Supplemental Online Content

Enhanced diagnostic work-up increases pathological findings in patients with acute ischaemic stroke – Results of the prospective HEBRAS study

Supplemental Methods

Inclusion and exclusion criteria of the HEBRAS study:

Inclusion criteria

• Written informed consent by patient

• Age ≥ 18 years.

• Acute ischaemic stroke (with matching brain lesion on MR imaging).

• Admission to the stroke unit at the Charité, Campus Benjamin Franklin

• Enrolment within 144 h after onset of stroke-related symptoms.

Exclusion criteria

• Known AF by past medical history before hospital admission.

• Atrial fibrillation (AF) according to 12-lead ECG on hospital admission.

• AF according to inpatient ECG recording / stroke unit monitoring before enrolment.

• Pre-stroke life expectancy less than 1 year.

• Participation in an interventional study.

• Pregnancy and / or breast-feeding.

• Contraindications to undergo MRI (i.e., mechanic heart valve, cardiac pacemaker) • History of adverse response to MRI contrast agents

• Clinically severe heart failure (NYHA III-IV)

• Renal insufficiency (creatinine > 1.3 mg/dl (females); creatinine > 1.7 mg/dl (males))

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Magnetic resonance imaging

All examinations were performed on a 3 Tesla MR scanner (MAGNETOM® TIM TRIO, Siemens, Erlangen, Germany) *Brain MRI* consisted of a T2*-sensitive sequence (repetition time (TR) 620 ms, echo time (TE) 20 ms, flip angle 20°, field-of-view (FOV) 220 mm, slice thickness (ST) 5 mm) to exclude intracerebral haemorrhage, a diffusion weighted (DWI) sequence (TR 7600 ms, TE 93 ms, FOV 230 mm, ST 2.5 mm) for detection of ischemic tissue, and a 3D time-of-flight (TOF) angiographic sequence (TR 22 ms, TE 3.86 ms, flip angle 18°, FOV 200 mm, ST 0.65 mm) for imaging of intracranial vessels and a fluid-attenuated inversion recovery (FLAIR) sequence (TR 8000 ms, TE 96 ms, FOV 220 mm, ST 5 mm) for the detection of chronic infarction.

Cardiac MRI was performed using ECG and pulse triggering. Localization and planning were based on a dark-blood-prepared HASTE sequence (half-Fourier acquisition single-shot turbo spin-echo) with the following parameters: TR 750 ms, TE 49 ms, Flip angle 160°, ST 5 mm, FOV/matrix. Two chamber, three chamber and four chamber view were acquired using double-oblique cine SSFP (steady state free precession) sequence (TR 40.56 ms, TE 1.48 ms, flip angle 50°, slice thickness 5 mm) with prior scouting for individual frequency adjustment. Short axis examinations of the left ventricle were obtained parallel to the atrioventricular plane with prior individual frequency adjustment (TR 40.32 ms, TE 1.48 ms, flip angle 50°, matrix size 256x216 pixel, ST 8 mm (2 mm interslice gap)). Integrated parallel imaging techniques (iPAT) with two-fold acceleration were used for short axis stack acquisition. Following standard k-space segmented SSFP imaging, a fast SSFP real-time sequence (CRT) was used for short axis cine imaging (TR 48.6 ms, TE 1.21 ms flip angle 55°, matrix size 128x54 pixel, ST 8 mm (2 mm interslice gap) with three-fold acceleration (iPAT). ECG triggering was performed every second heartbeat to capture the entire diastole. Post-contrast imaging is performed after intravenous injection of 0.1 mmol/kg body weight Gadobutrol (Gadovist®, Bayer Schering Pharma, Berlin, Germany) with post-contrast cine imaging parameters held constant. For angiographic imaging

of the aortic arch as well as extracranial cerebral arteries, a three-dimensional TWIST (Time-resolved angiography With Interleaved Stochastic Trajectories) sequence was used (TR 2.36 ms, TE 0.99 ms, Flip angle 20°, ST 1.40 mm). Finally, myocardial late enhancement is characterized employing a turbo FLASH (Fast Low Angle SHot) sequence (TR 750.00 ms, TE 1.97 ms, Flip angle 20°, ST 8 mm).

Assessment of systolic cardiac function

To determine systolic cardiac function, left ventricular ejection fraction (LVEF) was determined based on TTE or CMR. In case of TTE, biplane method of disks (modified Simpson's rule) was used to determine LVEF according to guideline recommendations[22]. In case of CMR, LVEF was derived from volume-time-curves generated by delineating endocardial contours of the left ventricle in each short axis stack whenever possible or using enddiastolic and endsystolic volumes on long axis four chamber views[15]. As it is regarded to be the gold standard for LVEF estimation, CMR-derived LVEF was used for analysis whenever available[23]. CMR analysis was performed on cvi42® software (Circle Cardiovascular Imaging Inc., Calgary, Canada).

Detailed description of stroke aetiology after routine work-up

Analysing all 356 patients after routine clinical work-up, aetiology of AIS was categorized as "large-artery atherosclerosis" in 70 (19.7%) patients. Fifteen (4.2%) patients were categorized as "cardioembolic" (for the following reasons: newly detected atrial fibrillation (n=5), akinetic left ventricular segment (n=6), bioprosthetic aortic valve (n=2), paradoxical embolism (patent foramen ovale and deep venous thrombosis) (n=1) and systolic dysfunction (LVEF = 35%); n=1).

With regard to small-artery occlusion, 85 patients (23.9%) presented with a subcortical infarct with a diameter <2 cm on DWI imaging and a corresponding lacunar syndrome. A total of ten patients (2.8%) was found to have another determined aetiology.

Fourteen patients (3.9%) presented with competing aetiologies (lacunar syndrome with DWI lesion <2 cm and ipsilateral stenosis >50% or occlusion n=11, lacunar syndrome with DWI lesion <2 cm and aortic plaque of 7 mm (TEE) n=1 and ipsilateral stenosis >50% and concomitant atrial fibrillation n=2).

The remaining 162 patients (45.5%) were classified as “cryptogenic”. Of those, 45 (12.6%) patients did not receive echocardiography in clinical routine (therefore labelled as “cryptogenic because of incomplete workup”).

Comparison of baseline characteristics in patients with and without echocardiography

Comparison of study participants who received echocardiography with those who did not revealed that patients not undergoing echocardiography were older, more often severely affected and more dependent on the help of others on hospital admission, suffered more frequently from hypertension, were discharged earlier, and were more often on ACE inhibitors (**supplemental table 1**).

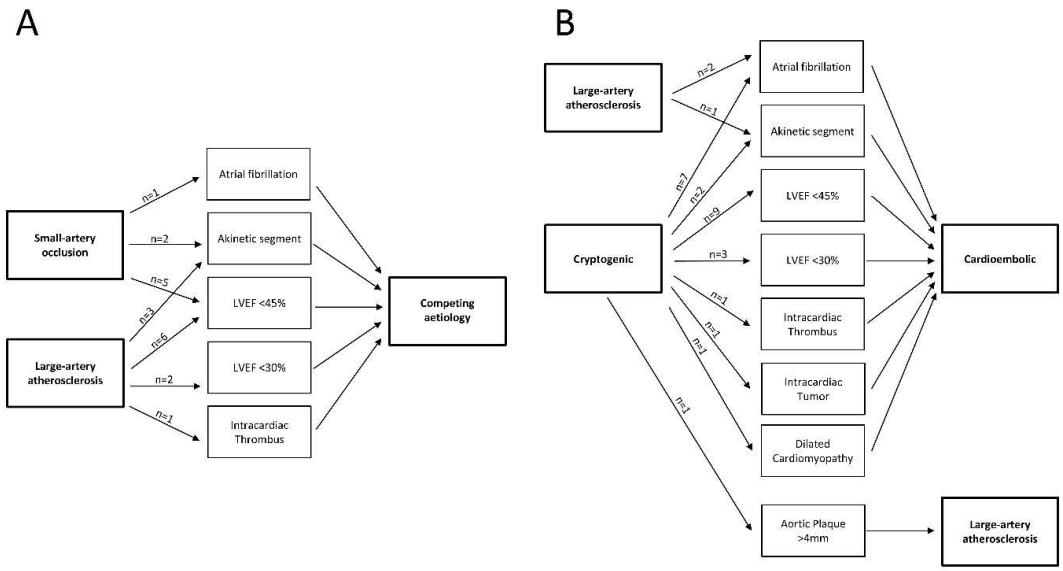
Reasons not to undergo CMR and comparison of baseline characteristics in these patients

In our study, 42 patients (11.8%) refused to undergo the CMR examination after enrolment, 11 patients (3.1%) were discharged prior to planned CMR, three patients (0.8%) were unable to follow breath-hold commands, three patients (0.8%) suffered worsening of the symptoms which precluded the examination, two patients (0.6%) had claustrophobia, two patients (0.6%) were unable to undergo CMR because of severe adipositas, and one patient (0.3%) suffered a panic attack immediately prior to the examination.

Comparing patient groups with and without CMR revealed that patients who did not undergo CMR were older, more often female, more often severely affected and more dependent on the help of others on hospital admission, suffered more frequently from hypertension and less likely from hypercholesterolemia (**supplemental table 2**).

Supplemental Figure 1

Detailed depiction of changes in aetiological classification after routine diagnostic work-up and after enhanced diagnostic work-up, resulting in competing aetiologies (A), cardioembolic (B) or large-artery atherosclerosis (B).



LVEF, left ventricular ejection fraction

Supplemental Table 1 – Baseline characteristics of all 356 HEBRAS patients and the subgroup of 228 AIS patients who received echocardiography (TTE and/or TEE) and the subgroup of 128 patients who did not receive echocardiography. Data are given as mean (STD), median [IQR] or n (%).

	Whole cohort (n=356)	**Echocardio-graphy (n=228)**	**No echocardio-graphy (n=128)**	**p**
Female sex	134 (37.6)	86 (37.7)	48 (37.5)	0.967
Age (years)	66 (12)	64 (13)	71 (9)	<0.001
Length of in-hospital stay (days)	5 [4-6]	5 [4-6]	4 [3-6]	0.032
NIHSS on admission (points)	2 [1-4]	2 [0-3]	2 [1-4]	0.002
mRS on admission (points)	2 [1-3]	2 [1-3]	2 [1-3]	0.008
Barthel Index on admission (points)	100 [75-100]	100 [80-100]	90 [70-100]	0.005
Intravenous thrombolysis	66 (18.5)	45 (19.7)	21 (16.4)	0.438
Diabetes mellitus	65 (18.3)	41 (18.0)	24 (18.8)	0.857
Arterial hypertension	212 (59.6)	123 (53.9)	89 (69.5)	0.004
High blood lipids	125 (35.1)	77 (33.8)	48 (37.5)	0.479
Current tobacco use	96 (27.0)	60 (26.3)	36 (28.1)	0.712
Previous ischaemic stroke or TIA	58 (16.3)	32 (14.0)	26 (20.3)	0.124
Coronary artery disease	42 (11.8)	27 (11.8)	15 (11.7)	0.972

Prior myocardial infarction	31 (8.7)	17 (7.5)	14 (10.9)	0.264
Chronic heart failure	7 (2.0)	3 (1.3)	4 (3.1)	0.238
Oral anticoagulation on admission*	6 (1.7)	4 (1.8)	2 (1.6)	0.893
Antiplatelet(s) on admission	114 (32.0)	69 (30.3)	45 (35.2)	0.342
Acetylsalicylic acid (ASA)	97 (27.2)	60 (26.3)	37 (28.9)	
Clopidogrel	11 (3.1)	6 (2.6)	5 (3.9)	
Dual antiplatelets	6 (1.7)	3 (1.3)	3 (2.3)	
Beta blocker on admission	100 (28.1)	60 (26.3)	40 (31.3)	0.320
ACE inhibitor on admission	64 (18.0)	34 (14.9)	30 (23.4)	0.044
Angiotensin II receptor antagonist on admission	73 (20.5)	43 (18.9)	30 (23.4)	0.305
Calcium channel blocker on admission	65 (18.3)	38 (16.7)	27 (21.1)	0.299
Statin on admission	98 (27.5)	60 (26.3)	38 (29.7)	0.494

AIS, Acute ischemic stroke; TTE, transthoracic echocardiography; TEE, transesophageal echocardiography; STD, Standard deviation; IQR, Interquartile range; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin scale; TIA, transient ischemic attack; ACE, angiotensin converting enzyme

Supplemental Table 2 – Baseline characteristics of all 356 HEBRAS patients and the subgroup of 292 AIS patients who received cardiovascular MRI (CMR) and the subgroup of 64 patients who did not receive CMR. Data are given as mean (STD), median [IQR] or n (%).

	Whole cohort (n=356)	**CMR (n=292)**	**No CMR (n=64)**	**p**
Female sex	134 (37.6)	103 (35.3)	31 (48.4)	0.049
Age (years)	66 (12)	65 (12)	70 (12)	0.003
Length of in-hospital stay (days)	5 [4-6]	5 [4-6]	5 [3-6]	0.891
NIHSS on admission (points)	2 [1-4]	2 [1-3]	3 [2-4]	0.004
mRS on admission	2 [1-3]	2 [1-3]	2 [1-3]	0.004
Barthel Index on admission (points)	100 [75-100]	100 [80-100]	90 [70-100]	0.008
Intravenous thrombolysis	66 (18.5)	53 (18.2)	13 (20.3)	0.687
Diabetes mellitus	65 (18.3)	52 (17.8)	13 (20.3)	0.639
Arterial hypertension	212 (59.6)	164 (56.2)	48 (75.0)	0.005
High blood lipids	125 (35.1)	110 (37.7)	15 (23.4)	0.031
Current tobacco use	96 (27.0)	77 (26.4)	19 (29.7)	0.588
Previous ischaemic stroke or TIA	58 (16.3)	47 (16.1)	11 (17.2)	0.830
Coronary artery disease	42 (11.8)	38 (13.0)	4 (6.3)	0.129
Prior myocardial infarction	31 (8.7)	28 (9.6)	3 (4.7)	0.208
Chronic heart failure	7 (2.0)	6 (2.1)	1 (1.6)	0.797

Oral anticoagulation on admission*	6 (1.7)	4 (1.4)	2 (3.1)	0.323
Antiplatelet(s) on admission	114 (32.0)	95 (32.5)	19 (29.7)	0.658
Acetylsalicylic acid (ASA)	97 (27.2)	79 (27.1)	18 (28.1)	
Clopidogrel	11 (3.1)	10 (3.4)	1 (1.6)	
Dual antiplatelets	6 (1.7)	6 (2.1)	-	
Beta blocker on admission	100 (28.1)	78 (26.7)	22 (34.4)	0.217
ACE inhibitor on admission	64 (18.0)	49 (16.8)	15 (23.4)	0.209
Angiotensin II receptor antagonist on admission	73 (20.5)	63 (21.6)	10 (15.6)	0.286
Calcium channel blocker on admission	65 (18.3)	53 (18.2)	12 (18.8)	0.911
Statin on admission	98 (27.5)	81 (27.7)	17 (26.6)	0.849

AIS, Acute ischemic stroke; STD, Standard deviation; IQR, Interquartile range; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin scale; TIA, transient ischemic attack; ACE, angiotensin converting enzyme

Supplemental Table 3 – Detailed description of the pathological findings detected by CMR defined as relevant for stroke aetiology a priori per study protocol of the HEBRAS trial. CMR detected 60 pathological findings in 47 patients (LVEF<45%: n=40 [LVEF<30%: n=8]; at least on akinetic myocardial segment: n=14; intraventricular thrombus: n=3; dilated cardiomyopathy: n=2; intracardiac tumour: n=1).

Study ID	Major risk cardioembolic source detected by CMR
H003	Ischemic LGE, WMA, LVEF=33%
H007	LVEF=33%
H016	Ischemic LGE, WMA. Transmural chronic MI with hypo-/dyskinesia. Left ventricular thrombus in apex.
H018	LVEF=43%
H022	Ischemic LGE. Chronic MI RIVA / Apex. Akinesia. 13 mm intraventricular thrombus. LVEF=41%
H023	LVEF=43%
H029	Chronic, non-transmural MI with hypokinesia. LVEF=42%
H035	LVEF=30%
H045	LVEF=36%
H061	Ischemic LGE; transmural chronic MI with lateral hypo-akinesia. LVEF=25%
H075	Global hypokinesia. LVEF=41%
H086	Transmural wall thinning with akinesia.
H112	LVEF=38%
H119	Chronic MI anterior wall / septum. Apical aneurysm with thrombus. Ischemic LGE. LVEF=40%
H128	Transmural chronic MI apical / inferior with dyskinesia. LVEF=41%

H146	Ischemic LGE. Inferolateral chronic MI with akinesia. LVEF=44%
H147	LVEF=30%
H159	Ischemic LGE. Almost transmural chronic MI; wall thinning, akinetic myocardium. Biatrial dilatation.
H162	Dilated cardiomyopathy with severely impaired LVEF (LVEF=14%). Diffuse hypokinesia with septal akinesia. Left atrial dilatation.
H165	Ischemic LGE. Chronic MI with wall thinning. Septal-basal to midventricular akinesia. LVEF=31%
H185	LVEF=35%
H189	LVEF=21%
H196	Ischemic LGE; Basal-septal chronic MI. LVEF=15%
H197	Ischemic LGE; Transmural chronic MI inferolateral with hypokinesia. LVEF=36%
H207	Concentric left ventricular hypertrophy; Biventricular lipoma.
H215	Non-ischemic LGE; LVEF=41%
H216	Ischemic LGE; chronic MI with septal hypokinesia. LVEF=39%
H255	Ischemic LGE; lateral-basal chronic MI with akinesia.
H258	LVEF=44%
H260	LVEF=44%
H264	LVEF=42%
H267	Non-ischemic LGE inferolateral/septal. Dilated cardiomyopathy. Left ventricular hypertrophy, borderline mitral valve prolapse. LVEF=38%
H273	Marked biatrial dilatation. LVEF=37%
H288	Ischemic LGE; chronic MI with hypokinesia anterior, anterolateral and inferolateral. LVEF=17%

H290	Ischemic LGE; transmural chronic MI with septal and apical akinesia.
H298	LVEF=20%
H306	Ischemic LGE; right atrial dilatation. LVEF=40%
H307	Ischemic LGE; Akinesia apical inferior wall and apex.
H318	Ischemic LGE; circumscript scarring left ventricular outflow tract DD infarction DD non-compaction cardiomyopathy. LVEF=41%
H328	Ischemic LGE; inferior wall akinesia. LVEF=38%
H329	Ischemic LGE; left atrial and left ventricular dilatation. Non-transmural chronic MI; inferolateral hypokinesia. Non-ischemic fibrosis septal basal. LVEF=36%
H330	LVEF=43%
H339	Chronic MI with akinesia inferior / inferolateral wall basal to midventricular. LVEF=39%.
H345	LVEF=42%
H360	Ischemic LGE; 50% transmural chronic MI inferolateral wall. Global hypo-akinesia of the left ventricle. LVEF=16%. Suspected non-compaction cardiomyopathy.
H363	Apical to lateral hypo-akinesia. Chronic MI inferolateral wall basal to midventricular. LVEF=28%.
H368	Gross vegetations of the mitral valve with mitral valve prolapse and regurgitant jet. LVEF=40%. (Endocarditis was not confirmed on TEE).

LGE, late gadolinium enhancement; WMA, wall motion abnormalities; LVEF, left ventricular ejection fraction; MI, myocardial infarction; RIVA, ramus interventricularis anterior