Final

Statistical Analysis Plan

Psychological implications and health-related quality of life in women experiencing a pregnancy of unknown location: a multicentre cohort study

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Event
SAE	Serious Adverse Event
PUL	Pregnancy of Unknown Location
TVU	Transvaginal Ultrasound
EP	Ectopic Pregnancy
IUP	Intrauterine Pregnancy
Failed PUL	Spontaneously resolving pregnancy of unknown location
PPUL	Persisting Pregnancy of Unknown Location
RCT	Randomised Controlled Trial
NICE	National Institute for Health and Care Excellence
M4	Prediction Model M4
hCG	Human Chorionic Gonadotrophin
HADS	Hospital anxiety and depression scale
SF-36	36-Item Short Form Health Survey
LMP	Last Menstrual Period
CI	Confidence Interval
Mtx	Methotrexate
HRQoL	Health-related quality of life

1 STUDY DETAILS

1.1 Study Objectives

To evaluate the presence and level of psychological morbidity and health-related quality of life (HRQoL) among women with a PUL, details of the study can be found at www.clinicaltrials.gov (NCT 03461835).

Primary objective

- To test the difference in the hospital anxiety and depression scale (HADS) score ≥ 8 between all women with PUL one week after their first clinic visit and a control group of women attending antenatal care that had a gestation ≤12 weeks and no scan done in the present pregnancy.

Secondary objective

- To test the difference in the HADS score ≥ 8 among women with viable IUP starting as PUL compared with control subjects (women having a normal early pregnancy without complications) after one week.
- To compare HADS between women with a viable IUP and women with an early pregnancy loss (EP, non-Viable IUP, miscarriage or a failed PUL) among women with PUL after one week and four weeks.
- To compare HADS between women with an early pregnancy loss (EP, non-Viable IUP, miscarriage or a failed PUL) and controls subjects after one week.
- To assess if there is an early onset and continued psychological morbidity among women with PUL between one week and four weeks.
- To study predictors at baseline investigation given in section 3.5 to predict HADS Anxiety ≥8 at four weeks and both Anxiety and Depression ≥8 at four weeks.
- To evaluate the standard version of the 36-Item Short Form Health Survey (SF-36) scores among women with PUL (Group variable B in section 3.1).

1.2 Study Design

Multicentre prospective cohort study of women with PUL. Psychological morbidity and HRQoL was evaluated in conjunction with an RCT. Women attending maternity care serve as control subjects.

1.3 Intervention

Participants filled out two internationally validated self-reporting questionnaires, the HADS and the SF-36 [1, 2]. The HADS was completed twice, one and four weeks after randomisation in the RCT, and the SF-36 was completed once, four weeks after randomisation. Control subjects filled out the HADS questionnaire after their first appointment in antenatal care. Also, RCT participants and control subjects filled out a health questionnaire.

1.4 Sample Size

We have 170 women in the RCT population. We calculated that we would need 105 control women to reach a power of 80 % to detect an absolute difference of 15 percentage points after one week, with a 15 % incidence of HADS anxiety score ≥ 8 among control subjects and 30 % among PUL at a two-sided alpha level of 0.05 (20), using Chi-2 test. For protection against that 10 % of patients will be lost to follow-up a total of 115 women will be included in the control group.

In a second calculation based on using HADS anxiety subscale as a continuous scale, with 160 women in RCT group and 105 in the control group we will achieve a power of 80% with two-sample T-test to find a mean difference of 1.2 HAD units between the two groups with a standard deviation of 3.4 in both groups, at a significance level 0.05. A difference of 1.2 HAD units is regarded as minimally important difference based on mean scores on the HADS anxiety subscale reported for women in early pregnancy in previous studies [3-5].

2 STUDY POPULATIONS

2.1 Definition of Study Populations

Total study population (primary study population)

All participants completing the RCT with available data from self-reporting psychometric questionnaires and health questionnaire. Control subjects with available data from self-psychometric questionnaire and health questionnaire.

RCT population

All participants completing the RCT with available data from self-reporting psychometric questionnaires and health questionnaire.

3 STUDY VARIABLES

3.1 Group variables

A. RCT Participants vs control subjects

B. Viable IUP vs Early pregnancy loss (EP/Failed PUL/Non-viable IUP (incl. Miscarriage) /Persistent PUL) within the RCT population

C. RCT Participants with viable IUP vs control subjects

3.2 Baseline Variables (Participants in RCT and control subjects)

- Age (years)
- BMI (kg/m^2) measured upon enrollment in RCT and self-reported by control subjects in health-questionnaire.
- BMI ≥ 30 (Y/N)
- Bleeding (Y/N)
- Smoking (Never smoked/Former smoker/current smoker<10/11-20/21-30/>30)
- Investigated for infertility (Y/N)
- Assisted reproductive technology in the present pregnancy (Y/N)
- Time to conceive (<6 months/6-12 months/>12 months)
- Gestational weeks
- Ethnicity (Caucasian/African/Asian/Other)

- Born in Sweden (Y/N)
- At least one parent from Sweden (Y/N)
  - If N, which country (Text)
- Desired pregnancy (Y/N)
- Partner (Y/N)
  - If Y (Married/Unmarried)
- Partner support (None/Low/Moderate/High)
- Care satisfaction (Very dissatisfied/Dissatisfied/Very satisfied/Satisfied)
- Morning sickness (Y/N)
  - If Y Prescription drug (Y/N)
- Pregnant before (Y/N)
- Previous miscarriage ≤ 12 weeks (Y/N)
- Previous miscarriage >12 weeks (Y/N)
- Previous pregnancy loss (Y/N), (created from Prev EP, prev miscarriage < 12 w, prev miscarriage > 12 w.)
- Legal abortion (Y/N)
- Live birth (No/Vaginal birth/Caesarean section)
- Previous ectopic pregnancy (Y/N)
- Miscarriage worries (Strongly disagree/Disagree/Agree/Strongly agree)
- General health status (Very good/Good/Acceptable/Poor/Very poor)
- Worries for mental health (Never/>12 months/<12 months)
- Ever received care for mental illness (Never/Yes)
  - If Y what type of illness (Text)
- Medication for mental illness (Never/>12 months/<12 months)
- Diabetes (Y/N)
- Asthma (Y/N)
- Hypertension (Y/N)
- Education level (Elementary/Secondary/Bachelor or master/Doctoral)
- Bleeding symptoms (Y/N)

3.3 Efficacy Variables

Week 1 variables are applicable to group comparisons A, B and C and week 4 variables are applicable to group comparison B.

Primary Efficacy Variables

HADS-scores (see Appendix 9.1) after one week

- HADS Anxiety ≥8

Secondary Efficacy Variables

HADS-scores after 1 week

- HADS Depression ≥8

HADS-scores after 4 weeks

- Anxiety ≥8
- Depression ≥8
- Both depression and anxiety ≥8

HADS-scores after 1 week and 4 weeks

- Anxiety (continuous)
- Anxiety category (0–7 normal, 8–10 mild, 11–14 moderate, and 15–21 severe), only for descriptive purpose
- Depression (continuous)
- Depression category (0–7 normal, 8–10 mild, 11–14 moderate, and 15–21 severe), only for descriptive purpose

Change in HADS score between 1 and 4 weeks

- Anxiety, continuous
- Anxiety, ≥81w to ≥84w, ≥81w to <84w, <81w to ≥84w and <81w to <84w, only for descriptive purpose
- Depression, continuous
- Depression, ≥81w to ≥84w, ≥81w to <84w, <81w to ≥84w and <81w to <84w, only for descriptive purpose

SF-36 scales (HRQoL, see appendix 9.2) at 4 weeks

1. Physical functioning
2. Role limitations due to physical health
3. Role limitations due to emotional problems
4. Energy/fatigue
5. Emotional well-being
6. Social functioning
7. Pain
8. General health

SF-36 (HRQoL) at 4 weeks

- Summary measures of physical health (Scales 1–4)
- Summary measures of mental health (Scales 5–8)

3.4 Confounders

- Age

- Investigated for infertility (Y/N)
- Gestational weeks
- Desired pregnancy (Y/N)
- Previous pregnancy loss (Y/N), (created from previous EP, previous miscarriage <12 w, previous miscarriage >12 w).

3.5 Predictor variables (Only RCT participants), associated with HADS ≥8 on anxiety and with both anxiety ≥8 and depression ≥8 after one and four weeks.

- Abdominal pain and bleeding (Y/N)
- Diagnosis first ultrasound (Unknown/Probable IUP/Probable EP)
- Risk category (High risk of EP/Low risk of EP)
- Ever investigated for infertility (Y/N)
- Time to conceive (<6 months/6-12 months/>12 months)
- Desired pregnancy (Y/N)
- Previous pregnancy loss (Y/N), (created from Prev EP, prev miscarriage < 12 w, prev miscarriage > 12 w).
- General health status (Very good/Good/Acceptable/Poor/Very poor), äv bedömt I SF-36.
- Psychological vulnerability (Y/N), (Created from Worries for mental health, received care for mental illness and medication for mental illness). To have responded positive on ≥1 of the three variables qualify for Y otherwise N.
- Education level (Elementary/Secondary/Bachelor or master/Doctoral)

4 STATISTICAL METHODOLOGY

4.1 General Statistical Methodology

Descriptive statistics will be presented by mean, standard deviation (SD), median, interquartile ranges (continuous variables) and number and percentage (categorical variables).
All efficacy variables measured at week 1 will be analysed and described for all group variables A, B, and C. (See section 3.1 Group variables. All efficacy variables measured at week 4 will only be analysed within the PUL group comparison B

Adjusted analyses of dichotomous outcome variables between two groups will be performed with multivariable logistic regression and the result will be given as adjusted odds ratio with 95% CI and p-value. Adjusted analyses of continuous outcome variables between two groups will be performed using ANCOVA and the result will be given as adjusted mean difference with 95% CI and p-value. The adjusted analyses with all confounders will be the main analyses. The unadjusted analyses will be performed with Fisher's exact test presented as mean percentage points difference with 95% CI using Farrington-Mannings method for dichotomous outcome variables and T-test for continuous variables. For all outcome variables and two-groups comparison we will give analyses: unadjusted, adjusted for all confounders. If

the missing values of combination of the covariates are more than 10% in primary analysis multiple imputations will be performed using fully conditional specification (FCS) with m=100 with seed 4976 as a sensitivity analysis.
If the missing values of combination of the covariates are more than 20% in selected important analysis multiple imputations will be performed using fully conditional specification (FCS) with m=100.
Important proportions will be given with 95% CI with Wilson's method.

Because it is an observational study, we will give both p-values and standardised mean difference in the baseline tables.

When analysing changes within groups paired t-test will be used to test changes in HADS-scores between 1 and 4 weeks and McNemar's test for changes in HADS-scores ≥ 8 between 1 and 4 weeks.

In the prediction study we start with unadjusted logistic regression, with OR (95% CI), p-value, AUC and probability for outcome variable in subgroups of the predictors.
In next step we take all predictors with a unadjusted p-value less than 0.10 into a multivariable logistic model.
The outcome variables in the prediction analyses are:
- HADS Anxiety ≥8
- Both HADS Anxiety≥8 and HADS Depression≥8

The goal with the prediction study is to find risk factors for anxiety and depression not to build a validated prediction model.

Fixed-sequence test will be performed for primary analysis followed by the three first secondary variables on the same group comparison and with multivariable logistic regression with the same adjusted covariates.
- HADS Depression >= 8 after one week
- HADS Anxiety >= 8 after 4 weeks
- HADS Depression >= 8 after 4 weeks

If a significant superiority on primary efficacy analysis, then probability mass will be transformed to the first secondary analysis. If this analysis also is significant this analysis is also confirmative and so on. After the first non-significant result this analysis and all following are not confirmative. All other analysis will be exploratory.

To study if some baseline variables interact with primary analysis, exploratory interaction analyses will be performed.

All tests will be two-tailed and conducted at the 0.05 significance level. All analyses will be performed by using SAS® v9.4 (Cary, NC).

4.2 Patient Disposition and Data Sets Analysed

Present the number of patients in the different populations:

- In total study population:
  EP, non-Viable IUP, miscarriage or a failed PUL, viable IUP, RCT subjects and control subjects

4.3 Baseline

Summary tables will be produced totally and for the different group variables A, B and C with p-values and standardized mean differences.

4.4 Efficacy Analyses

Primary Efficacy Analysis

Analyses of difference in HADS score (anxiety) ≥8 among women with PUL compared with control subjects (women having a normal early pregnancy without complications) after one week will be made using adjusted odds ratios (AOR) with 95% confidence interval (CI) and p-value using logistic regression. Adjustment will be performed for confounders listed in section 3.4. If the missing of combination of the covariates are more than 10% a first sensitive analysis using multiple imputations will be performed using fully conditional specification (FCS) with m=100.

Additionally unadjusted crude odds ratio (OR) with 95% CI and p-value along with unadjusted absolute percentage difference with 95% CI and p-value (Fisher exact test) will also be presented.

Summary of primary variables will also be presented totally and by the two groups as described in general methods above.

Secondary Efficacy Analyses

The secondary efficacy analyses will be the analyses of the secondary efficacy variables given in section 3.3.2 using the methods in General Statistic Methodology section 4.1.

Exploratory Interaction Analyses

To find subgroups of baseline variables where the proportion of HADS Anxiety ≥8 differs more between the PUL group and the control group an exploratory interaction will be performed between the following baseline variables and PUL/Control using the logistic model:

HADS (Anxiety ≥8) = PUL/Control BaselineVar PUL/Control * BaselineVar.

- Age
- Partner support (None/Low/Moderate/High)
- Care satisfaction (Very dissatisfied/Dissatisfied/Very satisfied/Satisfied)
- Investigated for infertility (Yes/No)
- Assisted reproductive technology (IVF) (Yes/No)
- Time to conceive (<6 months/6-12 months/>12 months)
- Gestational weeks
- Live birth (No/Vaginal birth/Caesarean section)
- Previous pregnancy loss (Y/N), (created from Prev EP, prev miscarriage < 12 w, prev miscarriage > 12 w).
- Miscarriage worries (Strongly disagree/Disagree/Agree/Strongly agree)
- General health status (Very good/Good/Acceptable/Poor/Very poor)
- Worries for mental health (Never/>12 months/<12 months)
- Received care for mental illness (Never/Yes)
    - If Y what type of illness (Text)
- Medication for mental illness (Never/>12 months/<12 months)

For baseline variables with interactions p<0.15 primary analyses will be performed for suitable subgroups.

5 INTERIM ANALYSES

No interim analysis will be made.

6 CHANGES OF ANALYSIS FROM ORIGINAL STUDY PROTOCOL

7 LISTING OF TABLE, FIGURES AND LISTINGS

7.1 Listing of Tables

Table Number	Table Title
14.1.1	Patient Disposition
14.1.2.1	Baseline characteristics (RCT Participants vs control subjects)
14.1.2.2	Baseline characteristics (Viable IUP vs Early pregnancy loss)
14.1.2.3	Baseline characteristics (RCT Participants with viable IUP vs control subjects)
14.2.1	Primary analysis (RCT Participants with PUL vs control subjects)
14.2.1.1	Sensitivity primary analysis (PUL vs control subjects) with multiple imputation
14.2.2.1	Secondary analyses (RCT Participants with PUL vs control subjects)
14.2.2.2	Primary variable and Secondary analyses (Viable IUP vs Early pregnancy loss)
14.2.2.3	Primary variable and Secondary analyses (RCT Participants vs control subjects)
14.2.2.4	Exploratory Interaction analyses between group variable (RCT Participants vs control subjects) and baseline variables.
14.2.2.5.1	Relationship between predictor variables and HADS anxiety ≥8 at four weeks. Univariable logistic regression
14.2.2.5.2	Relationship between predictor variables and HADS at four weeks. Multiple logistic regression
14.2.2.5.3	Relationship between predictor variables and both HADS anxiety ≥8 and HADS depression ≥8 at four weeks. Univariable logistic regression
14.2.2.5.4	Relationship between predictor variables and both HADS anxiety ≥8 and HADS depression ≥8at four weeks. Multiple logistic regression

7.2 Listing of Figures

Decided as needed.

7.3 Listing of Listings

Decided as needed

8 REFERENCES

1. Zigmond, A.S. and R.P. Snaith, *The hospital anxiety and depression scale.* Acta Psychiatr Scand., 1983. **67**(6): p. 361-70.
2. Sullivan, M., J. Karlsson, and J.E. Ware Jr, *The Swedish SF-36 Health Survey--I. Evaluation of data quality, scaling assumptions, reliability and construct validity across general populations in Sweden.* Soc Sci Med., 1995. **41**(10): p. 1349-58.
3. Do, N.C., et al., *Quality of life, anxiety and depression symptoms in early and late pregnancy in women with pregestational diabetes.* Acta Obstet Gynecol Scand, 2017. **96**(2): p. 190-197.
4. van de Loo, K.F.E., et al., *Depression and Anxiety during Pregnancy: The Influence of Maternal Characteristics.* Journal of Mood Disorders and Therapy, 2019. **1**(1).
5. Farren, J., et al., *Posttraumatic stress, anxiety and depression following miscarriage and ectopic pregnancy: a multicenter, prospective, cohort study.* Am J Obstet Gynecol, 2020. **222**(4): p. 367 e1-367 e22.

9 APPENDIX

9.1 Calculating HADS

- Questions 2, 4, 7, 9, 12 and 14 are translated into numbers as a=0, b=1, c=2, d=3.
- Questions 1, 3, 5, 6, 8, 10, 11 and 13 and 14 are translated into numbers as a=3, b=2, c=1, d=4.
- For the anxiety subscale sum the question number 1, 3, 5, 7, 9, 11 and 13. This sum can be between 0 and 21.
- For the depression subscale sum the question number 2, 4, 6, 8, 10, 12 and 14. This sum can be between 0 and 21.

9.2 Calculating SF-36

Step 1: Recoding Items

Item numbers	Change original response category *	To recoded value of:
1, 2, 20, 22, 34, 36	1 →	100
	2 →	75
	3 →	50
	4 →	25
	5 →	0
3, 4, 5, 6, 7, 8, 9, 10, 11, 12	1 →	0
	2 →	50

Item numbers	Change original response category *	To recoded value of:
	3 →	100
13, 14, 15, 16, 17, 18, 19	1 →	0
	2 →	100
21, 23, 26, 27, 30	1 →	100
	2 →	80
	3 →	60
	4 →	40
	5 →	20
	6 →	0
24, 25, 28, 29, 31	1 →	0
	2 →	20
	3 →	40
	4 →	60
	5 →	80
	6 →	100
32, 33, 35	1 →	0
	2 →	25
	3 →	50
	4 →	75
	5 →	100

* Precoded response choices as printed in the questionnaire.

Table 2

Step 2: Averaging Items to Form Scales

Scale	Number of items	After recoding per Table 1, average the following items
Physical functioning	10	3 4 5 6 7 8 9 10 11 12
Role limitations due to physical health	4	13 14 15 16
Role limitations due to emotional problems	3	17 18 19
Energy/fatigue	4	23 27 29 31
Emotional well-being	5	24 25 26 28 30
Social functioning	2	20 32
Pain	2	21 22
General health	5	1 33 34 35 36

Table 3

Reliability, Central Tendency, and Variability of Scales in the Medical Outcomes Study

Scale	Items	Alpha	Mean	SD
Physical functioning	10	0.93	70.61	27.42
Role functioning/physical	4	0.84	52.97	40.78
Role functioning/emotional	3	0.83	65.78	40.71
Energy/fatigue	4	0.86	52.15	22.39
Emotional well-being	5	0.90	70.38	21.97
Social functioning	2	0.85	78.77	25.43
Pain	2	0.78	70.77	25.46
General health	5	0.78	56.99	21.11
Health change	1	—	59.14	23.12

Note: Data is from baseline of the Medical Outcomes Study (N=2471), except for "Health change," which was obtained one year later.