Statistical analysis plan – CEBA

AIM AND STUDY DESIGN

Design overview

Chronic Fatigue following acute Epstein-Barr virus infection in Adolescents (CEBA) is a prospective study aiming at identifying risk factors for chronic fatigue 6 months after the acute infection. A group of healthy controls will also be included, providing an opportunity for a cross-sectional comparison as well.

A total of 200 adolescents with acute Epstein-Barr virus (EBV) infection will be included and followed prospectively for 6 months (Figure 1). A similar investigational program is to be conducted at baseline (0 months) and 6 months. A total of 70 healthy controls will be included.

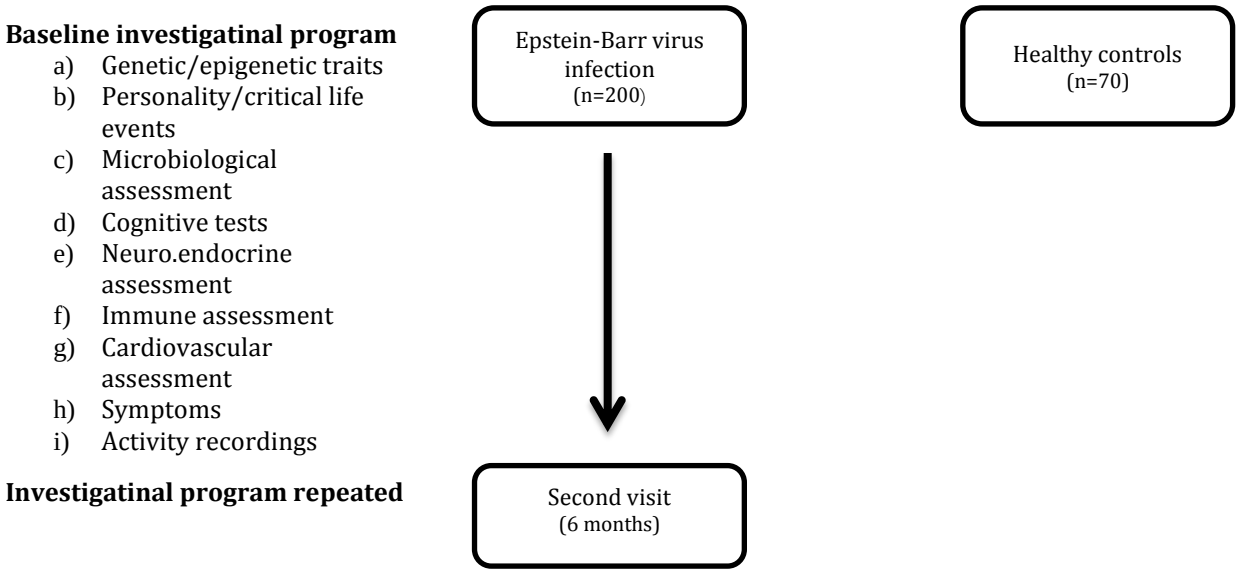

Figure 1. Design overview

Aims

The primary aim is to explore risk factors for chronic fatigue and physical disability 6 months after an acute EBV infection. A secondary aim is to explore the pathophysiology of chronic fatigue by comparing disease markers across fatigued patients and healthy controls.

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Possible risk factors for chronic fatigue 6 months after acute EBV-infection includes:

- Severity of the initial infection
- Immune response characteristics
- Neuro-endocrine stress response characteristics
- Cognitive functioning
- Emotional disturbances
- Genetics/ epigenetics of candidate genes
- Personality traits (perfectionism, strong focus on bodily sensations, worries about coping abilities)
- Life events

Recruitment, inclusion and exclusion

The Microbiological Laboratory at AHUS University Hospital and Fürst laboratory provides microbiological analyses for almost all General Practitioners in the hospital's population area. Adolescents with acute EBV-infection will be consecutively identified and invited to participate in the study through telephonic contact with the patient himself or one of the parents (depending on the age of the patient). Healthy controls will be recruited among the patients' peers

Table 1. Criteria for inclusion and exclusion

Criteria for inclusion and exclusion	
Inclusion criteria	*Exclusion criteria*
Patients	
Age ≥ 12 years and < 20 years Serological confirmation of acute EBV infection Lives in one of the following Norwegian counties: Oslo, Akershus, Buskerud, Vestfold, Østfold	Medical treatment for another disease (hormonal conterception and antibiotics against tonsillitis/pharyngitis are accepted) Pregnancy Debut of illness > 6 weeks ago (anamnestic)
Healthy controls	
Age ≥ 12 years and < 20 years Lives in one of the following Norwegian counties: Oslo, Akershus, Buskerud, Vestfold, Østfold	Medical treatment for another disease (hormonal conterception is accecpted) Pregnancy

POWER CALCULATION

In a cross-sectional-design, a total number of 200 EBV patients and 70 healthy controls will give a power of at least 80% to detect mean differences between the two groups of ≥ 0.5 standard deviations (i.e. effect size ≥0.5). Previous studies indicate that the study is thus sufficiently large to detect clinically important differences in pathophysiology(1).

For the prospective part there are two primary endpoints; the total sum score in the Chalder Fatigue Questionnaire (0-33) and the mean number of steps per day measured over 7 consecutive days. The primary statistical analysis will be a linear regression analysis. With 200 EBV patients and significance level 5% the power to detect that a variable explains 5% of the total variance (R^2=0.05) is at least 80%. Correspondingly, the power would be close to 95% to detect R^2=0.075. That implies that the study has sufficient power to detect small to medium effect sizes.

Previous studies indicate that up to 1/3 of all adolescents with EBV-infection might suffer from chronic fatigue after one year(defined as a sum score of dichotomized responses ≥ 4 on the Chalder Fatigue questionnaire(2)). The NorCAPITAL project suggests a drop-out rate of approximately 10 %, leaving 60 patients with a significantly different endpoint score. With 60 chronic fatigued patients followed over time, the power to detect an effect size of ≥0.4 is ≥87%. This effect size is slightly smaller than the change in fatigue score observed in the NorCAPITAL project, thus the sample size is regarded as sufficiently large. For all the measurable risk factor values an effect size of 0.4 seems reasonable (0.4 times the standard deviation).

200 EBV patients will according to the table below give sufficient power to detect effects of different dichotomous (present or not present) risk factors (Table 2).

Table 2. Power calculation. Following table present the power calculation of the bionomic risk factors. n=number of participants in each group, p = stipulated presence of risk factor in each group. Level of significance is set to 0,05.

Risk of chronic fatigue (p)				Risk difference	Relative risk	Power %
Present		Not present				
n	p	n	p			
100	0.4	100	0.2	0.2	2	85
50	0.4	150	0.2	0.2	2	86
100	0.25	100	0.1	0.15	2.5	80
40	0.25	160	0.1	0.15	2.5	76

VARIABLES

The primary endpoints in this study are fatigue at 6 months (assessed by Chalder Fatigue Questionnaire total score)(2) and physical activity (mean steps per day over 7 consecutive days) at 6 months (assessed by accelerometer recordings)(3).

Variables constituting possible risk factors include

- Severity of the initial infection
  - ALAT
  - Viral load (quantitative PCR for Epstein-Barr virus)
- Immune responses characteristics
  - Concentration of monocytes
  - Concentration of CRP
  - Concentration of total IgG
- Neuro-endocrine stress response characteristics
  - Norepinephrine concentration in venous forearm plasma
  - Cortisol concentration/creatinine concentration ratio in morning spot urine
  - Autonomic cardiovascular control; heart rate, blood pressure and cardiac output during rest, 5 minutes recording.
- Cognitive functioning
  - WISC test of digit span, backward order
  - HVIL-T word memory
  - STROOP-test(color-word interference, all 4 conditions)
- Emotional disturbance
  - The two subscales Difficulty Describing Feelings (DDF) and Difficulty Identifying Feelings (DIF) from the Toronto Alexithymia Scale (TAS-20)(4).
  - The total score of the two subscales depression and anxiety in the Hospital Anxiety and Depression Scale (HADS)(5)
- Personality traits (perfectionism)
  - Children and Adolescents Perfectionism Scale (CAPS)(6)
- Life events
  - Life Event Checklist (LEC)(7)

ANALYSIS SETS

Full analysis set

The 'full analysis set' is defined as all participants who were subsequently included (n = 200) (Figure 2). Missing values will be imputed based on the principle of 'multiple imputation' (MI).

Per protocol analysis set
The 'per protocol analysis set' is defined as all patients in the 'full analysis set' that completed the observation period (attended and completed consultation at baseline and at 6 months) without any of the following protocol deviations:

- Lost to follow-up
- Chalder fatigue score or mean steps per day measurements missing (primary endpoints)
- Diagnosed with another chronic disorder during the study period.

Missing data will not be imputed in the per protocol analysis set. The 'per protocol analysis set' will be used for per protocol assessment (cf. below).

Healthy controls analysis set
The 'healthy control analysis set' is defined as all healthy controls that were included in the project based upon the criteria specified under (Table 1). Missing values will not be imputed.

STATISTICAL METHODS

General considerations
Continuous variables will be reported with parametric (mean/standard deviation) or non-parametric (median, quartiles) descriptive statistics, depending on the distribution. Ordinal/nominal variables will be reported as frequency tabulation. All statistical tests will be carried out two-sided. A p-value ≤ 0.05 is considered statistically significant.

Population characteristics
Patients with acute EBV-infection (full analysis set) will be compared with healthy controls for background variables applying the Student t-test or the Mann-Whitney U test as appropriate. The null hypothesis is no differences between patients and healthy controls.

Outcome 6 months after infectious mononucleosis
The changes within the acute EBV-infection patients (full analysis set) over time will be analyzed using (multiple) linear regression analysis. The two endpoints are set as the dependent variables in separate analyses, and all the different potential risk factors as independent (or explanatory) variables. In each analysis the null hypothesis is that the dependent variable is not associated with the independent variables (the potential risk factors). The primary endpoints are Chalder fatigue score and mean number of steps/day count during 7 consecutive days at 6 months.

The potential relationship between each risk factor variable and the two end points are first explored in separate linear regression analyses including one risk factor. The distribution of residuals will be assessed for normality. Risk factors with $p<0.1$ will be included in a multivariable linear regression analyses. P-values <0.05 will be regarded as statistically significant.

REFRENCES

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