Guidelines for collaborating studies on biomarkers within the BIOMS-eu network

Please fill in the following points and sustain your arguments with data and references.

To be completed by BioMS-eu:
Date of receipt proposal by BioMS-eu:_____
BioMS-eu project number: BIOMS-year-_____

1. Name and address of applicant:

Harald Hegen, MD, PhD, PD

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2. Application date: ___25.11.2022_____

3. Title and project description

Title:

A Prospective Multicenter Study to Evaluate and Validate Kappa Free Light Chains for Diagnosis and Prediction of Disease Activity in People with Multiple Sclerosis

Project description:

Objective:

i) To provide a universal cut-off for k-FLC index to diagnose MS, to determine the impact of different assays and platforms on k-FLC index and to compare its diagnostic accuracy to oligoclonal bands (OCB) (Work Package 1 [WP1])

ii) To reproduce the independent prognostic value of k-FLC index in patients with a first demyelinating event of the central nervous system (CNS) suggestive of MS (Work Package 2 [WP2])

Methods:

Inclusion Criteria

Disease group (WP1, WP2)

- Patients presenting with a first demyelinating event of the CNS suggestive of MS (i.e. diagnosed as clinically isolated syndrome [CIS] or MS according to revised McDonald 2017 criteria)
- Aged 18-60 years
- Available sample volume of CSF and serum (each 3 ml) collected for routine diagnostic purposes within 3 months after disease onset
- Normal renal function (GFR >60 ml/min)

Control group (WP1)

- Patients with a non-inflammatory neurological disease or inflammatory neurological disease (NIND, SC, IND) according to Teunissen et al. (Mult Scler. 2013 Nov;19(13):1802-9)
- Aged 18-60 years
- Available sample volume of CSF and serum (each 3 ml) collected for routine diagnostic purposes within 3 months after disease onset
- Normal renal function (GFR >60 ml/min)

Exclusion Criteria (WP1, WP2)

- Pregnant or breast-feeding
- Participants with monoclonal gammopathy of unknown significance (MGUS), multiple myeloma and plasmacytoma
- Participants unable to provide informed consent
- Unwillingness or inability to comply with the requirements of this protocol including the presence of any condition (physical, mental, or social) that, in the opinion of the principal investigator, is likely to affect the participant's ability to comply with the study protocol.

Endpoints

WP1 – Primary endpoints

Diagnostic accuracy of k-FLC index in MS compared to controls as assessed by

- Sensitivity/ specificity
- Between-center variability of optimal k-FLC index cut-off
- Non-inferiority of k-FLC index compared to OCB

WP2 – Primary endpoints

- Time to relapse [Time Frame: From date of baseline visit until the date of first documented relapse, up to end of follow-up]

WP2 – Secondary endpoints

- Time to disability progression, confirmed in at least two consecutive visits, 6 months-confirmed [Time Frame: From date of baseline visit until the date of first documented sustained disability progression, up to end of follow-up]
- Disability progression, confirmed in at least two consecutive visits, 6 months-confirmed [Time Frame: Baseline to end of follow-up]
- Progression to EDSS ≥3.0, confirmed in at least two consecutive visits, 6 months-confirmed [Time Frame: Baseline to end of follow-up]
- Occurrence of new T2 lesions on MRI at month 12, if available
- Time to 'McDonald MS' [Time From data of baseline visit until fulfillment of diagnostic criteria]

Study design

WP1

Each center includes a minimum of 30 patients with CIS/ MS and 30 controls (15 NIND/SC and 15 IND). Controls should be age- and sex-matched to CIS/MS patients per center. Accordingly, a total of 180 CIS/ MS patients and 180 controls (total 360 patients) will be included.

Aliquots of CSF and serum (each 400 μl) are prepared. One aliquot each is sent to each of the participating centers.

After recruiting patients, each center measures k-FLC and albumin concentration in all CSF and serum samples and calculates the k-FLC index which is used for final statistical analysis (see Appendix).

WP2

Each center follows the patients with CIS/MS after first manifestation for at least 2 years in order to record the occurrence of a second clinical attack (primary endpoint).

Data collected

WP1

- Age (years)
- Sex
- Body-mass-index
- Diagnosis (CIS/ MS, other [of controls])
- Disease duration (days)
- Type of symptoms (optic neuritis, myelitis, brainstem/ cerebellum, other CNS topography) at the time of disease onset
- EDSS
- High-dose corticosteroid treatment (within last 4 weeks before CSF collection)
- Date (start and stop) of high-dose corticosteroid treatment

- Dosage of high-dose corticosteroid treatment (g)
- Date cerebral MRI
- Field strength of cerebral MRI
- Number of cerebral T2 lesions
- Number of cerebral contrast-enhancing lesions
- Infratentorial lesions
- Date spinal MRI
- Field strength of spinal MRI
- Number of spinal T2 lesions
- Number of spinal contrast-enhancing lesions
- CSF RBC count (µl)
- CSF WBC count (µl)
- CSF total protein (mg/dl)
- CSF albumin (mg/dl)
- Serum albumin (mg/dl)
- CSF IgG (mg/dl)
- Serum IgG (mg/dl)
- CSF IgM (mg/dl)
- Serum IgM (mg/dl)
- OCB status (positive, negative)
- OCB pattern, if available (Pattern II or III)
- OCB applied cut-off
- OCB applied laboratory method (in house assay, Sebia, other)
- Renal function (GFR ml/min)
- SDMT ('optional')
- MSFC ('optional')

WP2

- Relapses [if yes: Date, administration of corticosteroids, start and stop date of corticosteroids, dosage]
- EDSS [every 6 months]
- New T2 lesion on cerebral MRI at month12, if available
- New T2 lesion on spinal MRI at month 12, if available
- Start of DMT before 2nd relapse
- Type of DMT
- Date (start and stop) of DMT
- SDMT ('optional')
- MSFC ('optional')

<u>Statistical analysis</u>

WP1

Agreement of k-FLC index between different centers will be analyzed by Spearman correlation analysis and Passing-Bablok regression. A priori power analysis for the paired Wilcoxon signed-rank test (significance level of 0.0033, power 0.9) revealed a necessary sample size of 176 in order to detect a k-FLC index difference of 1. The k-FLC index difference was determined as relevant considering a coefficient of variation of 10% in the low range of k-FLC index values (where the cut-off denoting positive or negative test result is located). We estimated a standard deviation of 3 for the difference between two k-FLC index values (using a k-FLC index range between 0 and 500 and a correlation of agreement between centers or assays of 0.93). This resulted in an effect size of 0.33. We considered a significance level of 0.003 in order to correct for the number (15) of post-hoc tests.

Each center will determine a best cut-off to discriminate MS/CIS from controls by receiver operating characteristic (ROC) analysis and maximizing Youden index. This allows to determine the variability of k-FLC index cut-off introduced by laboratory, center-specific characteristics, and to determine borderline values which delineate clearly negative or positive patients.

Above-mentioned regression analysis is also used to assess the impact of different assays (Freelite, N Latex) and platforms (nephelometry, turbidimetry) on k-FLC index values.

Diagnostic accuracy of k-FLC index and OCB will be compared by kappa statistics and Chi-square test. A priori power analyses (power 0.8, significance level 0.05, small effect size [Cohen f2 0.15] revealed a total of 349 samples. This small effect size guarantees to detect a 5% difference in diagnostic sensitivity/ specificity between k-FLC index and OCB.

Similarly, diagnostic accuracy of k-FLC index will be determined comparing OCB positive and OCB negative patient groups.

WP2

To identify predictors of the time to relapse (primary endpoint), Cox regression will be employed including k-FLC index as the variable of interest and age, sex, type of symptoms [optic neuritis, myelitis, brainstem/ cerebellar, other], remission of symptoms, number of MRI T2 lesions and number of contrast-enhancing lesions, prior use of corticosteroids, DMT during follow-up (and optional OCB) as co-variables. Checks for collinearity will be performed (VIFs).

A priori computed power analysis with a significance level of 0.05, a power of 0.90, a total of 12 independent variables including an interaction effect (prior corticosteroid treatment*k-FLC index), and a medium effect size of the parameters of interest (according to previous findings a Cohen f2 of 0.15 was used) revealed a necessary sample size of 157 for testing all parameters. We consider a drop-out rate of 15%; thus, a total of approx. 180 patients are aimed to be recruited.

4. Description of methods:

k-FLC and albumin measurement will be performed out of CSF and serum by nephelometry or turbidimetry as available in the participating centers (e.g. for k-FLC: Freelite on Optilite [Binding Site] or N Latex on BN [Siemens]) according to the manufacturers' specifications.

Sample volume for CSF and serum (per "measurement"): 400 µl

Samples should be stored at -80°C (not more than 2 freezing/thawing) until analysis.

5. Sample handling at the investigating center:

Aliquots of CSF and serum will be sent to the others centers for k-FLC and albumin measurement. When relevant sample volumes remain, samples will be sent back to the respective centers if requested.

6. Names of researchers and/or technicians participating.

- Medical University of Innsbruck, Department of Neurology, Innsbruck, Austria

  Harald Hegen, Florian Deisenhammer

- Medical University of Graz, Department of Neurology, Graz, Austria

  Michael Khalil

- Mayo Clinic, Department of Laboratory Medicine and Pathology, Rochester, MN, USA

  Maria Alice Willrich

- Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK

  Sharmilee Gnanapavan

- University of Ulm, Department of Neurology, Ulm, Germany

  Hayrettin Tumani

- Charles University, Department of Neurology, Prague, Czech Republic

  Petra Nytrova

- Centre d'Esclerosi Múltiple de Catalunya, Department of Neurology/ Neuroimmunology, Hospital Universitari Vall d'Hebron, Universitat Autónoma de Barcelona, Barcelona, SpainPetra Nytrova

  Georgina Arrambide

Statistical analysis done by:

Prof. Janette Walde

Department of Statistics, University of Innsbruck

Universitätsstraße 15, A-6020 Innsbruck, Austria

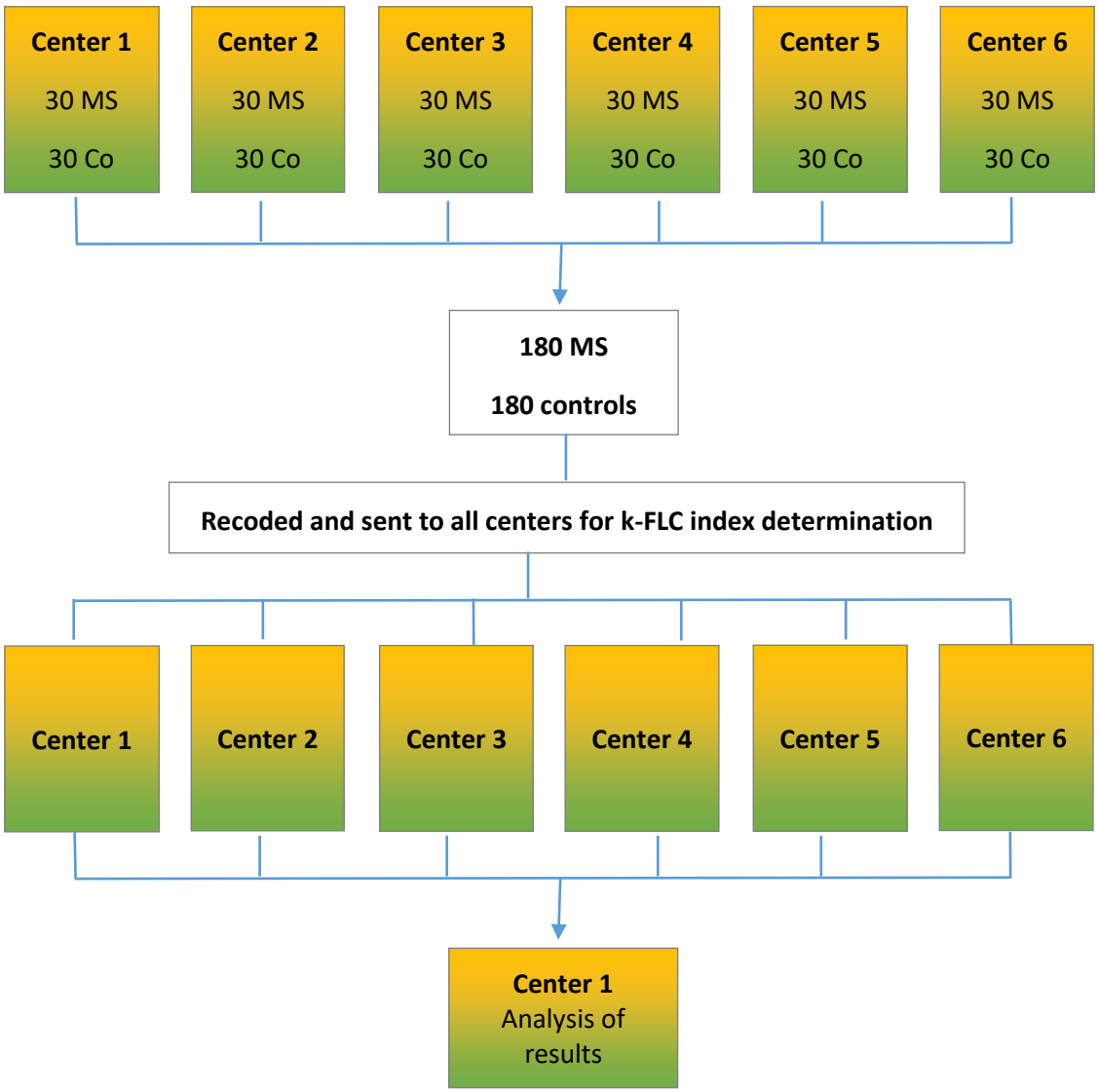
7. Please describe in detail how the participating centers will be compensated for their input (e.g. financial compensation (price per sample?), compensation in natura, sharing intellectual property, co-authorship (which position?)).

Collaborating/ contributing researches of above-mentioned centers will qualify for co-authorship on all publications evolving out of this proposal.

8. Intention to publish in international refereed journals.

The findings of this study should result in two publications in international, peer-reviewed journals.

Appendix



Flow chart