Circulating microRNAs as biomarkers in Multiple Sclerosis

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Primary Objective: The primary aim of this proposal is to identify miRNA profiles that can be used as novel MS biomarkers and to design a test, based on these profiles, that can be used in clinical practice.Key objectives are:(1) deep-sequencing of...

Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Demyelinating disorders
Study type	Observational invasive

Summary

ID

NL-OMON38705

Source
ToetsingOnline

Brief title
miRNAs in MS

Condition

- Demyelinating disorders

Synonym
Multiple Sclerosis

Research involving
Human

Sponsors and support

Primary sponsor: Universitair Medisch Centrum Groningen
Source(s) of monetary or material Support: Stichting MS Research

Intervention

Keyword: biomarker, microRNAs, Multiple Sclerosis, progression

Outcome measures

Primary outcome

The main study parameter is the miRNA profle in blood or CSF. The main clinical enpoint in the longitudinal study is the conversion from RR-MS to SP-MS. These data will be used to develop and validate a miRNA profile based test for the accurate identification of conversion to SPMS.

Secondary outcome

In addition analysis of observed miRNA profiles in different MS-types will provide insight in the (differences in) underlying disease mechanisms.

Study description

Background summary

Multiple Sclerosis
Based on pathological hallmarks, Multiple Sclerosis (MS) can be subdivided in 4 phases: 1) a pre-clinical phase, 2) relapsing/remitting MS (RRMS), 3) secondary progressive MS (SPMS), and 4) a burnt-out phase. During RRMS the immune system is responsible for the pathology, while during SPMS neuro-degeneration is the predominant pathological factor. It is important to distinguish these two stages as during the first inhibition of inflammation is a viable treatment option, while this has no proven efficacy during the latter. Presently there are no biomarkers available to discern these two phases. Interestingly, 10-15% of MS patients are diagnosed with another form of MS, called primary progressive MS (PPMS), without evidence for a preceding relapsing/remitting phase. As it is conceivable that the biomarker profile of PPMS resembles that of SPMS, this will be investigated as well.

Genetics of MS
MS is a multifactorial disease with a strong genetic component. A recent high-profile study describes >50 genetic variants associated with MS. Most of these affect genes encoding proteins that are known to play a role in

immunity-related cytokine pathways, co-stimulatory pathways, and signal transduction pathways. Surprisingly, only two genes could be associated with neuro-degeneration. Interestingly, several genetic variants associated with autoimmune diseases cannot be pinpointed to protein-encoding genes but localize to regions containing non-coding RNAs (ncRNAs) or to regulatory regions containing putative binding sites for transcription factors or ncRNAs (e.g. miRNA binding sites). Moreover, in some cases the localisation of these disease susceptibility markers suggests that they affect the expression or sequence of non-coding RNAs, rather than that of protein-encoding genes.

Circulating miRNAs as biomarkers for MS
A recently identified class of small ncRNAs, miRNAs, can provide novel biomarkers for MS. miRNAs are small (~19 to 24 nucleotides in length) ncRNAs that are stable and detectable in different body fluids, including plasma and cerebrospinal fluid (CSF). The biological function of miRNAs is to fine-tune gene expression and they do so by inducing the degradation of target mRNAs or by inhibition of translation of these. Excitingly, circulating miRNA profiles are disease and disease-stage specific.

The role of miRNAs in MS
A recent review summarizes several studies in which miRNAs have been profiled in MS lesions or in whole blood. From this a shortlist of miRNAs has emerged that have been identified in multiple studies. All of the MS miRNA profiles published to date were obtained by array- or PCR technology and none of them have investigated circulating miRNAs. We will profile circulating miRNAs using next generation sequencing (NGS) technology, providing unprecedented quantification accuracy and sequence depth. Moreover, in contrast to other techniques NGS allows for identification of novel miRNAs.

Research questions
Although widely studied in cancer research the concept of using circulating miRNAs as biomarkers for autoimmune diseases is relatively new. Presently, no papers have been published on MS-specific circulating miRNA profiles (nor on using the extremely sensitive NGS approach to detect MS-specific miRNA profiles). It is unknown to date whether CSF or plasma provides the best source of MS stage-specific miRNA profiles. We will investigate this in the proposed study.

Study objective

Primary Objective:
The primary aim of this proposal is to identify miRNA profiles that can be used as novel MS biomarkers and to design a test, based on these profiles, that can be used in clinical practice.

Key objectives are:
(1) deep-sequencing of circulating miRNAs in plasma and CSF of MS patients with

relapsing disease or progressive disease,
(2) quantification of known and novel miRNAs as performed previously,
(3) correlation of miRNA expression profiles with the investigated MS stages, and
(4) the design and validation of a test for the aforementioned MS stages.

Study design

In the explorative phase of the study circulating miRNAs will be profiled in samples already obtained from the *Nederlandse Hersenbank (NHB)* (Netherlands Brain Bank). Plasma and CSF of MS patients diagnosed with RRMS (n=30), SPMS (n=30), PPMS (n=10; the number of patients with PPMS is relatively limited in the NHB), and control samples (non-MS patients; n=30) have been obtained. In total this study design adds up to 200 paired samples of plasma and CSF.

The technical steps include:
• RNA collection from the samples using the commercially available miRNA isolation kit of choice (see section 6 of this proposal). These methods are described in literature; most researchers follow the instructions provided by the manufacturer (with small modifications).
• Preparation of miRNA sequencing libraries using the respective kit available from Illumina and subsequent deep sequencing performed on the HiSeq 2000 sequencer (Illumina).
• Analysis of the sequencing data (as published) and determination of stage specific miRNAs profiles.

Currently, it cannot be predicted whether a single miRNA will be enough to distinguish between the two MS stages under. It is just as likely that a panel of miRNAs will be needed to obtain sufficient specificity. Using the stage specific profiles predictive models will be developed based on stringent criteria.

This METc application is only relevant to the validation phase of the study:
In the validation phase of the project the profiles selected in the explorative phase will be validated and for this purpose we will use commercially available validated single-miRNA PCR assays for the miRNAs of interest. The *MS-centrum Noord Nederland* (Northern Netherlands MS-center) is an initiative from the University Medical Center and the Martini Hospital, both in Groningen. Within the center ~800 patients are regularly visiting the outpatient department of the two hospitals Of these, 200 RR-MS patients will be sampled and followed clinically in time. It can be estimated that ~10% of these patients will convert to SPMS within the next 2-3 years. The predictive value of the stage-specific miRNA-profiles will be investigated in two different ways.
First, as the samples obtained from the NHB have been obtained over the past decade, it is essential to investigate whether the profiles found in this material are also indicative of disease-stage in freshly obtained samples from an independent cohort of MS patients. To study this the test will be applied to

plasma obtained from 30 RRMS, 30 SPMS and 30 PPMS patients from Groningen, thereby measuring only a limited set of miRNAs that are representative for the disease-stage profile.
Secondly, we will also apply the test to samples collected twice a year from ~20 converted patients that were obtained before and after conversion and compare these to controls that did not convert from RRMS to SPMS during this period. Conversion to SPMS from RRMS will be defined retrospectively as confirmed progression of disability without concomitant relapses.

Study burden and risks

This study only involves routine venapuncture and study related adverse events are therefore not anticipated.
Individual participants will not benefit from participating in this study.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)

Elderly (65 years and older)

Inclusion criteria

A diagnosis of Relapsing Remitting Multiple Sclerosis (RR-MS) according to the revised McDonald criteria, >5 years after diagnosis. (n=200)
A diagnosis of Secondary Progressive Multiple Sclerosis (SP-MS) according to the revised McDonald criteria (n=30)
A diagnosis of Primary Progressive Multiple Sclerosis (PP-MS) according to the revised McDonald criteria (n=30)
Age 18-55 years.

Exclusion criteria

recent diagnosis of RR-MS (low probability of converting to Secondary Progressive MS (SP-MS)).

Study design

Design

Study type: Observational invasive

Masking:	Open (masking not used)
Control:	Uncontrolled
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	24-01-2014
Enrollment:	260
Type:	Actual

Ethics review

Approved WMO

Date:	23-07-2013
Application type:	First submission
Review commission:	METC Universitair Medisch Centrum Groningen (Groningen)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL43107.042.13