Utility of plasma drug level monitoring and *CYP2C19* genotyping in dose personalization of escitalopram therapy

PsyCise_E

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Clinical trial protocol

Test groups:

PsyCise trial is designed to quantify changes in clinical outcomes in participants treated for depression using personalized antidepressant dosing regimen, based on therapeutic drug monitoring (TDM) and CYP2C19 genotyping; as compared to the standard, "one size fits all" dosing. In order to reach this goal, we have defined two test groups based on the TDM findings. Namely, all participants who enter the trial (Visit 0) will begin taking standard escitalopram dose of 10 mg/day for two weeks when their blood samples will be taken for the purpose of TDM, and based on their steady state escitalopram concentrations they will be allocated to one of two groups at week 4 of the trial (visit 1):

1) "Standard dose" group: Participants will be allocated to this group if their TDM results at week 2 show their steady state escitalopram plasma concentrations were inside "therapeutic window" (25-50 ng/ml). For these participants, initial standard escitalopram dose of 10 mg/day is estimated to be optimal so they will continue to receive the same dose until the end of the trial at week 8 (visit 2).
2) "Adjusted dose" group: If TDM results at week 2 suggest that participant taking standard dose is underdosed or overdosed, given participant is allocated to this group. New, personalized dosing regimen will be produced for this participants based on steady state escitalopram plasma concentrations measured at week 2, and they will receive personalized escitalopram dose from week 4 (visit 1) until the end of the trial at week 8 (visit 2).

Course of the trial:

Week 0
Initial Visit (Visit 0 – V0)

- Participant will be enrolled at this point if inclusion criteria are met.
- Escitalopram monotherapy for Major Depressive Disorder in previously untreated patient will be initiated at standard dose of 10 mg/day.
- Outcomes measured:
    1. General and socio-demographic information
    2. Clinical questionnaires
    3. Anthropometric measurements
    4. Cardiological assessment
    5. Serum samples for biochemical analyses
    6. Buffy coat samples for DNA extraction and *CYP2C19* genotyping*

(*Sample is not time dependent, it can be taken at any visit)

Week 2
Mid-Visit (Vk)

- Escitalopram plasma concentrations reach steady state.
- Plasma sample taken for the purpose of TDM and group allocation.

- Percentages of participants who are underdosed and overdosed will be measured and used to confirm previous findings [Jukić 2018]

Week 4
Visit 1 (V1)

- Participants are allocated to the “Standard dose” or “Adjusted dose” group
- Independent clinician prescribes new dosing regimen to the participants in “Adjusted dose” group, and informs patients in “Standard dose” group to continue taking the same standard escitalopram dose.
- Clinician who usually monitors given patient is unaware of the group patient is allocated to, or the dose prescribed to a patient at Visit 1.
- Outcomes measured:
    1. Clinical questionnaires
    2. Anthropometric measurements
    3. Cardiological assessment
    4. Serum samples for biochemical analyses
    5. Plasma samples to reconfirm TDM findings in VK

Week 8
Visit 2 (V2)

- Final visit of the trial and the end of the follow-up.
- Outcomes measured:
    1. Clinical questionnaires
    2. Anthropometric measurements
    3. Cardiological assessment
    4. Serum samples for biochemical analyses
    5. Plasma samples to reconfirm TDM findings in VK and V1 for Standard dosing group, and to determine whether personalized dose is optimal for the patients in Personalized dosing group.

If needed, alternative course will be used:

Week 0
Initial visit + Mid-visit (V0+k)

- Participant on the stable (longer than 2 weeks) monotherapy of Major Depressive Disorder with standard dose of escitalopram (10 mg/day) will be enrolled if other inclusion criteria are met.
- Outcomes measured:
    1. General and socio-demographic information
    2. Clinical questionnaires
    3. Anthropometric measurements
    4. Cardiological assessment
    5. Serum samples for biochemical analyses
    6. Plasma sample for the purpose of TDM and group allocation.
    7. Buffy coat samples for DNA extraction and *CYP2C19* genotyping*

(*Sample is not time dependent, it can be taken at any visit)

Week 2
Visit 1 (V1) – *Identical to the standard protocol*

- Participants are allocated to the "Standard dosing" or "Personalized dosing" group
- Independent clinician prescribes new dosing regimen to the participants in "Personalized dosing" group, and informs patients in "Standard dosing" group to continue taking the same standard escitalopram dose.
- Clinician who usually monitors given patient is unaware of the group patient is allocated to, or the dose prescribed to a patient at Visit 1.
- Outcomes measured:
    1. Clinical questionnaires
    2. Anthropometric measurements
    3. Cardiological assessment
    4. Serum samples for biochemical analyses
    5. Plasma samples to reconfirm TDM findings in Vk

Week 6
Visit 2 (V2) – *Identical to the standard protocol*

- Final visit of the trial and the end of the follow-up.
- Outcomes measured:
    1. Clinical questionnaires
    2. Anthropometric measurements
    3. Cardiological assessment
    4. Serum samples for biochemical analyses
    5. Plasma samples to reconfirm TDM findings in Vk and V1 for Standard dosing group, and to determine whether personalized dose is optimal for the patients in Personalized dosing group.

Hypotheses:

1) More than one quarter of participants will be overdosed or underdosed under standard escitalopram dosing regimen (10 mg/day), as determined by TDM data 2 weeks after the beginning of the therapy.

2) Participants with escitalopram steady state plasma concentration outside the therapeutic window ("Adjusted dose" group) will experience reduced efficacy and more severe side effects of escitalopram therapy before dose personalization (at V1) compared to the participants with escitalopram levels within therapeutic window ("Standard dose" group) at standard dosing.

3) Participants with escitalopram steady state plasma concentration outside the therapeutic window ("Adjusted dose" group) will show improved efficacy and less severe side effects of escitalopram therapy after dose personalization (at V2) as compared to before (at V1), and will reach clinical outcomes comparable to the "Standard dose" group (at V2).

4) Decisions on whether to use personalized or standard dosing, and how to adjust personalized dose based on TDM data could have been determined before the beginning of the treatment using predictive model which, besides other predictors also includes *CYP2C19* genotyping data.

Statistical Analysis Plan

Primary outcomes:

To assess the efficacy of the escitalopram therapy under different dosing regimens we will use 21-item Hamilton rating scale for depression (HAM-D). Depression severity score will be measured at baseline (V0), at week 4 (V1) and week 8 (V2) after the beginning of the treatment. Since there is the need to compare depression severity scores between two test groups as well as between the visits V1 and V2, mixed analysis of covariance (Mixed ANCOVA) will be used if all assumptions associated with ANCOVA are met. Variables will be assigned as follows:

1. Dependent variable: Depression severity score measured by HAM-D scale
2. Independent variable - Fixed factor: Test group (Personalized vs Standard dosing)
3. Independent variable - Repeated measurements: Visit (Visit 1 vs Visit 2)
4. Covariates: Baseline HAM-D score (V0), Childhood trauma questionnaire score at V0, Age, Sex (Male=0, Female=1), Smoking status (0=Non-smoker, 1=Smoker), Alcohol consumption (0=No, 1=Yes), Metabolic syndrome diagnosed using siMS score [Soldatović 2016] (0=No, Yes)

Positive outcome: interaction between group and visit is significant (α=0.05)

Besides using absolute HAM-D scores, we will also compare relative changes in HAM-D scores between V1 and V2 visits as another way to express the potential differences in therapy efficacy between the two test groups. If all needed assumptions are met, statistical test used will be analysis of covariance (ANCOVA) with variables assigned as follows:

1. Dependent variable: Relative change in HAM-D score between visits V1 and V2 calculated by formula:
$$\frac{HAMD_{V1} - HAMD_{V2}}{HAMD_{V1}}$$
2. Independent variable: Test group (Pesonalized dosing vs Standard dosing)
3. Covariables: Baseline HAM-D score (V0), Childhood trauma questionnaire score at V0, Age, Sex (Male=0, Female=1), Smoking status (0=Non-smoker, 1=Smoker), Alcohol consumption (0=No, 1=Yes), Metabolic syndrome (0=No, Yes)

Positive outcome: Test group is a significant factor (α=0.05)

Other primary outcome we will use to quantify changes in clinical outcomes between test groups will be severity of the treatment side effects measured using clinician reported UKU (*Udvalg for Kliniske Undersogelser*) side effect rating scale. UKU scale rates 48 potential adverse reactions with intensity score ranging from 0 to 3 where score 3 signifies maximal intensity and score 0 signifies absence of the adverse rection. Adverse drug reaction intensity scores for all 48 adverse reactions monitored with UKU scale will be measured at weeks 4 (V1) and 8 (V2) after the initiation of the escitalopram therapy. Due to large number of comparisons, significance measures will be adjusted using False Discovery Rate (FDR) correction. Absolute values of all 48 Adverse reaction scores will be compared between test groups and between two time points using mixed model analysis of covariance (Mixed ANCOVA) where variables will be assigned as follows:

1. Dependent variable: Adverse drug reaction score for the given adverse reaction measured by UKU scale
2. Independent variable - Fixed factor: Test group (Personalized vs Standard dosing)
3. Independent variable - Repeated measurements: Visit (Visit 1 vs Visit 2)
4. Covariables: Age, Sex (Male=0, Female=1), Smoking status (0=Non-smoker, 1=Smoker), Alcohol consumption (0=No, 1=Yes), Coffee consumption (0=No, 1=Yes), Use of psychoactive substances (0=No, 1=Yes), Metabolic syndrome (0=No, Yes), Creatinine clearance, AST/ALT ratio.

Positive outcome: interaction between group and visit is significant (α=0.05)

Alternatively, proportions of patients with intensity score greater than 0 (UKU scale intensity score >0 signifies the presence of the given adverse reaction) will be compared between the groups for all 48 adverse effect monitored with UKU scale at two time points: 4 weeks after the enrolment (V1) and 8 weeks after the enrolment (V2). Due to large number of comparisons, significance measures will be adjusted using False Discovery Rate (FDR) correction. Proportion of individuals in two test groups affected with the given adverse reaction will be compared using binary logistic regression analysis separately for visit V1 and visit V2 as follows:

1. Dependent variable: Frequency of the individuals affected with the given adverse drug reaction measured by UKU scale
2. Covariables: Test group (Personalized vs Standard dosing), Age, Sex (Male=0, Female=1), Smoking status (0=Non-smoker, 1=Smoker), Alcohol consumption (0=No, 1=Yes), Coffee consumption (0=No, 1=Yes), Use of psychoactive substances (0=No, 1=Yes), Metabolic syndrome (0=No, Yes), Creatinine clearance, AST/ALT ratio.

Positive outcome: Test group is a significant factor (α=0.05)

Secondary outcomes:

Retrospective TDM data on 2087 patients taking escitalopram [Jukić 2018] suggests that, in the clinical setting, about 40% of patients are expected to have their plasma escitalopram concentrations under the lower limit of the therapeutic window (25 ng/ml), and for about 10% of patients we expect to have plasma escitalopram levels above the upper limit of the therapeutic window (50 ng/ml). One of our aims is to try to confirm these findings using prospective study design which allows for the better control of the confounding factors compared to the retrospective data. Number of the patients with plasma escitalopram levels outside the therapeutic window under standard dosing regimen (10 mg/day) will be assessed using TDM data at Mid-Visit (VK), 2 weeks after the beginning of the therapy.

Big disadvantage of TDM guided establishment of the optimal drug exposure in the given patient is that it takes 4 weeks to personalize escitalopram dose and 2 more weeks to achieve optimal steady state drug concentrations. Alternative approach would be to build model that could predict optimal dose for every patient even before the initiation of the treatment. This model would have to include patient data on factors that could impact escitalopram exposure such as body weight, liver and kidney functions and genotype for the *CYP2C19* gene which is responsible for the interindividual variability in the rate of escitalopram metabolism in liver [Jukić 2018]. After all participants have finished the trial we will retrospectively build model using data collected during the trial, and then validate our model comparing it to the TDM method of dose personalization. For this purpose, all patients will be genotyped for *CYP2C19* gene and divided into 4 groups based on the enzymatic capacity of CYP2C19 enzyme:

1. Poor metabolizer (PM): Carries *2/*2, *2/*3 or *3/*3 *CYP2C19* genotype. Almost absent CYP2C19 enzyme activity
2. Intermediate metabolizer (IM): Carries *1/*2 or *1/*3 *CYP2C19* genotype. Reduced CYP2C19 enzyme activity
3. Normal metabolizer (NM): Carries *1/*1 *CYP2C19* genotype. Normal CYP2C19 enzyme activity
4. Ultra rapid metabolizer: Carries *1/*17 or *17/*17 *CYP2C19* genotype. Increased CYP2C19 enzyme activity

Multiple linear regression model will be build using selected variables:

1. Dependent variable: Plasma escitalopram concentrations at Week 2 (Vk) after the initiation of the treatment
2. Independent variables: Body mass index, PM status (0=Yes, 1=No), IM status (0=Yes, 1=No), NM status (0=Yes, 1=No), UM status (0=Yes, 1=No), Creatinine clearance, AST/ALT ratio

Other outcome measures/observations:

To further validate our primary outcome measures: escitalopram treatment efficacy and severity of adverse effects, we will use Clinical Global Impression (CGI) scales. Scores will be determined for all 3 CGI scales: for Severity of illness (CGI-S) scale at baseline, visit 1 and visit 2, and for Global improvement (CGI-I) scale and for Efficacy Index (CGI-E) at visits 1 and 2. Differences in all 3 CGI scales between visits 1 and 2 will be compared between test groups separately using ANCOVA tests with the equivalent sets of covariates to the ones used for HAM-D scale.

In all patients at baseline and at visits 1 and 2, electrocardiography will be performed in order to monitor potential QT interval prolongation. We will test for correlation between QT interval length and escitalopram plasma levels at various visits, and also we will compare changes in QT interval length between visit 1 and visit 2 in both test groups using mixed ANCOVA test adjusting for the baseline QT length.

Due to high comorbidity of anxiety and depression, and since escitalopram is frequently prescribed in anxiety disorders, we will explore severity of anxiety symptoms in all participants at baseline (V0), Week 4 (V1) and Week 8 (V2) of the treatment by using clinician reported Hamilton anxiety rating scale (HAM-A). Severity of anxiety symptoms will be compared between two test groups at two time points (V1 and V2) using mixed analysis of covariance (Mixed ANCOVA). Variables to be considered as covariates will be: Baseline HAM-A score (V0), Childhood trauma questionnaire score at V0, Age, Sex (Male=0, Female=1), Smoking status (0=Non-smoker, 1=Smoker), Alcohol consumption (0=No, 1=Yes), Metabolic syndrome (0=No, Yes).

Alongside with HAM-A scale we will also use serum cortisol levels as a biomarker for anxiety disorders in our population. Cortisol levels will be analyzed in a similar way to HAM-D and HAM-A scores using mixed analysis of covariance (Mixed ANCOVA) with covariates being: Baseline Cortisol serum levels (V0), Childhood trauma questionnaire score at V0, Age, Sex (Male=0, Female=1), Smoking status (0=Non-smoker, 1=Smoker), Alcohol consumption (0=No, 1=Yes), Metabolic syndrome (0=No, Yes). Additionally, correlation between HAM-A score and cortisol levels will be tested.

Since stress, trauma and socio-economic status can all be a part of depressive disorder pathogenesis, we will explore these environmental factors in order to determine their role in the clinical pictures of our participants. Outcome measures used will be:

1. Perceived stress score measured at baseline, visit 1 and visit 2,
2. Childhood trauma score measured at baseline,
3. Employment status (1=Unemployed, 2=Employed; monthly incomes <40 000 RSD, 3=Employed; monthly incomes 40 000-80 000 RSD, 4=Employed; monthly incomes >80 000RSD).
4. Marital status (1=Unmarried, 2=Married, 3=Divorced, 4=Widowed)
5. Education level (1=Primary education (8 years), 2= High school diploma (8+3 or 8+4 years), 3= Associate's degree (8+4+2 or 8+4+3 years) 4= Bachelor's degree (8+4+4 years or higher)

We will separately test for the correlation between these variables and HAM-D score, HAM-A score and CGI-S score.