Version 28/01/2002

VITATOPS-Magnetic Resonance Imaging Substudy

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Background

The Vitamins To Prevent Stroke Study (VITATOPS) is based on observational investigations reporting an independent and linear relationship between increasing homocysteine levels and stroke and the possibility of lowering homocysteine concentrations by supplementation of folic acid, vitamin B6 and vitamin B12 (1-3). Most previous research on the association between hyperhomocysteinemia and cerebrovascular disease did not differentiate between different stroke subtypes. Yet, meanwhile there have been two studies using neuroimaging to differentiate between various stroke pattern (4,5). Both investigations demonstrated an association between homocysteinemia and strokes due to small vessel disease, while there existed no significant relationship with large vessel disease-related cerebrovascular attacks. The study of Faßbender and coworkers (5) used clinical and imaging findings to define a group of patients with subcortical vascular encephalopathy (SVE) with white matter lesions or lacunes representing imaging evidence of cerebral small vessel disease. Study participants with SVE had homocysteine levels of 18.24 µmol/L as opposed to levels of 13.81 µmol/L in patients with large vessel disease-related strokes and 13.06 µmol/L in controls. The differences between SVE patients and large vessel strokes and controls were statistically significant and hyperhomocysteinemia =>15 µmol/L was the most important predictor for

SVE in logistic regression analysis followed by arterial hypertension, age and smoking. Another study by van den Berg (6) also reported an association between mild hyperhomocysteinemia and diffuse periventricular white matter lesions or multiple small infarcts. We plan an MRI substudy of VITATOPS which provides the unique opportunity to study if lowering of homocysteine levels may reduce the evolution of diffent types of vascular brain ischemia including both large and small vessel disease.

It is particularly interesting as to whether vitamin supplementation with subsequent reduction of homocyteine levels results in slowing of the progression of small vessel disease-related brain lesions. This is a pertinent question as progression of such brain abnormalities may result in cognitive decline and dementia but also in gait disturbances and falls both major sources of disability in our aging societies. Implementation of MRI in a subsample of VITATOPS may allow to answer this clinically important question. Our own group has only recently published first results on the progression rate of white matter changes in middle-aged and elderly normals. It has been shown that even in „normals“ such changes progress at a considerable rate of 17.9% over a time period of 3 years (7). Conceivably, symptomatic individuals with more pronounced cerebral arteriolosclerosis as studied in VITATOPS will present even more rapid progression of small vessel disease related brain lesions. Based on the association of hyperhomocysteinemia and cerebrovascular disease we plan to use ischemic brain lesions as a surrogate marker for efficacy of multivitamin supplementation in a subset of the VITATOPS cohort.

Patients

VITATOPS patients who underwent brain MRI at the baseline evaluation can be included in the substudy regardless of their clinical stroke classification.

Diagnostic Work-up

The diagnostic work-up is identical to VITATOPS, however fasting homocysteine levels and MMSE as well as Hamilton Depression scale should be performed as this enables to perform also a cross-sectional data analysis of homocysteine levels and MRI small vessel disease at baseline and may allow to assess if a presumed slowing of lesion progression under therapy is paralleled by less pronounced cognitive decline. The investigators should also state at baseline at follow-up if study participants fulfill a diagnosis of dementia based on the DSM IV criteria.

Sample size

Sample size calculation can only roughly be estimated based on our previous findings in normal individuals which probably results in an overestimation of the sample size. Based on quantitative assessments of lesion load in the Austrian Stroke Prevention Study one would need approximately 200 subjects per treatment arm when focusing on white matter abnormalities alone. This number applies when considering that multivitamin supplementation reduces WMH progression by 30% over 2 years at an alpha-value of 0.05 and a beta-value of 0.80. For the analysis focusing on change of total vascular burden the estimates are somewhat lower.

MR Studies

VITATOPS participants would have to be re-scanned after 2 years or if patients withdraw from the study at the the time of withdrawl. The scanning protocol will be as follows:

MR scanning

Baseline and follow-up scans should be performed according to protocol A or B as outlined in Appendix I (may still be modified by the Steering Group). Two scanning options are provided to allow for greater flexibility at different sites. However, every site has to decide for either protocol A or B. This protocol has then to be used throughout the study. Besides the use of similar sequences at all sites, exact repositioning at follow-up will also be of crucial importance and the following procedure should be adopted: Three coronal scout views serve to determine the interhemispheric fissure and to angulate subsequent sagittal scout views accordingly. The mid-axial plane is then defined on the mid-sagittal section by a line which passes just inferior to the genu of the corpus callosum and below its splenium (examples will be provided). For quality assurance it would be desirable to send a “dummy run” with the chosen sequences including repositioning to the MR Analysis Center in Graz (see address below). This should be done at the start of the study and such a “dummy run” can well be performed on the first patient.

Image analysis

Image analysis will be performed centrally in Graz. For this purpose the MR examinations are sent **both** on hard copy and in electronic format. Appendix B outlines the formats that are currently supported by our site. Specific other solutions can be discussed with Dr. Ropele and his e-mail will be provided.

Lesion progression will be assessed both by visual inspection (7) and quantitatively (8).

Visual Inspection and Rating

Scans will be assessed by one experienced reader for presence of white matter hyperintensities (WMH) and lacunes. The baseline and follow-up scans of each study participant will be read blinded to the clinical data of study participants. WMH will be specified and graded according to our scheme (9,10) into absent (grade 0), punctate (grade1), early confluent (grade2), and confluent (grade 3) abnormalities. The number of WMH will be recorded and categorized into 0, 1-4, 5-9, and >9 lesions. We will disregarded caps and „pencil-thin" periventricular lining as they represent normal anatomical variants. Lacunes will be defined as focal lesions isointense to CSF involving the basal ganglia , the internal capsule, the thalamus or the brainstem not exceeding a maximum diameter of 10mm. The number of lesions will be recorded. Change of WMH in grade and number from baseline will first be determined by direct scan comparison. The change in number will again be categorized into 0, 1-4, 5-9 and > 9 lesions. Newly occurring lacunes will be registered. Regression or progression of small vessel disease-related brain changes will be graded as absent, minor or marked. A change from baseline by 1 to 4 punctate WMH will be defined as minor. Differences between both scans exceeding 4 punctate foci or newly occurring lacunes will be considered to be marked. We will also record evidence for thromboembolic infarcts.

Quantitative Assessment

For volumetric analysis signal hyperintensities will first be marked by an experienced investigator on a transparency fixated to the hard copy of the scan. With this tracing as a guide, the technical assistant displays each slice containing an abnormality on the monitor of a work station. In general, measurements are performed on proton density weighted scans or FLAIR scans of the entire brain. Using a mouse-controlled cursor the perimeter of the lesions is indicated manually on the screen. The computer program then examines the image in a region close to where the mouse was clicked to find the strongest local intensity gradient. This is considered to be the edge of the lesion. Then the lesion is outlined by following a contour of isointensity from this initial edge point thereby defining the lesion as a region of the image, where the signal intensity is locally above the signal intensity at the initial edge position. In case of errors in the automatic lesion definition because of structures with a similar signal intensity, the lesions are traced manually by the operator. At the end of lesion definition on all slices, the lesion volume is calculated as the total lesion area multiplied by the slice thickness. This results in the total volume of hyperintense lesions.

The software program for these semi-automated local thresholding technique has been developed and provided by David Plummer (11), University College London, UK, and is

implemented on three computer work stations in the Department of Neurology, Karl-Franzens University Graz. The software program was already successfully used for determining the lesion load in MS patients and the intra-observer variability of this technique was reported to be as low as 3%. Costs for this analysis will be covered by a grant application or local funding at the Department of Neurology in Graz.

Homocysteine measurements

Homocysteine measurements will be performed at the Department of Laboratory Medicine of the Karl-Franzens University Graz. Measurements will be done by a fluorescence polarization immunoassay on an Imx autoanalyzer (Abott). Blood samples will be obtained at the time of the first and second MRI study. Blood has to be drawn in fasting condition. It has to be centrifuged as soon as possible but at least within 60 minutes. A total of 750µl has to be pipetted to plastic vials and stored at –20°Celsius.Frozen samples at –20°Celsius are stable for at least 8 months. We request for batchwise shipment of samples on dry ice. There is no restriction of the number of samples on our part. The maximum transportation time allowed depends on the isolation of the container and the amount of dry ice. According to our experience, 3-5 kilogram of dry ice keeps the samples frozen for at least 3 days. The use of an international delivery servive will therefore be necessary. The shipment address is

Dr. Andreas Tiran
Department of Laboratory Medicine
Auenbruggerplatz 15
A-8036 Graz
Austria-Europe

Shipment will have to announced in advance to

Dr.Andreas Tiran
Phone: 0043 (0) 316 385 81829
Fax: 0043 (0) 316 385 3430
e-mail: andreas.tiran@kfunigraz.ac.at

Alternatively, centers may measure homocysteine levels on their own but then the inter-laboratory variability between centers will be evaluated prior to the start of the study.

Data Analysis

We will correlate the fasting homocysteine levels of study participants with evidence and severity of vascular brain lesion particularly small vessel disease-related brain abnormalities. The longitudinal study will compare the rates of lesion progression and the increase in lesion

load over time between the vitamin and the placebo groups. Analyses will be adjusted for possible between group differences of baseline characteristics.

Finances

The Department of Neurology at the Karl-Franzens University Graz, Austria will perform all scan assessments but is unable to cover any costs for performing MRIs or shipping of hardcopies. Tapes or hard copies will be returned to the respective centers. The costs of homocysteine measurements will be covered by the Central Laboratory in Graz but again no shipment cost will have to be covered by the participating centers.

Publication of MRI Substudy Results

To be clarified with the VITATOPS Steering Committee. Depending upon the number of participating centers one could decide to publish in the name of a group or as a list of authors for the VITATOPS Study Group with a certain number of investigators being listed per contributing group depending upon the magnitude of contribution by a given center. Before submission of the main result paper of the MRI substudy all investigators will have the opportunity to comment. As for the whole study investigators may publish on patients in their own centers after the main results have been published

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VITATOPS

MRI PROTOCOL

Positioning and Repositioning

All transversal images should be planned from the midsagittal localizer (which follows transversal and coronal scouts). Two internal landmarks are used to determine the position and angulation of the central images, the inferior borders of the corpus callosum in front (rostrum) and back (splenium). Both landmarks have to be clearly visible on the midsagittal view otherwise repeat the localizer scan. Identical position has to be achieved at follow-up visits.

Scans / Examination Procedure

1.) Scout / Localizer
2.a) PDW/T2W-Sequence or **2.b)**Turbo T2 and TurboFLAIR
3.) T1W-Sequence
4.) T1W 3D-GE

Imaging Geometry and Orientation for all Sequences

APPENDIX I

- FOV: 250 mm
- RECT_FOV: 70-80 % (optional)
- THK: 5 mm contiguous slices
- SLICES: 20-28 (whole brain coverage)
- ORIENT: axial
- MATRIX: 256^2
- PHASE ENCODING DIRECTION: RL (not for scout localizer)
- HALF_FOURIER: only if SNR is reasonable
- BANDWIDTH: optimize

1 T1W-Scouts/Localizer (SE or GE) *Duration: ~ 1-3 min.*

- Axial and coronal scout
- Sagittal survey (localizer)
- Fast imaging technique can be used

- Central scout slice should match the cerebral falx

- From the sagittal localizer axial scans are planned parallel to the corpus callosum line (i.e through the rostrum of the CC anteriorly and just below the splenium of the CC posteriorly)

2a PDW/T2W-Sequence *Duration: ~ 6 - 10 min*

- TE (first echo): 10-30 msec.
- TE (sec. echo): 60-120 msec.
- TR: 2000-3000 msec.
- AVERAGES: 1
- FLOWCOMP: yes
- SATURATION SLAB: inferior with a gap of at least 5 mm (if possible)

2b Fast Spin Echo T2 *Duration: ~ 2 - 3 min*

- TE: 100-120 msec.
- TR: 4000-5000 msec.
- AVERAGES: 2
- echoes per shot (Turbofactor): 16-24

Turbo FLAIR *Duration: ~ 3 - 4 min*

- TE: 100-140 msec.
- TR: 8000-11000 msec.
- TI: 2200-2400 msec.
- AVERAGES: 2
- echoes per shot (Turbofactor): 16-24

3 T1W-Sequence *Duration: ~ 6 - 8 min*

- TE: 10-20 msec
- TR: 500-700 msec.
- AVERAGES: 2
- FLOWCOMP: yes
- SATURATION SLAB: inferior with a gap of at least 5 mm (if possible)

4 T1W-3D GE (should be discussed) – atrophy measurenments ???

APPENDIX II

The following data formats and transfer media are currently supported by the MR Analysis Center in Graz:

MR system	Electronic medium	Format
GE	4mm DAT	GE archive format
	4mm DAT	images extracted with ximg utility (GE Advantage)
	CDROM	images extracted with ximg utility (GE Advantage)
Siemens	Optical Disc $5^1/_4$" (Pioneer DEC-702 only, no WORMs !)	Siemens private format
	4mm DAT	ACR-NEMA images, UNIX-Tar
	CDROM	ACR-NEMA images
Philips	Optical Disc $5^1/_4$" and older one	Philips private format
	TAPE (TK70)	Philips private format
	4mm DAT	ACR-NEMA images, UNIX-Tar
	CDROM	ACR-NEMA images

Shipping address: Franz Fazekas, M.D., Department of Neurology and MR Analysis Center, Karl-Franzens University Graz, Auenbruggerplatz 22, A-8036 Graz, Austria