in whom undertaking an (otherwise unnecessary) endometrial biopsy may worsen a pre-existing condition (e.g. Asherman's syndrome, intra-uterine adhesions)

Women with an unwanted / non-viable pregnancy will also be excluded, as the potential risks of undertaking a dilatation and curettage in that context are significantly higher than in a non-gravid (not pregnant) uterus. A urinary pregnancy test will be completed immediately pre-operatively (i.e. on the day of the planned operation, in the pre-operative / admission area of the relevant hospital), to exclude any pregnant women.

6.3 Informed Consent Process

Potential participants will be given the attached 'Patient Information and Consent' form at least two weeks prior to their planned procedure to read at their leisure (please see Appendix 13.2). This outlines the potential risks of undertaking an endometrial biopsy.

Prior to their planned procedure, they will be given the opportunity to clarify their understanding with a clinician not associated with this study (e.g. surgical assistant or consultant anaesthetist). They will then be invited to sign the attached 'Patient Information and Consent' form.

6.4 Enrolment Procedure

Participants will be enrolled into the study after the informed consent process has been completed (as above), and the participant has met all inclusion criteria and none of the exclusion criteria. The participant will receive a study enrolment number; thereafter, all of their data will be linked with this enrolment number only.

6.5 Blinding Arrangements

The surgeons who classify participants' endometriosis intra-operatively will not yet have the result of their BCL6 endometrial biopsy. The pathologists who determine participants' BCL6 result will be blinded as to participants' endometriosis severity.

6.6 Participant Withdrawal

Participants are able to withdraw from the endometrial biopsy part of the study up until they are anaesthetised. Should any participants ask to withdraw from the study pre-operatively, endometrial biopsy will not be undertaken.
Should they later decide that they want to withdraw from the study, their data can be removed from the database up until the point of analysis.

6.7 Trial Closure

All participants will receive follow-up through our private gynaecology clinic. This involves: a telephone call from our clinic nurse at approximately 1 week post-operatively (henceforth known as 'follow-up 1'); and two phone calls at approximately 4 weeks post-operatively ('follow-up 2'). One phone call will be from the Principal Investigator, to explain to the patient the stage of endometriosis (if present), and their BCL6 HSCORE. The other phone call will be from the clinic nurse, to ask if they have experienced any complications, such as uterine perforation, fever, or post-operative haemorrhage. If any patient reports any complications, the nurse will escalate immediately to the Principal Investigator.

Should any patient have any concerns post-operatively, they are welcome to call our clinic to discuss these concerns over the phone, and/or to come in for consultation.

7 STUDY VISITS AND PROCEDURES SCHEDULE

List of interventions	**Enrolment Visit**	**Day of procedure**	**Follow-up 1**	**Follow-up 2**
Patient information sheet discussed	✓	✓		
Consent signed		✓		
Inclusion / exclusion criteria discussed	✓	✓		
Stage of endometriosis classified		✓		
Adverse event and serious event assessment			✓	✓
Stage of endometriosis discussed with participant				✓

8 CLINICAL AND LABORATORY ASSESSMENTS

Patient's BCL6 results on their endometrial biopsy samples will be analysed at Douglass Hanly Moir pathology laboratories, based in Macquarie Park.

Formalin-fixed, paraffin-embedded tissue blocks will be sectioned at 4 mm. Slides will be stained with haematoxylin–eosin, and consecutive sections stained with antibodies against BCL6 (clone GI191E/A8; Cell Marque, Rocklin, California). Immunohistochemistry will be performed on an automated system by a certified Pathology Laboratory (Douglass Hanly Moir, Macquarie Park, Sydney) using the Ventana Benchmark Ultra Auto-immunostainer platform (Roche Diagnostics). Antigen retrieval will be performed with Tris based buffer (CC1, Roche Dianostics, Arizona). Positive immunoreactivity (Brown precipitate) will be detected using the OptiView DAB Detection Kit (Roche Diagnostics, Arizona) A positive control of lymph node will be included. The semiquantitative assessment of expression will be made using the HSCORE (0-4), calculated using the following equation: HSCORE . P Pi (i . 1)/100, where i . intensity of staining with a value of 1, 2, or 3, (weak, moderate, or strong, respectively) and Pi is the percentage of stained epithelial cells for each intensity, varying from 0% to 100%.

The use of an automated system for immunostaining will reduce a potential bias in immunohistochemical analysis; this automated system will be read by a gynaecologic pathologist [either Adj. Prof Annabelle Farnsworth (AF), or Dr Sophie Corbett-Burns (SCB)].

Participants' endometriosis severity will be classified intra-operatively according to the r-ASRM: this is the standard scoring system for endometriosis[14]. This involves documenting and scoring patients': endometriotic implants based on their location and size, the location and thickness of any adhesions (internal scarring), plus the extent to which the 'Pouch of Douglas' (the area between the uterus and bowel) is involved (please see Appendix 13.1). Patients will then be classified as having either: no endometriosis; stage I (minimal); stage II (mild); stage III (moderate); or stage IV (severe) endometriosis[14].

9 ADVERSE EVENT REPORTING

9.1 Definition

An 'adverse event' following a procedure is also referred to as an adverse experience. It refers to any untoward medical occurrence in a clinical investigation participant which may or may not have a causal relationship with that procedure. An adverse event can therefore be any unfavourable and unintended sign, symptom, or disease associated with the procedure.

9.2 Eliciting Adverse Event Information

Included in the 'Patient Information and Consent' form is an outline of potential adverse events post-operatively (such as infection) (please see Appendix 13.2). Patients will be encouraged to contact the research team should they develop any concerning symptoms post-operatively (e.g. temperature ≥ 38.0, malodorous vaginal discharge, worsening abdominal pain etc).

An adverse event checklist will be completed at both the first and second follow-up consultation post-operatively, along similar lines.

9.3 Assessment and Documentation of Adverse Events

Should there be concern that a patient is developing or has sustained an adverse event, they will be assessed clinically by ABS and/or AL. All possible adverse events will be documented in the study log.

9.4 Serious Adverse Event (SAE) Reporting

9.4.1 SAEs

For this study, a 'serious adverse event' is defined as any adverse event that occurs in the course of clinical research, and that: results in death; is life-threatening; requires hospitalisation or prolonged existing hospitalisation; or results in persistent or significant disability or incapacity.

9.4.2 Suspected Unexpected Serious Adverse Reaction (SUSAR)

For this study, a 'suspected unexpected serious adverse reaction' is defined as adverse events that are suspected to be related to an investigational procedure, and that are both unexpected and serious.

10 STATISTICAL METHODS

Regarding participants' answers to questions in the EHP-30 questionnaire: RedCap automatically exports data to statistical software SPSS, which will be used to perform the statistical analysis. Participants' BCL6 levels will be uploaded into the relevant section of the SPSS document used for this research, as will participants' endometriosis severity (based on the r-ASRM classification system).

Participants' BCL6 results will be continuous data, with their BCL6 HSCORE ranging from 0 to 4. Participants' endometriosis severity will be ordinal data, in that all participants will be categorised into one of five distinct groups: no endometriosis; stage I (minimal) endometriosis; stage II (mild) endometriosis; stage III (moderate) endometriosis; or stage IV (severe) endometriosis. It is expected that participants' BCL6 results by endometriosis severity will be presented in a boxplot.

Receiver-operator characteristic analysis will be performed to assess the usefulness of the biomarker and to find the optimal cut-off score for BLC6 that differentiates best between cases and controls.

Students t test will be used to compare mean BLC6 levels between cases and controls.

Regarding participants' EHP-30 questionnaire results: logistic regression analysis and a multi-variable model will enable us to ascertain whether including particular aspects of the long-form EHP-30 improves the predictive value of BCL6 in finding endometriosis at laparoscopy. If any such relationships are found, it is expected that these results could be presented as a nomogram, or classification tree analysis.

The statistical comparisons will be performed using statistical software SPSS (IBM), with P values < 0.05 considered significant.

10.1 Sample Size Estimation

Our estimated sample size of 184 patients is based on the following assumptions:

(1) For approximately one-third of our patient population, endometriosis is not suspected pre-operatively (i.e. laparoscopy performed for another indication, such as laparoscopic myomectomy)

(2) Approximately 10% of these patients will be found to have endometriosis at laparoscopy

(3) For approximately two thirds of our patient population, endometriosis is expected to be found at laparoscopy

(4) Approximately 50% of these patients will be found to have endometriosis at laparoscopy

This gives an overall prevalence of endometriosis of approximately 37% amongst our patient population. Assuming the sensitivity and specificity of BCL6 testing for endometriosis are at least 85% (from Evans-Hoeker et al[1]), the sample size of 184 will provide precision of +/- 10% for 95% confidence intervals. We will need to recruit 68 patients who are found to have endometriosis at laparoscopy, and 116 who are not.

The sample size of 184 patients will provide 80% power to detect a difference of 0.374 in average BLC6 levels between controls (women without endometriosis at laparoscopy) and cases (women with endometriosis at laparoscopy) using a two-sided 5% significance level.

10.2 Population to be analysed

Women aged 18-50yo with regular menstrual cycles (21 – 35 days' duration, inclusive), who are planned to have laparoscopic surgery with the research team for any indication.

10.3 Statistical Analysis Plan

Regarding participants' answers to questions in the EHP-30 questionnaire: RedCap automatically exports data to statistical software SPSS, which will be used to perform the statistical analysis. Participants' BCL6 levels will be uploaded into the relevant section of the SPSS document used for this research, as will participants' endometriosis severity (based on the r-ASRM classification system).

Participants' BCL6 results will be continuous data, with their BCL6 HSCORE ranging from 0 to 4. Participants' endometriosis severity will be ordinal data, in that all participants will be categorised into one of five distinct groups: no endometriosis; stage I (minimal) endometriosis; stage II (mild) endometriosis; stage III (moderate) endometriosis; or stage IV (severe) endometriosis. It is expected that participants' BCL6 results by endometriosis severity will be presented in a boxplot.

Receiver-operator characteristic analysis will be performed to assess the usefulness of the biomarker and to find the optimal cut-off score for BLC6 that differentiates best between cases and controls.

Students t test will be used to compare mean BLC6 levels between cases and controls.

Regarding participants' EHP-30 questionnaire results: logistic regression analysis and a multi-variable model will enable us to ascertain whether including particular aspects of the long-form EHP-30 improves the predictive value of BCL6 in finding endometriosis at laparoscopy. If any such relationships are found, it is expected that these results could be presented as a nomogram, or classification tree analysis.

The statistical comparisons will be performed using statistical software SPSS (IBM), with P values < 0.05 considered significant.

11 DATA MANAGEMENT

11.1 Data Collection

'RedCap' will be used to develop an online version of the long-form EHP-30 questionnaire. Participants will complete this questionnaire online, pre-operatively.

Participants' BCL6 results will be analysed and collated at the Douglass Hanly Moir pathology laboratories by AF and SCB, and forwarded on to ABS in due course.

Participants' endometriosis severity will be classified intra-operatively by the relevant surgeon, according to the r-ASRM classification system. These results will be documented immediately post-operatively into the participant's 'case report form' (CRF). The hard copy of the CRF will be given to the Principal Investigator by hand, and the data entered into 'RedCap'.

11.2 Data Storage

All data will be stored in an Excel spreadsheet, on a NSLHD server. Only two authors (ABS and AL) will have access to the source data and the Excel spreadsheet.

Each participant will be allocated a 'enrolment number' in chronological order, based upon their operation date and time. Thereafter, their data (EHP-30 results, BCL6 result, endometriosis severity) will be stored based on their enrolment number only.

The Excel spreadsheet that links each participant's name with their unique enrolment number will be stored on a password-protected computer on a NSLHD served, and will only be accessible to ABS and AL.

The hard copies of the CRFs will be stored in a locked filing cabinet at the Centre for Advanced Reproductive Endosurgery, for fifteen years after the last related publication.

11.3 Data Confidentiality

Once participants are allocated an enrolment number, all of their data will be stored based on their enrolment number (and not any identifying features). Records will be de-identified thereafter.

For publication, participants' data will be grouped according to the severity of their endometriosis (for example: no endometriosis / stage I / II / III / IV), without any identifying features.

11.4 Study Record Retention

We will retain the data for fifteen years after the last publication.

12 ADMINISTRATIVE ASPECTS

We have applied for registration in the Australian and New Zealand Clinical Trials Registry.

12.1 Independent HREC approval

We patiently await approval by the North Sydney Local Health District Human Research Ethics Committee (HREC).

12.2 Amendments to the protocol

Any amendments will be submitted to the HREC for review prior to implementation, as per HREC guidelines.

12.3 Protocol deviations

Any protocol deviations will be submitted to the HREC for review.

12.4 Participant reimbursement: N/A

12.5 Financial disclosure and conflicts of interest

A/Prof Condous reports grants from Australian Women and Children's Research Foundation, BUPA, personal fees from Roche Diagnostics and personal fees from GE Healthcare.

No other authors have reported any financial disclosures or conflicts of interest.

13 USE OF DATA AND PUBLICATIONS POLICY

We hope to write at least two publications based on this research, which we hope will be published in peer-reviewed medical journals (e.g. Journal of Minimally Invasive Gynecology, British Journal of Obstetrics and Gynaecology, The Australian and New Zealand Journal of Obstetrics and Gynaecology). ABS will take the lead in writing any publications that result from this research, while her co-authors will provide guidance and editorial expertise.

14 REFERENCES

1. Evans-Hoeker E, Lessey BA, Jeong JW, Savaris RF, Palomino WA, Yuan L, et al. Endometrial BCL6 Overexpression in Eutopic Endometrium of Women with Endometriosis. Reprod Sci. 2016;23(9):1234–41.

2. Schenken RS. Endometriosis: pathogenesis, clinical features and diagnosis. [Internet]. UpToDate. 2020 [cited 2020 Aug 7]. Available from: https://www.uptodate.com.acs.hcn.com.au/contents/endometriosis-pathogenesis-clinical-features-and-diagnosis?search=endometriosis&source=search_result&selectedTitle=1~150&usage_type=default&display_rank=1#H2

3. Singh N, Rashid M, Herath RP. How can we reduce negative laparoscopies for pelvic pain? J Obstet Gynaecol (Lahore). 2011;31(1):62–8.

4. Hsu AI, Khachikyan I, Stratton P. Invasive and non-invasive methods for the diagnosis of endometriosis. Clin Obstet Gynecol. 2011;53(2):413–9.

5. Barnhart K, Dunsmoor-Su R, Coutifaris C. Effect of endometriosis on in vitro fertilization. Fertil Steril. 2002;77(6):1148–55.

6. Tokushige N, Markham R, Russell P, Fraser IS. High density of small nerve fibres in the functional layer of the endometrium in women with endometriosis. Hum Reprod. 2006;21(3):782–7.

7. Al-Jefout M, Dezarnaulds G, Cooper M, Tokushige N, Luscombe GM, Markham R, et al. Diagnosis of endometriosis by detection of nerve fibres in an endometrial biopsy: A double blind study. Hum Reprod. 2009;24(12):3019–24.

8. Bokor A, Kyama CM, Vercruysse L, Fassbender A, Gevaert O, Vodolazkaia A, et al. Density of small diameter sensory nerve fibres in endometrium: A semi-invasive diagnostic test for minimal to mild endometriosis. Hum Reprod. 2009;24(12):3025–32.

9. Newman TA, Bailey JL, Stocker LJ, Woo YL, MacKlon NS, Cheong YC. Expression of neuronal markers in the endometrium of women with and those without endometriosis. Hum Reprod. 2013;28(9):2502–10.

10. Ellett L, Readman E, Newman M, McIlwaine K, Villegas R, Jagasia N, et al. Are endometrial nerve fibres unique to endometriosis? A prospective case-control study of endometrial biopsy as a diagnostic test for endometriosis in women with pelvic pain. Hum Reprod. 2015;30(12):2808–15.

11. Liutkevičienė R, Mečėjus G, Žilovič D, Bumbulienė Ž. Endometrial biopsy and density of nerve fibers in eutopic endometrium. Looking for easier ways to diagnose endometriosis. Gynecol Endocrinol [Internet]. 2019;35(12):1107–10. Available from: https://doi.org/10.1080/09513590.2019.1640198

12. Yoo JY, Kim TH, Fazleabas AT, Palomino WA, Ahn SH, Tayade C, et al. KRAS Activation and over-expression of SIRT1/BCL6 Contributes to the Pathogenesis of Endometriosis and Progesterone Resistance. Sci Rep. 2017;7(1):1–12.

13. Lessey BA, Kim JJ. Endometrial receptivity in the eutopic endometrium of women with endometriosis: it is affected, and let me show you why. Fertil Steril [Internet]. 2017;108(1):19–27. Available from: http://dx.doi.org/10.1016/j.fertnstert.2017.05.031

14. American Society for Reproductive Medicine. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril. 1997;67(5):817–21.

15. Australian Government: National Health and Medical Research Council. Conduct in human research national statement on ethical conduct in human research [Internet]. Vol. 2007. 2018. 104 p. Available from: www.nhmrc.gov.au/guidelines/publications/e72%0Ahttps://www.nhmrc.gov.au/about-us/publications/national-statement-ethical-conduct-human-research-2007-updated-2018

16. Jones G, Kennedy S, Barnard A, Wong J, Jenkinson C. Development of an Endometriosis Quality-of-Life Instrument. Obstet Gynecol. 2001;98(2):258–64.

17. Farooq F, Javed L, Mumtaz A, Naveed N. Comparison of manual vacuum aspiration, and dilatation and curettage in the treatment of early pregnancy failure. J Ayub Med Coll Abbottabad. 2011;23(3):28–31.

18. Tunçalp Ö, Gülmezoglu AM, Souza JP. Surgical procedures for evacuating incomplete miscarriage. Cochrane Database Syst Rev. 2010;

19. Braaten KP, Dutton C. Dilatation and curettage. UpToDate. 2020.

20. Deans R, Abbott J. Review of Intrauterine Adhesions. J Minim Invasive Gynecol. 2010;17(5):555–69.

21. Levy B. Uterine perforation during gynecologic procedures. UpToDate. 2020;

22. Cedars M, Adeleye A. Intrauterine adhesions: Clinical manifestation and diagnosis. UpToDate. 2020;

23. Pryer A, Mann W, Bates A. Complications of laparoscopic surgery. UpToDate. 2020;

24. Lim C, Mowers E, Mahnert N, Skinner B, Kamdar N, Morgan D, et al. Risk Factors and Outcomes for Conversion to Laparotomy of Laparoscopic Hysterectomy in Benign Gynecology. Obstet Gynecol. 2016;128(6).

15 APPENDICES

15.1 Revised ASRM classification system for endometriosis

American Society for Reproductive Medicine revised classification of endometriosis

Patient's name _____ Date _____

Stage I (minimal) _____ 1 to 5 Laparoscopy _____

Stage II (mild) _____ 6 to 15 Laparotomy _____

Stage III (moderate) _____ 16 to 40 Photography _____

Stage IV (severe) _____ >40 Recommended treatment _____

Total _____ Prognosis _____

Peritoneum	Endometriosis	<1 cm	1 to 3 cm	>3 cm
	Superficial	1	2	4
	Deep	2	4	6
Ovary	R superficial	1	2	4
	Deep	4	16	20
	L superficial	1	2	4
	Deep	4	16	20
	Posterior cul-de-sac obliteration	Partial		Complete
		4		40
Ovary	Adhesions	<1/3 enclosure	1/3 to 2/3 enclosure	>2/3 enclosure
	R filmy	1	2	4
	Dense	4	8	16
	L filmy	1	2	4
	Dense	4	8	16
Tube	R filmy	1	2	4
	Dense	4*	8*	16
	L filmy	1	2	4
	Dense	4*	8*	16

* If the fimbriated end of the fallopian tube is completely enclosed, change the point assignment to 16. Denote appearance of superficial implant types as red ([R], red-pink, flamelike, vesicular blobs, clear vesicles), white ([W], opacifications, peritoneal defects, yellow-brown), or black ([B], black, hemosiderin deposits, blue). Denote percent of total described as R__ percent, W__ percent, and B __ percent. Total should equal 100 percent.

Original figure modified for this publication. American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril 1997; 67:817. Illustration used with the permission of Elsevier Inc. All rights reserved.

1.2 15.2 Patient Information and Consent form, for patients already planning to have an endometrial biopsy

Participant Information and Consent form

Study title: B-cell lymphoma 6 protein (BCL6) as a potential biomarker for endometriosis: can it be used to predict endometriosis' presence and/or severity?

Principal investigator: Dr Alison Bryant-Smith

AGES Accredited Training Program trainee, Centre for Advanced Reproductive Endosurgery (CARE)

You are invited to take part in this study, which is called 'B-cell lymphoma 6 protein (BCL6) as a potential biomarker for endometriosis: can it be used to predict endometriosis' presence and/or severity'? You have been invited because you are planning to have keyhole surgery with one of the following gynaecologists: A/Prof Alan Lam, A/Prof George Condous, or Dr Jessica Lowe. This Participant Information and Consent Form tells you about the research project. It explains the processes involved in taking part. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand, or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend, or local health worker.

Participation in this research is voluntary. If you don't wish to take part, you don't have to.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it, you are telling us that you:

- Understand what you have read
- Consent to take part in the research project
- Consent to be involved in the research described
- Consent to the use of your personal and health information as described

You will be given a copy of this Participant Information and Consent form to keep.

What is the purpose of the study?

This research is trying to find a way of screening for a condition called 'endometriosis', using samples of the lining of the womb (an 'endometrial biopsy'), rather than keyhole surgery. This research has been initiated by the Principal Investigator, Dr Alison Bryant-Smith.

Purpose of this study

Endometriosis is a common condition, affecting approximately 10% (1 in 10) of women of reproductive age. Endometriosis leads to a build-up of old period blood collecting around a woman's internal organs (e.g. ovaries, Fallopian tubes, womb). Endometriosis can cause problems such as: severe period pain, difficulty falling pregnant, and pain during sex.

Currently, the best way to diagnose endometriosis is by having keyhole surgery. Imaging (e.g. ultrasound or 'MRI') is helpful in severe endometriosis, however keyhole surgery remains the only way to diagnose mild endometriosis. There are currently no less invasive ways to diagnose or screen for endometriosis, such as blood tests.

Some researchers overseas have found that taking a tiny sample of tissue from the lining of a woman's womb can be helpful: measuring the level of a substance called 'B-cell lymphoma 6 protein' (or 'BCL6') in this tissue can help predict whether or not endometriosis is present at keyhole surgery.

Our current research project aims to determine if there is a link between how abnormal a woman's BCL6 result is, and how bad her endometriosis is.

Selection

All women aged 18 – 50 years old who are planning to have keyhole surgery with A/Prof Alan Lam, A/Prof George Condous, or Dr Jessica Lowe will be invited to participate. Participants need to have a regular menstrual cycle (i.e. no shorter than 21 days, and no longer than 35 days).

What will my participation in the study involve?

You have been invited to participate in this research because you have been offered keyhole surgery ('laparoscopy') with A/Prof Alan Lam, A/Prof George Condous, or Dr Jessca Lowe.

You will be asked to complete a questionnaire regarding possible symptoms of endometriosis. This validated questionnaire is called the 'Endometriosis Health Profile Questionnaire', or 'EHP-30'. This takes approximately five minutes to complete.

As you are already planning to have a sample of the lining of the womb taken as part of your planned operation, there will be no additional risk to you: we will undertake your operation as planned, while you are asleep (or 'anaesthetised'). Once the endometrial biopsy sample has been collected, we will send one part of the biopsy tissue to the laboratory for the usual analysis, and another part of the tissue to a different laboratory to measure the BCL6 level. During your operation, your surgeon will classify how bad your endometriosis is, by looking closely at all your pelvic organs with our keyhole surgery camera.

Endometrial biopsy procedure

As part of your planned operation, you will be given a general anaesthetic (i.e. you won't feel or remember anything during the operation). As originally planned, you will have both keyhole surgery and an endometrial biopsy performed (i.e. a sample of the lining of the womb taken).

After the endometrial biopsy tissue has been sent to the pathology laboratory, it will be analysed to assess the BCL6 level. The pathology company then routinely stores all pathology slides and paraffin block for ten years. After this point, samples are incinerated.

Data collection

In addition to your answers for the 'EHP-30' questionnaire, and the endometrial biopsy sample, your surgeon will assess how bad your endometriosis (if you have any) is, during your keyhole surgery. This would be done during all laparoscopies in any case, whether or not you participate in this research. We use the standard endometriosis classification system, which is called the 'revised American Society of Reproductive Medicine' classification system for endometriosis. This involves looking closely at your pelvic organs using the the keyhole surgery camera, and classifying any endometriosis present by its location, size and depth.

Follow-up

Your follow-up consultations will be similar, whether or not you participate in this research. Our clinic nurse will call you approximately 1 week after your operation, to see how you are recovering, and clarify whether you have suffered any complications of the surgery. The clinic nurse will call you again at approximately four weeks after your operation, to ask if you have suffered any complications of the surgery. The Principal Investigator will also call you at approximately four weeks after your operation, to explain what stage of endometriosis was found (if any), and the results of the BCL6 test that was done on the sample of your endometrial lining.

What are the possible benefits and risks of this study?

If this research can help develop a way to diagnose the presence and severity of endometriosis using only an endometrial biopsy, there would be significant benefits to many other women in future. For example: women who may have endometriosis based on their symptoms could have an endometrial biopsy taken (while awake, in a clinic room). The result may help guide whether or not she would benefit from laparoscopic surgery. It may also help to guide whether keyhole surgery or *in vitro* fertilisation (IVF) is the next best step.

There will be no clear benefits to you from your participation in this research.

In terms of the potential risks to you if you choose to participate: as you are already planning to have an endometrial biopsy as part of your operation, there will be no additional risks to you. The potential complications of having an endometrial biopsy done include:

- Uterine perforation (small hole in the muscle of the uterus) (approximately 1 in 300 women)
- Formation of scar tissue inside the cavity of the uterus (approximately 1 in 60 women)
- Injury to the cervix (neck of the womb) (approximately 1 in 25 women)
- Infection (approximately 1 in 20 women)
- Blood loss > 100mLs (approximately 1 in 10 women)

By and large, having an endometrial biopsy performed is a very safe procedure.

The potential implications of the above possible complications and their management include:

- Uterine perforation: collection of blood tests for full blood count; injury to bladder or bowel (1 in 15 patients who have a uterine perforation); intra-abdominal bleeding (1 in 6 patients who have a uterine perforation); need for open surgery (very rare)[21]
- Formation of scar tissue inside the cavity of the uterus: lighter periods (4 in 5 women with intra-uterine scarring); decreased fertility (1 in 8 women with intra-uterine adhesions); cyclical pelvic pain (1 in 30 women with intra-uterine adhesions); recurrent pregnancy loss (1 in 8 women with intra-uterine adhesions). Management of the above issues involves hysteroscopy (a procedure performed under general anaesthetic to visualise the lining of the uterus with a small video camera), and resection of the intra-uterine adhesions (separating the intra-uterine scarring, using electrosurgical devices).[22]
- Injury to the cervix: repair of any cervical injury is performed immediately, using absorbable stitches.
- Infection: mild infection (the majority) are treated with tablet antibiotics; severe infections (very rare) require admission to hospital and intravenous antibiotics.
- Blood loss > 100mLs: observation; collection of blood tests for full blood count; administration of medications to help control the bleeding; in the cases of very rare major haemorrhage (> 1000mLs), open surgery may be needed

The potential risks of keyhole surgery include:

- Conversion to an open operation ('laparotomy') (approximately 1 in 70 women)
- Injury to urinary tract (bladder or ureters) (approximately 1 in 100 women)
- Injury to major (big) blood vessel (approximately 1 in 200 women)
- Injury to minor (small) blood vessel (approximately 1 in 350 women)
- Later development of a hernia through one of the scars (approximately 1 in 500 women)
- Injury to gastrointestinal tract (stomach or bowels) (approximately 1 in 1000 women)

Who pays for the study?

Pathology Company 'Douglass Hanly Moir' has agreed to perform the BCL6 tests on endometrial biopsy samples free of charge. In exchange for funding, fertility company 'Genea' will be acknowledged in publications. No study data or personal information will be sent to either of these companies.

What happens if I suffer injury or complications as a result of the study?
If you suffer any injuries or complications as a result of this study, you should contact the study doctor as soon as possible, who will assist you in arranging appropriate medical treatment. You may have a right to take legal action to obtain compensation for any injuries or complications resulting from the study. Compensation may be available if your injury or complication is caused by the drugs or procedures, or by the negligence of any of the parties involved in the study. If you receive compensation that includes an amount for medical expenses, you will be required to pay for your medical treatment from those compensation monies.
If you are not eligible for compensation for your injury or complication under the law, but are

eligible for Medicare, then you can receive any medical treatment required for your injury or complication free of charge as a public patient in any Australian public hospital. If applicable The parties to this study agree to follow the Medicines Australia Guidelines for Compensation for Injury Resulting from Participation in an Industry-Sponsored Clinical Trial. These Guidelines allow for some claims for compensation to be settled without the need for legal action to be taken. The fact that the parties have agreed to abide by these guidelines in respect of the clinical trial does not affect your rights to pursue a legal remedy in respect of any injury you may suffer as a result of participation. You can obtain a copy of these Guidelines from the Secretary of the Human Research Ethics Committee.

What are my rights?

Participation in this research is completely voluntary: you are free to decline to participate, or withdraw from the study at any time. You do not need to provide a reason. Choosing not to participate, or withdrawing from the study, will not affect the care or treatment you receive from our team.

As a participant in this research, you have a right to access all information collected about you for the purposes of the study.

By signing the consent form, you consent to the research team collecting and using personal information about you for the research project. Any information obtained in connection with this research project that can identify you will remain confidential. All information collected about you will be stored in a secure Excel spreadsheet, which is password-protected, and only accessible by investigators on the study. No identifiable information about any research participants will be published or provided to anyone outside of this research.

It is anticipated that the results of this research project will be published and/or presented in a variety of ways. In any publication and/or presentation, information will be provided in such a way that you cannot be identified.

What happens after the study, or if I change my mind?

De-identified data collected about you will be stored electronically for 15 years, and then destroyed. When the research is completed, you will receive information about results of the study in a brief publication sent to the email address you provide to us.

If you change your mind, and decide not to participate in the study, please contact the Principal Investigator (whose contact details are listed below). If you have not yet had your surgery, your original surgery will be undertaken, without any additional components that were only needed because of your participation in this research. If you have already had your operation, any data relating to your involvement will be removed from the data to be analysed if possible. (This step is only possible up to the point of completion of data collection. Once the results from all participants start being analysed, it will not be possible to remove your data from other participants' data.)

You are welcome to decline involvement in the study: doing so will not have any impact on how the team of surgeons, nurses, and anaesthetists treat you.

Who has reviewed the research project?

All research in Australia involving humans is reviewed by an independent group of people called Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the Northern Sydney Local Health District HREC. This project will be carried out according to the *National Statement on Ethical Conduct in Human Research*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

Who do I contact for more information if I have concerns?

This research is being led by clinicians from the Centre for Advanced Reproductive Endosurgery: A/Prof Alan Lam and Dr Alison Bryant-Smith. Other clinicians involved in this study include A/Prof George Condous, Dr Jessica Lowe, pathologists from Douglass Hanly Moir pathology company, and an infertility specialist (Dr Alison Gee).

If you have any questions, concerns or complaints about the research at any stage, you can contact:

Principal investigator Alison Bryant-Smith at alison.bryant-smith@sydneycare.com.au or

(02) 9966 9121.

Associate Investigator Alan Lam at alanlam@sydneycare.com.au or (02) 9966 9121.

If you would like to talk to someone who is independent of this research, you can contact the Human Research and Ethics Committee (HREC) that approved this study on:

Phone: (02) 9926 4590

Email: NSLHD-Research@health.nsw.gov.au

The Northern Sydney Local Health District HREC approved this study. If you would like to contact the HREC with any feedback or concerns, please quote reference number 2020/ETH02388.

Confidential

Consent form

Study title: B-cell lymphoma 6 protein (BCL6) as a potential biomarker for endometriosis: can it be used to predict endometriosis' presence and/or severity?

Principal investigator: Dr Alison Bryant-Smith MBBS/BA MPH MSurgEd MRCOG FRANZCOG

AGES Accredited Training Program trainee, Centre for Advanced Reproductive Endosurgery (CARE)

408 / 69 Christie St, St Leonards, 2065, alison.bryant-smith@sydneycare.com.au

Declaration by Participant

I have read the Patient Information Sheet (or someone has read it to me in a language that I understand).

I understand the purposes, procedures, and risks of the research described in the project.

My surgeon will be: A/Prof Alan Lam ☐

A/Prof George Condous ☐

Dr Jessica Lowe ☐

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described, and understand that I am free to withdraw at any time during the project without affecting my future care.

I understand that I will be given a signed copy of this document to keep.

Name of Participant (please print): _____

Signature: _____ Date: _____ / _____ / _____

Declaration by Researcher / associate

I have given a verbal explanation of the research project, its procedures and risks, and I believe that the participant has understood that explanation.

Name of Researcher (please print): _____

Signature: _____ Date: _____ / _____ / _____

1.3 15.4 Patient Information and Consent form, for patients not planning to have an endometrial biopsy

Participant Information and Consent form

Study title: B-cell lymphoma 6 protein (BCL6) as a potential biomarker for endometriosis: can it be used to predict endometriosis' presence and/or severity?

Principal investigator: Dr Alison Bryant-Smith

AGES Accredited Training Program trainee, Centre for Advanced Reproductive Endosurgery (CARE)

You are invited to take part in this study, which is called 'B-cell lymphoma 6 protein (BCL6) as a potential biomarker for endometriosis: can it be used to predict endometriosis' presence and/or severity'? You have been invited because you are planning to have keyhole surgery with one of the following gynaecologists: A/Prof Alan Lam, A/Prof George Condous, or Dr Jessica Lowe. This Participant Information and Consent Form tells you about the research project. It explains the processes involved in taking part. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully. Ask questions about anything that you don't understand, or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend, or local health worker.

Participation in this research is voluntary. If you don't wish to take part, you don't have to.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it, you are telling us that you:

- Understand what you have read
- Consent to take part in the research project
- Consent to be involved in the research described
- Consent to the use of your personal and health information as described

You will be given a copy of this Participant Information and Consent form to keep.

What is the purpose of the study?

This research is trying to find a way of screening for a condition called 'endometriosis', using samples of the lining of the womb (an 'endometrial biopsy'), rather than keyhole surgery. This research has been initiated by the Principal Investigator, Dr Alison Bryant-Smith.

Purpose of this study

Endometriosis is a common condition, affecting approximately 10% (1 in 10) of women of reproductive age. Endometriosis leads to a build-up of old period blood collecting around a woman's internal organs (e.g. ovaries, Fallopian tubes, womb). Endometriosis can cause problems such as: severe period pain, difficulty falling pregnant, and pain during sex.

Currently, the best way to diagnose endometriosis is by having keyhole surgery. Imaging (e.g. ultrasound or 'MRI') is helpful in severe endometriosis, however keyhole surgery remains the only way to diagnose mild endometriosis. There are currently no less invasive ways to diagnose or screen for endometriosis, such as blood tests.

Some researchers overseas have found that taking a tiny sample of tissue from the lining of a woman's womb can be helpful: measuring the level of a substance called 'B-cell lymphoma 6 protein' (or 'BCL6') in this tissue can help predict whether or not endometriosis is present at keyhole surgery.

Our current research project aims to determine if there is a link between how abnormal a woman's BCL6 result is, and how bad her endometriosis is.

Selection

All women aged 18 – 50 years old who are planning to have keyhole surgery with A/Prof Alan Lam, A/Prof George Condous, or Dr Jessica Lowe will be invited to participate. Participants need to have a regular menstrual cycle (i.e. no shorter than 21 days, and no longer than 35 days).

What will my participation in the study involve?

You have been invited to participate in this research because you have been offered keyhole surgery ('laparoscopy') with A/Prof Alan Lam, A/Prof George Condous, or Dr Jessca Lowe.

You will be asked to complete a questionnaire regarding possible symptoms of endometriosis. This validated questionnaire is called the 'Endometriosis Health Profile Questionnaire', or 'EHP-30'. This takes approximately five minutes to complete.

As you were not originally planning to have a sample of the lining of your womb taken as part of your procedure, this would be an additional part of your operation, done only for these research purposes, after you have been anaesthetised. The potential risks of having this sample taken are outlined below.

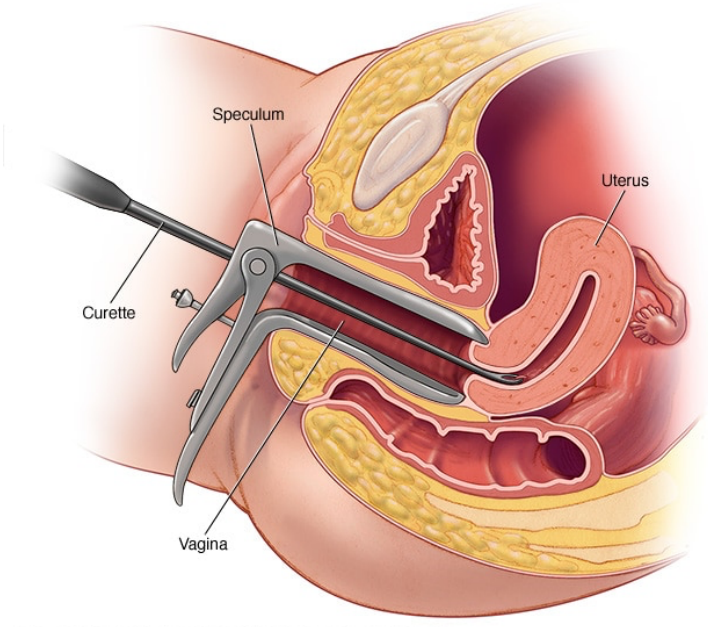
Once the endometrial biopsy sample has been collected, we will send one part of the biopsy tissue to the laboratory for the usual analysis, and another part of the tissue to a different laboratory to measure the BCL6 level. During your operation, your surgeon will classify how bad your endometriosis is, by looking closely at all your pelvic organs with our keyhole surgery camera.

Endometrial biopsy procedure

As part of your planned operation, you will be given a general anaesthetic (i.e. you won't feel or remember anything during the operation). If you agree to participate in this research, in addition to your planned keyhole surgery, an endometrial biopsy will be taken.

To take a sample of the layer of cells lining the inside of your womb, your surgeon will perform a simple procedure known as 'dilatation and curettage'. An instrument called a 'speculum' is inserted into the vagina, to allow us to see your cervix (the opening to the womb) (please see diagram below). The cervix muscle is dilated temporarily using rods called 'dilators'. A sample of the endometrium (the lining of the womb) is then scraped off, using a spoon-shaped instrument called a 'curette'. (This is like using a spatula to scrape the last bit of cake mix out of a mixing bowl when baking.) This procedure takes approximately 1-2 minutes.

The diagram below shows how an endometrial biopsy is taken, using the 'curettage' technique. This will be performed while you are anaesthetised ('asleep').



After the endometrial biopsy tissue has been sent to the pathology laboratory, it will be analysed to assess the BCL6 level. The pathology company then routinely stores all pathology slides and paraffin block for ten years. After this point, samples are incinerated.

Data collection

In addition to your answers for the 'EHP-30' questionnaire, and the endometrial biopsy sample, your surgeon will assess how bad your endometriosis (if you have any) is, during your keyhole surgery. This would be done during all laparoscopies in any case, whether or not you participate in this research. We use the standard endometriosis classification system, which is called the 'revised American Society of Reproductive Medicine' classification system for endometriosis. This involves looking closely at your pelvic organs using the the keyhole surgery camera, and classifying any endometriosis present by its location, size and depth.

Follow-up

Your follow-up consultations will be similar, whether or not you participate in this research. Our clinic nurse will call you approximately 1 week after your operation, to see how you are recovering, and clarify whether you have suffered any complications of the surgery. The clinic nurse will call you again at approximately four weeks after your operation, to ask if you have suffered any complications of the surgery. The Principal Investigator will also call you at approximately four weeks after your operation, to explain what stage of endometriosis was found (if any), and the results of the BCL6 test that was done on the sample of your endometrial lining.

What are the possible benefits and risks of this study?

If this research can help develop a way to diagnose the presence and severity of endometriosis using only an endometrial biopsy, there would be significant benefits to many other women in future. For example: women who may have endometriosis based on their symptoms could have an endometrial biopsy taken (while awake, in a clinic room). The result may help guide whether or not she would benefit from laparoscopic surgery. It may also help to guide whether keyhole surgery or *in vitro* fertilisation (IVF) is the next best step.

There will be no clear benefits to you from your participation in this research.

In terms of the potential risks to you if you choose to participate: the potential complications of having an endometrial biopsy done include:

- Uterine perforation (small hole in the muscle of the uterus) (approximately 1 in 300 women)
- Formation of scar tissue inside the cavity of the uterus (approximately 1 in 60 women)
- Injury to the cervix (neck of the womb) (approximately 1 in 25 women)
- Infection (approximately 1 in 20 women)
- Blood loss > 100mLs (approximately 1 in 10 women)

By and large, having an endometrial biopsy performed is a very safe procedure.

The potential implications of the above possible complications and their management include:

- Uterine perforation: collection of blood tests for full blood count; injury to bladder or bowel (1 in 15 patients who have a uterine perforation); intra-abdominal bleeding (1 in 6 patients who have a uterine perforation); need for open surgery (very rare)[21]
- Formation of scar tissue inside the cavity of the uterus: lighter periods (4 in 5 women with intra-uterine scarring); decreased fertility (1 in 8 women with intra-uterine adhesions); cyclical pelvic pain (1 in 30 women with intra-uterine adhesions); recurrent pregnancy loss (1 in 8 women with intra-uterine adhesions). Management of the above issues involves hysteroscopy (a procedure performed under general anaesthetic to visualise the lining of the uterus with a small video camera), and resection of the intra-uterine adhesions (separating the intra-uterine scarring, using electrosurgical devices).[22]
- Injury to the cervix: repair of any cervical injury is performed immediately, using absorbable stitches.
- Infection: mild infection (the majority) are treated with tablet antibiotics; severe infections (very rare) require admission to hospital and intravenous antibiotics.
- Blood loss > 100mLs: observation; collection of blood tests for full blood count; administration of medications to help control the bleeding; in the cases of very rare major haemorrhage (> 1000mLs), open surgery may be needed

The above potential complications of dilatation and curettage can be compared with the potential risks of keyhole surgery, which include:

- Conversion to an open operation ('laparotomy') (approximately 1 in 70 women)
- Injury to urinary tract (bladder or ureters) (approximately 1 in 100 women)
- Injury to major (big) blood vessel (approximately 1 in 200 women)
- Injury to minor (small) blood vessel (approximately 1 in 350 women)
- Later development of a hernia through one of the scars (approximately 1 in 500 women)
- Injury to gastrointestinal tract (stomach or bowels) (approximately 1 in 1000 women)

Who pays for the study?

Pathology Company 'Douglass Hanly Moir' has agreed to perform the BCL6 tests on endometrial biopsy samples free of charge. In exchange for funding, fertility company 'Genea' will be acknowledged in publications. No study data or personal information will be sent to either of these companies.

What happens if I suffer injury or complications as a result of the study?
If you suffer any injuries or complications as a result of this study, you should contact the study doctor as soon as possible, who will assist you in arranging appropriate medical treatment. You may have a right to take legal action to obtain compensation for any injuries or complications resulting from the study. Compensation may be available if your injury or complication is caused by the drugs or procedures, or by the negligence of any of the parties involved in the study. If you receive compensation that includes an amount for medical expenses, you will be required to pay for

your medical treatment from those compensation monies.
If you are not eligible for compensation for your injury or complication under the law, but are eligible for Medicare, then you can receive any medical treatment required for your injury or complication free of charge as a public patient in any Australian public hospital.
If applicable The parties to this study agree to follow the Medicines Australia Guidelines for Compensation for Injury Resulting from Participation in an Industry-Sponsored Clinical Trial. These Guidelines allow for some claims for compensation to be settled without the need for legal action to be taken. The fact that the parties have agreed to abide by these guidelines in respect of the clinical trial does not affect your rights to pursue a legal remedy in respect of any injury you may suffer as a result of participation. You can obtain a copy of these Guidelines from the Secretary of the Human Research Ethics Committee.

What are my rights?

Participation in this research is completely voluntary: you are free to decline to participate, or withdraw from the study at any time. You do not need to provide a reason. Choosing not to participate, or withdrawing from the study, will not affect the care or treatment you receive from our team.

As a participant in this research, you have a right to access all information collected about you for the purposes of the study.

By signing the consent form, you consent to the research team collecting and using personal information about you for the research project. Any information obtained in connection with this research project that can identify you will remain confidential. All information collected about you will be stored in a secure Excel spreadsheet, which is password-protected, and only accessible by investigators on the study. No identifiable information about any research participants will be published or provided to anyone outside of this research.

It is anticipated that the results of this research project will be published and/or presented in a variety of ways. In any publication and/or presentation, information will be provided in such a way that you cannot be identified.

What happens after the study, or if I change my mind?

De-identified data collected about you will be stored electronically for 15 years, and then destroyed. When the research is completed, you will receive information about results of the study in a brief publication sent to the email address you provide to us.

If you change your mind, and decide not to participate in the study, please contact the Principal Investigator (whose contact details are listed below). If you have not yet had your surgery, your original surgery will be undertaken, without any additional components that were only needed because of your participation in this research. If you have already had your operation, any data relating to your involvement will be removed from the data to be analysed if possible. (This step is only possible up to the point of completion of data collection. Once the results from all participants start being analysed, it will not be possible to remove your data from other participants' data.)

You are welcome to decline involvement in the study: doing so will not have any impact on how the team of surgeons, nurses, and anaesthetists treat you.

Who has reviewed the research project?

All research in Australia involving humans is reviewed by an independent group of people called Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the Northern Sydney Local Health District HREC. This project will be carried out according to the *National Statement on Ethical Conduct in Human Research*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

Who do I contact for more information if I have concerns?

This research is being led by clinicians from the Centre for Advanced Reproductive Endosurgery: A/Prof Alan Lam and Dr Alison Bryant-Smith. Other clinicians involved in this study include A/Prof George Condous, Dr Jessica Lowe, pathologists from Douglass Hanly Moir pathology company, and an infertility specialist (Dr Alison Gee).

If you have any questions, concerns or complaints about the research at any stage, you can contact:

Principal investigator Alison Bryant-Smith at alison.bryant-smith@sydneycare.com.au or

(02) 9966 9121.

Associate Investigator Alan Lam at alanlam@sydneycare.com.au or (02) 9966 9121.

If you would like to talk to someone who is independent of this research, you can contact the Human Research and Ethics Committee (HREC) that approved this study on:

Phone: (02) 9926 4590

Email: NSLHD-Research@health.nsw.gov.au

The Northern Sydney Local Health District HREC approved this study. If you would like to contact the HREC with any feedback or concerns, please quote reference number 2020/ETH02388.

Confidential

Consent form

Study title: **B-cell lymphoma 6 protein (BCL6) as a potential biomarker for endometriosis: can it be used to predict endometriosis' presence and/or severity?**

Principal investigator: Dr Alison Bryant-Smith MBBS/BA MPH MSurgEd MRCOG FRANZCOG

AGES Accredited Training Program trainee, Centre for Advanced Reproductive Endosurgery (CARE)

408 / 69 Christie St, St Leonards, 2065, alison.bryant-smith@sydneycare.com.au

Declaration by Participant

I have read the Patient Information Sheet (or someone has read it to me in a language that I understand).

I understand the purposes, procedures, and risks of the research described in the project.

My surgeon will be: A/Prof Alan Lam ☐

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described, and understand that I am free to withdraw at any time during the project without affecting my future care.

I understand that I will be given a signed copy of this document to keep.

Name of Participant (please print): _____

Signature: _____ Date: _____ / _____ / _____

Declaration by Researcher / associate

I have given a verbal explanation of the research project, its procedures and risks, and I believe that the participant has understood that explanation.

Name of Researcher (please print): _____

Signature: _____ Date: _____ / _____ / _____

15.5 Case report form (CRF)

Case report form

Participant ID number: I __ I __ I __ I __ I

Inclusion criteria (see below): Met ☐ Not met ☐

- Age between 18 – 50yo ☐
- Regular menstrual cycle (i.e. menstrual cycle length 21 - 35 days inclusive) ☐
- Already planning to undertake laparoscopy, for any indication ☐
- English speaking ☐
- Capacity and willingness to give written informed consent ☐
- Willingness to comply with the study ☐
- Willingness to have their planned procedure undertaken in the luteal phase of their menstrual cycle (see below) ☐

Date of last menstrual period: _____ / _____ / _____

Date of next expected menstrual period: _____ / _____ / _____

Date of informed consent: _____ / _____ / _____ **Date of procedure:** _____ / _____ / _____

Is the date of next expected menstrual period in the two weeks following the planned procedure?

Yes ☐ No ☐

NB: If 'no': this patient is not in the luteal phase of her menstrual cycle, so is ineligible for inclusion in this study.

Primary surgeon: A/Prof Lam ☐ A/Prof Condous ☐

Dr Lowe ☐

EHP-30 questionnaire completed ☐

Urine pregnancy test completed, and negative result reported ☐

BCL6 sample sent to DHM laboratory ☐

Severity of endometriosis:

Endometriosis		**< 1cm**	**1 to 3cm**	**> 3cm**
Peritoneum	**Superficial**	1	2	4
	Deep	2	4	6
Right ovary	**Superficial**	1	2	4
	Deep	4	16	20
Left ovary	**Superficial**	1	2	4
	Deep	4	16	20
PoD obliteration	**Partial**	4		
	Complete	40		
Adhesions		**< 1/3 enclosure**	**1/3 to 2/3**	**> 2/3 enclosure**
Adhesions right ovary	**Filmy**	1	2	4
	Dense	4	8	16
Adhesions left ovary	**Filmy**	1	2	4
	Dense	4	8	16
Adhesions right tube	**Filmy**	1	2	4
	Dense	4	8	16
Adhesions left tube	**Filmy**	1	2	4
	Dense	4	8	16

Points scored on rASRM classification: 0 / 1 to 5 / 6 to 15 / 16 to 40 / > 40

Severity of endometriosis: None / stage I / stage II / stage III / stage IV

BCL6 result: _____

Date of first follow-up: _____ / _____ / _____

Adverse events reported:

Uterine perforation	☐	Cervical injury	☐
Intra-operative blood loss > 100mLs			☐
Fever >38.0	☐	Post-op haemorrhage	☐

Date of second follow-up: _____ / _____ / _____

BCL 6 result given to patient ☐

Stage of endometriosis reported to patient ☐

Adverse events reported:

Uterine perforation	☐	Cervical injury	☐
Intra-operative blood loss > 100mLs			☐
Fever >38.0	☐	Post-op haemorrhage	☐