Thesis for the degree of Doctor of Philosophy (PhD)

CARDIOVACULAR AND CEREBROVASCULAR COMORBIDITIES IN RHEUMATOID ARTHRITIS

by Zsófia Kardos, MD

Supervisor: Prof. Zoltán Szekanecz, MD, PhD, DSc

UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF CLINICAL MEDICINE

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CONTENTS

1 ABBREVIATIONS

25-OH-vitD_3	25-hydroxy-vitamin D_3
ACA	anterior cerebral artery
anti-CCP	anti-Cyclic Citrullinated Peptide antibody
ACPA	Anti-Citrullinated Protein Antibodies
AHA	American Heart Association
Apo	apolipoprotein
ASE	American Society of Echocardiography
BA	basilar artery
BFV	blood flow velocity
biol	biologic treatment
BMD	bone mineral density
CBF	cerebral blood flow
CCA	common carotid artery
CI	confidence interval
cIMT	carotid intima-media thickness
CPP	cerebral perfusion pressure
CRC	cerebrovascular reserve capacity
CRP	C-reactive protein
CS	corticosteroids
CT	computed tomography
CTX	β-CrossLaps
CV	cardiovascular
DMARD	disease-modifying anti-rheumatic drug
DEXA	dual-energy x-ray absorptiometry
ELISA	enzyme-linked immunosorbent assay
EDFV	end diastolic flow velocity
ESC	European Society of Cardiology
EULAR	European League Against Rheumatism
FFT	Fast Fourier Transform
HDL	high density lipoprotein
HLA	human leukocyte antigen
ICA	internal carotid artery
ICP	intracranial pressure
IFX	infliximab
IL	interleukin

LDL	low density lipoprotein
MCA	middle cerebral artery
MFV	mean blood flow velocity
MMP	matrix metalloproteinase
MRI	magnetic resonance imaging
MS	metabolic syndrome
MTX	methotrexate
MVCC	mean flow velocity over one cardiac cycle
OC	osteocalcin
OPG	osteoprotegerin
P1NP	procollagen type 1 amino-terminal propeptide
PaCO2	carbon dioxide pressure
PCA	posterior cerebral artery
PI	pulsatility index
PSFV	peak systolic flow velocity
PTH	parathormone
RA	rheumatoid arthritis
RANK	receptor activator of nuclear factor-kappa B
RANKL	receptor activator of nuclear factor-kappa B ligand
RI	resistance index
RF	rheumatoid factor
sdLDL	small dense low density lipoprotein
SMR	standardized mortality ratio
SOST	sclerostin
SVM	Society for Vascular Medicine
TAW	temporal acoustic window
TAWF	temporal acoustic window failure
TBD	to be determined
TC	total cholesterol
TCD	transcranial Doppler
TCZ	tocilizumab
TNF-α	tumor necrosis factor alpha
TG	triglyceride
US	ultrasound

2 INTRODUCTION

2.1 Accelerated atherosclerosis in rheumatoid arthritis

Chronic inflammatory rheumatic diseases, such as rheumatoid arthritis (RA), mostly affect the joints but frequently other organ systems [1]. RA can also damage a wide variety of body systems, including the skin, eyes, lung, cardiovascular (CV) and even the central nervous system.

The life expectancy of patients with RA is reduced by at least 5-7 years. Their standardized mortality ratios (SMR) are between 0.87 and 3.0 [2]. The main causes of mortality in RA are CV and cerebrovascular diseases [1-3] primarily due to accelerated atherosclerosis associated with RA. Accelerated atherosclerosis can be observed even in early phase of the rheumatic disease [4].

Atherosclerosis is a systemic and also an immune-mediated disease. Accelerated atherosclerosis has also been designated as "autoimmune" or "inflammatory" atherosclerosis. The sustained "high-grade" inflammation in RA patients leads to accelerated atherosclerosis compared to general population, where atherosclerosis is associated only with "low-grade" inflammation. C-reactive protein (CRP) is an acute phase protein, its increased blood levels have been correlated with the activity of systemic inflammation. Elevated CRP levels in sera are more common in patients with RA. Moreover, CRP is an independent risk factor for atherosclerosis [5].

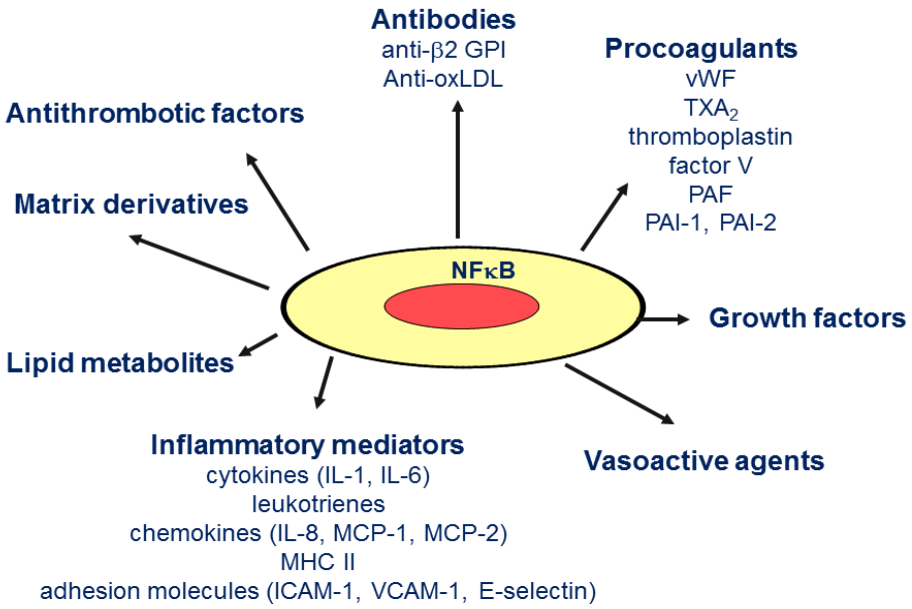
Similar immunological mechanisms have been recognized in the pathogenesis of both accelerated atherosclerosis and arthritis. These include endothelial activation (Figure 1), as well as interactions between immune cells and vascular components (Figure 2). During active arthritis, inflammatory cells, such as macrophages, T- and B-cells are activated. Soluble pro-inflammatory cytokines, chemokines are produced. These mediators are also involved in synovial and vascular inflammation (Table 1).

Tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) are the key cytokines in the pathogenesis of RA, as well as in vascular inflammation leading to atherosclerosis. Their serum concentrations correlated with carotid intima-media thickness (cIMT) and CV risk also in healthy individuals [6]. Rheumatoid factor (RF) and anti-citrullinated peptide/protein antibody (ACPA) positivity in RA indicate more progressive disease course. The levels of these antibodies have also been associated with endothelial dysfunction and atherosclerosis [7].

Fibrinogen in the vessel wall can be also citrullinated [8]. The release of collagen degrading enzymes, such as matrix metalloproteinases (MMP) also play a role in damaging the cartilage and bone in RA and in destabilization of atherosclerotic plaques.

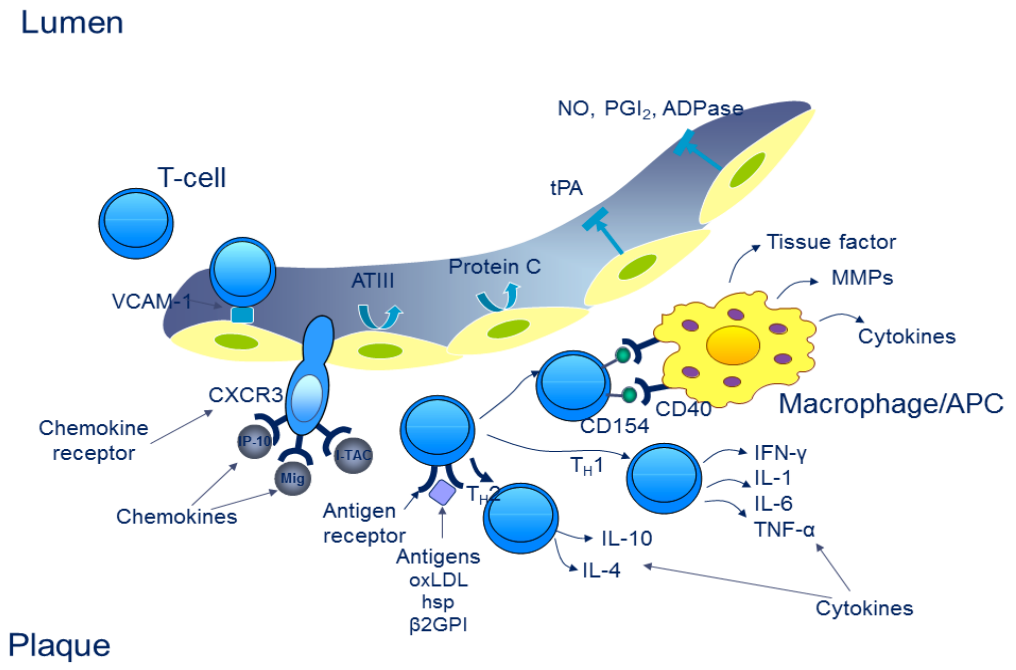
The RANK/RANKL/OPG system is also involved in both diseases. OPG is a decoy receptor, which play a crucial role in bone erosion, plaque calcification and rupture [9].

Figure 1 Endothelial activation during atherosclerosis. Upon activation, endothelial cells produce numerous mediators [10].



Abbreviations:β2GPI: β2 glycoprotein I; ICAM: intercellular adhesion molecule; IL: interleukin; MCP: monocyte chemoattractant protein; MHC: major histocompatibility complex; oxLDL: oxidized LDL; PAF: platelet-activating factor; PAI: plasminogen activator inhibitor; TXA2: thromboxane A2; VCAM: vascular cell adhesion molecule; vWF: von Willebrand factor.

Figure 2 Interactions between vascular components, inflammatory cells and mediators during the process of accelerated atherosclerosis [10]



Abbreviations: APC: antigen presenting cell; ATIII: antithrombin III; CXCR: CXC chemokine receptor; IFN: interferon; IL: interleukin; IP-10: interferon-inducible protein chemokine; MMP: matrix metalloproteinase; NO: nitric oxide; PGI2: prostacyclin; TNF: tumor necrosis factor; tPA: tissue plasminogen activator; VCAM: vascular cell adhesion molecule.

Table 1 Common immune-inflammatory mechanisms in RA and atherosclerosis.

Factor/Mediator	RA	Atherosclerosis
CRP	↑	↑
Pro-inflammatory cytokines (TNF- α, IL-6, IL-18, IL-20)	↑	↑
Chemokines (CCL2/MCP-1, fractalkine)	↑	↑
Autoantibodies (ACPA, anti-oxLDL, APA)	↑	↑
Proteases	↑	↑
RANKL	↑	↑
Adhesion molecules	↑	↑
CD40-CD40L	↑	↑
Adipokines: resistin, leptin	↑	↑
Adipokines: adiponectin	↓	↓
Insulin	↑	↑
Sex hormones	↑	↓
Microparticles	↑	↑

See the list of abbreviations.

There are also some genetic factors associated with both RA and atherosclerosis. RA-related HLA-DRB1*04 shared epitope alleles have also been implicated in endothelial dysfunction and atherosclerosis. Several other non-HLA polymorphisms were reported, which increase CV risk in patients with RA independently from traditional risk factors [11].

Carotid ultrasonography and intima-media thickness (cIMT) measurement -with or without plaque detection- are standard methods for detecting atherosclerosis. This technique has also been included in the recent European League Against Rheumatism (EULAR) recommendations for clinical practice [12]. This is based on the evidence that in RA, cIMT is increased and has been correlated with inflammatory activity and disease duration, again, independently from traditional risk factors [13-14].

RA may be a part of the metabolic syndrome (MS). The pro-atherogenic changes in lipid homeostasis are also quantitative and qualitative. RA has been associated with decreased high density lipoprotein cholesterol (HDL-C) levels and the presence of small dense low density lipoprotein (sdLDL) characterized by easy accumulation in atherogenic plaques, as well as increased LDL oxidation (oxLDL). Macrophages more readily ingest oxLDL and then they turn into foam cells. These cells are characteristic cell types of the atherosclerotic lesions. HDL is also a part of the innate immune system with its antioxidant and anti-inflammatory capacity. During permanent low-grade inflammation, HDL becomes dysfunctional [15].

Well-controlled disease activity by using conventional synthetic (csDMARD) or biologic disease-modifying antirheumatic drugs (bDMARD) can beneficially influence the vascular states of these patients with accelerated atherosclerosis [12, 16]. Corticosteroids (CS) are effective anti-inflammatory drugs, however, their atherogenic effect due to metabolic changes are more pronounced [17]. Methotrexate (MTX), the first-line treatment strategy in RA with robust anti-inflammatory effects, significantly reduces CV risk in RA patients [18]. MTX has beneficial effect on atherosclerosis even if it otherwise elevates the levels of homocysteine, a well-known pro-atherogenic factor. Supplementation with folic acid can reduce this harmful effect of the drug [19].

Systemic inflammation in RA leads to rheumatoid cachexia, dyslipidemia, a pro-inflammatory adipokine profile and insulin resistance. During TNF-α inhibitor treatment these metabolic changes can improve: HDL-C increase, insulin sensitivity improves and CRP concentrations decrease in RA patients [20]. Moreover, anti-TNF-α agents may ultimately reduce the rate of first CV events according to a longitudinal observational study and others [21-23].

However, biologics may increase lipid levels because of the „lipid paradox" associated with RA. There may be an inverse relationship between CRP and lipids. RA patients with high disease activity and elevated CRP levels are in a catabolic state and may have normal or lower total cholesterol (TC) and LDL-C levels. Upon treatment with biologics, in parallel with the normalization of CRP, lipid levels increase indicating the more potent anti-inflammatory effect of the treatment [24]. While TC, HDL-C and LDL-C levels usually increase during bDMARD treatment, TC/HDL-C or LDL-C/HDL ("Atherogenic Index"), as well as apolipoprotein-B/A1 (ApoB/ApoA1) ratios do not change significantly [25]. Tocilizumab (TCZ), an anti-IL-6 receptor antibody, has a more potent lipid increasing effect, however, it decreases the level of lipoprotein(a), another independent risk factor for atherosclerosis [26].

The risk management of atherosclerosis in RA is obviously complex. Traditional risk factors are also presented in RA patients, however, systemic inflammation may play a pivotal role in development of accelerated atherosclerosis [27].

2.2 Cerebrovascular diseases in rheumatoid arthritis

Accelerated atherosclerosis and increased CV comorbidity have been well-studied among RA patients. Over the past decade, several studies and meta-analyses evidenced a 50–60% increase in CV mortality in RA patients [28-29]. RA is an independent CV risk factor according to the European Society of Cardiology (ESC) guidelines [30].

An increased risk of stroke morbidity and mortality has been also associated with RA [31-33]. Cerebral ischemic diseases are the second most common vascular consequences in RA. Nevertheless, there are fewer data on stroke in comparison to CV diseases. Possibly, because of occurrence of cerebrovascular events is longer and often does not cause definite symptoms [20]. Yet, cerebrovascular mortality in RA cannot be dismissed. Meune et al. [34] reported a systematic review and meta-analysis on fatal and non-fatal stroke in patients with RA. The calculated pooled SMR for stroke was 1.46 (95% CI 1.31–1.63). In general, the risk of stroke is within the range of 1.51 and 2.13 in RA [33].

The two typical forms of stroke are ischemic and haemorrhagic. Ischemic stroke is the more common. It is due to embolism or caused by a blocked artery in the brain. Behind this phenomenon, there is usually cerebral thrombosis due to plaque formation in the arterial wall similar to heart attack. However, cerebral vessels are smaller than coronaries and the collateral circulation is advanced. An ischemic stroke can be silent when it impacts smaller vessels or less-functional areas of the brain.

These silent focal vascular lesions are actually far more common than strokes with symptoms [35, 36].

Atherosclerosis may affect the large vessels leads to ischemic strokes. These are more commonly in the middle cerebral artery territory. Occlusion of small intracranial vessels leads to lacunar infarction or smaller focal vascular lesions. Focal vascular lesions are often asymptomatic but indicate a high risk for ischemic stroke.

In this regard to examination of the intracranial vessels, even in asymptomatic patients help us to evaluate their cerebrovascular risk. To date no systematic evaluation of intracranial circulation has yet been performed in RA [37].

2.3 Transcranial Doppler ultrasonography

Diagnostic medical ultrasound (US) systems are based on the pulse-echo technique. The Doppler-effect was named after the physicist Christian Doppler, who described that the frequency or the wavelengths of a wave is changing for an observer who is moving relative to the wave source. In medical US, the difference between emitted and reflected sound frequencies is called Doppler-shift which is used to measure the velocity of organs or fluids. For example, using red blood cells as moving objects, the observer can measure the speed of blood in arteries by a method called Fast Fourier Transform (FFT) [38-39].

Rune Aaslid described first the transcranial Doppler (TCD) technique in 1982 [40]. TCD is also called as the doctor's stethoscope of the brain [41, 42]. It is a non-invasive, repeatable, portable, not too expensive examination, which allows dynamic monitoring of the cerebral vessels in real-time, adding not only anatomical but also (patho)physiological information like no other neuroimaging modalities [42-48].

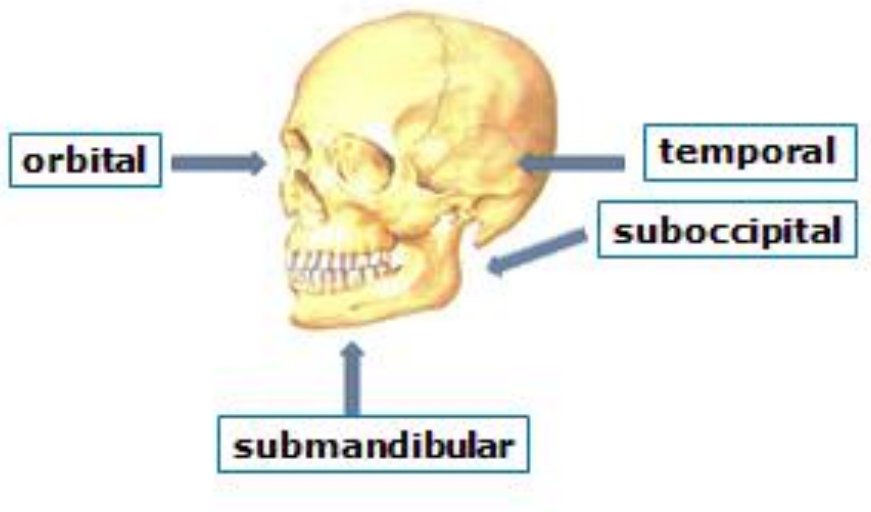
TCD is a high sensitivity measurement of intracranial vessels. This technique is able to determine the impaired function of cerebral arteries even in asymptomatic, healthy individuals with only risk for atherosclerosis, such as smoking or essential hypertension [49-53], but interestingly not in patients with hyperlipidemia [54].

TCD has a wide range of indications. It can be used in emergency cases, such as acute stroke, cerebral microembolism, right-left shunt detection, and subarachnoid hemorrhage but also in sickle cell disease, cerebral ischemia, brain stem death, head injury, raised intracranial pressure (ICP) or intraoperative monitoring [46-47]. In patients with cerebrovascular morbidity the most common indication for performing TCD is to diagnose the stenosis of intracranial vessels. TCD investigation results correlated with findings of invasive angiography in these patients [47, 55-56].

TCD is also ideal for cerebral autoregulatory testing, studying the pathophysiology of cerebrovascular ischemia or dynamic cerebrovascular responses, such as increased cerebrovascular resistance, vasospasm or hyperdynamic flow [43, 45, 47, 55]. Cerebral autoregulation means that the blood vessels of brain are able to maintain relatively constant cerebral blood flow (CBF) between mean arterial pressures of 50 to 170 mmHg. First, the dynamic autoregulation responds almost immediately, within seconds to sudden pressure changes. In addition, there is also a static autoregulation of response long-term fluctuation in cerebral perfusion pressure (CPP) [57]. TCD is able to identify patients with impaired autoregulatory function which poses high-risk for first-ever or recurrent stroke [47].

As attenuation is lower when using low frequency US, TCD uses ≤ 2 MHz US waves. Higher frequency probes are not able to adequately penetrate through the skull. As the bone attenuates about 90% of the US waves, TCD may only be performed at skull areas where the bone is thin enough or where there are preformed foramina in the bone. These regions are called acoustic windows. Different cerebral arteries can be examined through these windows (Figure 3).

Figure 3 Acoustic windows of the skull

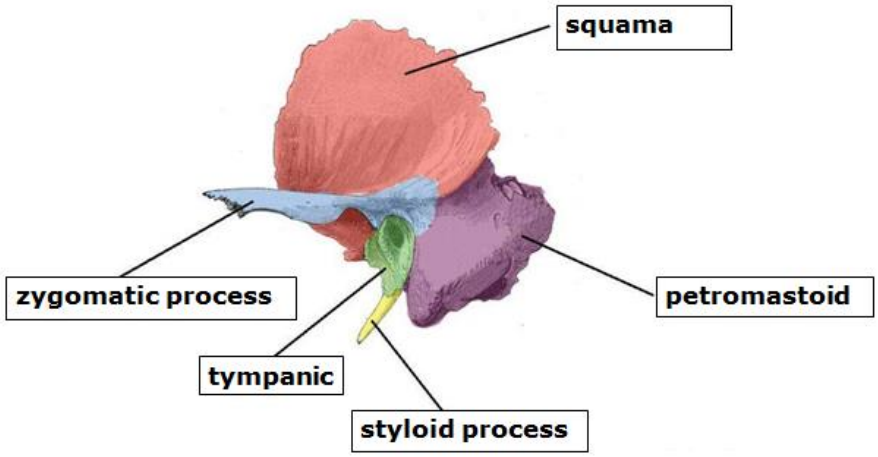


The blood supply of the brain is originating from the right and left internal carotid arteries (ICA) and from the vertebral arteries which vessels then become basilar artery (BA). The circle of Willis provides an important communication between them. There are three exiting arteries namely the anterior (ACA), middle (MCA) and posterior cerebral arteries (PCA), which are responsible for cerebral blood supply [37].

TCD assessment can also detect the collateral function of the anterior and posterior communicating arteries of the circle of Willis [36].

In adults, the orbital, temporal (TAW), suboccipital and submandibular foramina may be most commonly accessible for US investigations (Figure 3). The ophthalmic artery and the ICA siphons can be accessed through the transorbital window. TAW is the thinnest area of the lateral skull, located above the zygomatic arch and anterior to the earlobe (Figure 4). The squamous temporal bone is the thinnest here [58]. ACA, MCA, PCA, BA and the ICA bifurcation can be detected through the TAW. The suboccipital foramen is between the occipital bone and the atlas vertebrae. Through this window, the vertebral and basilar arteries can be detected. ICA can also be observed through the submandibular foramen (Figure 3) [41].

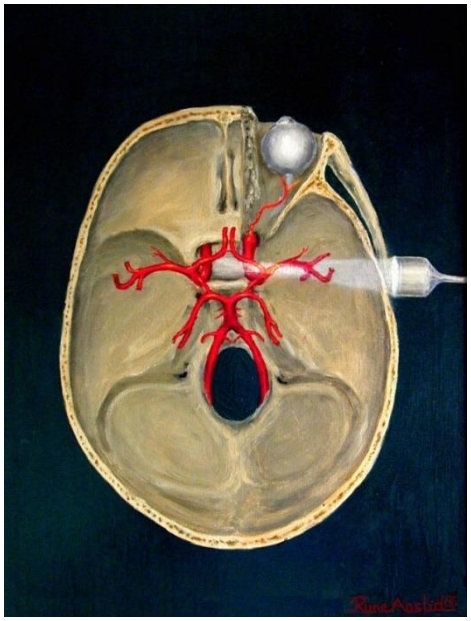
Figure 4 Anatomy of the temporal bone [59]



MCA has the largest blood supply territory. This artery is the most frequently affected by cerebral infarction because of the direct flow from ICA into the MCA and the size of the supplied territory. Therefore MCA is the most important intracranial vessel to be assessed by TCD and TAW is the most relevant acoustic window for this purpose [41] (Figure 5).

TCD parameters described later, such as MFV, PI and PI/MFV ratio of MCA are prognostic factors for recurrent cerebrovascular ischemia [36, 60]. TCD examination can evince even an asymptomatic stenosis of MCA, an acknowledged stroke risk factor [56, 61].

Figure 5 Transtemporal method of TCD (The original picture was painted by Rune Aaslid)



There are also some limitations of this technique. In healthy individuals, the temporal bone around the TAW is usually a thin cortical without trabecular structure [58, 62]. In approximately 10-20% of individuals, even in the general population, TAW may be inadequate [63-65]. TAW failure (TAWF) is more frequent in non-Caucasian, elderly women [63, 66-67]. In addition, when assessing TCD in patients with stroke, Deal et al. [68] found TAWF in 34% of patients.

Thickness of the temporal bone and the inhomogeneous texture of the bone have been correlated with TAWF [58, 68]. In a very elegant study Kollár et al. [58] examined moribund neurological patients with TCD. During autopsy, a sample from the area of TAW of the temporal bone was removed and determined thickness and texture by CT. They found significant correlation between TAWF and bone thickness and texture around TAW. TAWF, bone thickness and inhomogeneous temporal bone texture could be correlated with age, as well with each other.

Interestingly, also in stroke patients, there were no correlation between TAWF and vertebral, femoral or total bone mineral density (BMD). Only heterogeneous temporal bone texture was associated with lower BMD [68]. Presumably there are some correlation between TAWF, temporal bone texture and thickness and various bone markers, to data there is not any reports on this context.

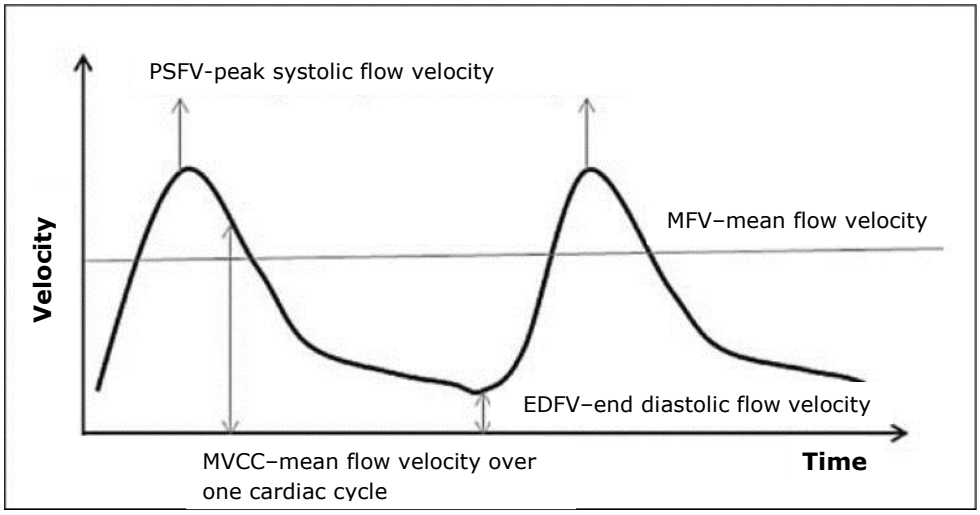
Another major limitation is that the TCD investigation is highly operator-dependent and requires a long learning period and practice [63].

2.4 TCD indices

TCD investigation provides many important indices that reflect vascular pathophysiology. Among TCD parameters, mean blood flow velocity (MFV, cm/sec), peak systolic flow velocity (PSFV, cm/sec) and end diastolic flow velocity (EDFV, cm/sec) are the most valuable indices [47] (Figure 6).

PSFV is the peak of the waveform during a cardiac cycle. Elevated PSFV indicates stenosis in the upstream arterial vessels. EDFV is normally 20-50% of PSFV value. MFV is the mean velocity within a cardiac cycle. It is calculated as (PSFV+[EDFVx2])/3 [69]. Obviously, MCA has the highest MFV among intracranial vessels. Increased value of MFV may indicate vasospasm, stenosis or hyperdynamic velocity flow. Its decrease may indicate decreased cerebral blood flow through hypotension, increased intracranial pressure or brain stem death [55, 70].

Figure 6 TCD indices



Pulsatility index (PI)	**PI = (PSFV-EDFV)/MFV**
Resistance index (RI)	**RI = (PSFV-EDFV)/PSFV**
Cerebrovascular reserv capacity (CRC)	**CRC = $(v_{max}.-v_0)/v_0$ x100**

Pulsatility index (PI) is a measure of the variability of blood velocity in the vessel, calculated as (PSFV-EDFV)/MFV. Its normal range is between 0.6 and 1.2. PI gives us information about the downstream cerebral vascular resistance. When PI is below 0.5, it indicates proximal stenosis or occlusion, while its value above 1.2 indicates distal occlusion or constriction [55, 70].

Resistance index (RI) represents flow resistance distal to the site of insonation. The normal range is between 0.4 and 0.7. Values >0.8 indicate downstream resistance. RI is calculated as (PSFV-EDFV)/PSFV [55, 70].

TCD is also capable to determine cerebrovascular reserve capacity (CRC), an indicator of vascular autoregulatory mechanisms and the function of precapillary (resistance) arteries. These arteries are responsible for the physiological protective mechanisms. They are able to contract and dilate in order to maintain the optimal cerebral blood flow even under changing cerebral pressure conditions. CRC is calculated as $(v_{max.}-v_0)/v_0 x100$. The result is expressed as percentage. After the administration of acetazolamide, the normal response is a 49.3% increase in MFV in women and a 38.9% increase in MFV in men [51, 55, 67, 70].

3 AIMS

Accelerated atherosclerosis associated with RA also involves the intracranial vessels which lead to ischemic cerebrovascular diseases. The assessment of these arteries by TCD is important to determine preclinical pathophysiological changes. Despite its importance there have been only very few publications on intracranial circulation in RA. The presence of adequate TAWs on both sides is essential for TCD investigation.

Our aims were:

3.1 Study 1

1. to assess the availability of TAWs in patients with RA undergoing various treatments in comparison to a control group representative for the general population,
2. to determine TAWF, temporal bone thickness and texture in RA,
3. to study the features of TAW in relation to BMD and bone biomarkers.

3.2 Study 2

1. to examine MCA and BA by TCD in order to determine the blood flow conditions in RA compared to healthy individuals,
2. to determine the abnormalities of circulation in intracranial vessels in RA compared to healthy controls,
3. to assess the effect of biologic therapy vs. MTX on TCD parameters,
4. to correlate TCD values with carotid US parameters (cIMT) and brain magnetic resonance imaging (MRI) scans.

To our knowledge Study 1 is the first one to assess TAWs, TAWFs and characteristics of temporal bone in RA. Study 2 is also the very first study to assess the intracranial vessels of RA patients by TCD. Moreover TCD was combined with traditional carotid ultrasonography and brain MRI investigation in order to explore the true cerebrovascular risk of these patients.

4 METHODS

4.1 Study 1

4.1.1 Patients and controls

Altogether 62 RA patients undergoing regular follow-ups at the Szent Ferenc Hospital (part of the Borsod County Central Teaching Hospital), Miskolc were recruited for the study.

RA patients were all females with a mean age of 60.7±9.5 (range: 27-78) years and with 11.5±7.6 (range: 1-36) years mean disease duration. Altogether 75% of these patients were immunoglobulin M (IgM) type RF positive, and 75% were ACPA positive. Forty-two patients had been receiving biologics (20 infliximab [IFX] and 22 tocilizumab [TCZ]) for a mean duration of 5.2±1.9 (range: 1-8) years. The other 20 patients were biologic-free. They only received methotrexate (MTX) for disease modification for a mean 6.8±4.7 (range: 1-15) years in an average dose of 14.1±4.5 (range: 5-20) mg per week. In this population, 32 patients had been receiving stable low-dose corticosteroids for at least 6 months. The characteristics of the MTX-, biologic-, IFX- and TCZ-treated RA subsets are also shown in Table 2.

For this study, a cohort of 60 non-RA women later undergoing detailed TCD investigations were chosen as control group with the mean age 60.43±9.48 (range: 31-90) years ($p=0.88$). Individuals in the control group did not have any known CV, cerebrovascular disease or diabetes.

Before the TCD assessment was carried out by the ultrasonographer (Sepsi M), the presence or failure of TAW had been determined by her. Bone structure and thickness of the temporal bone were assessed by computed tomography (CT) imaging in 43 RA patients and in 35 control subjects. Their mean age did not differ significantly (54.6±3.8 years (range: 43-67) in the RA subset and 53.7±4.0 years (range: 48-60) in the control group).

The Borsod County Central Teaching Hospital IRB approved this study (No. 13/2016). All patients gave informed consent to the study and all of them gave consent to publish.

Table 2 General characteristics and laboratory markers of RA patients in Study 1

	RA total	RA MTX	RA biol	RA IFX	RA TCZ
n	62	20	42	20	22
age (years)	60.7±9.5	61.2±9.1	60.4±9.8	61.8±8.2	59.1±11.0
disease duration (years)	11.5±7.6	11.4±8.6	11.5±7.1	11.5±7.5	11.6±6.9
RF positivity (%)	74	79	71	65	81
anti-CCP positivity (%)	75	86	73	67	76
MTX duration (years)	6.5±3.9	6.8±4.7	6.4±3.6	6.5±2.5	6.3±4.4
MTX dose (mg/week)	14.9±5.5	14.1±4.5	15.3±5.9	13.6±5.1	16.8±6.3
biologic duration (years)	-	-	5.2±1.9	5.2±1.8	3.0±1.6
DAS28	2.46±0.82	2.84±0.77	2.28±0.79	2.68±0.69	1.96±0.71
ESR (mm/h)	17.42±14.50	18.00±11.99	17.14±15.68	25.8±17.2	7.9±7.3
hsCRP (mg/l)	5.35±7.83	8.18±11.08	4.01±5.35	4.8±4.9	2.4±4.4
L1 BMD (g/cm²)	0.872±0.13	0.866±0.17	0.874±0.11	0.859±0.13	0.876±0.01
L1 T-score	-1.25±1.18	-1.52±1.49	-1.12±1.03	-1.20±1.16	-1.05±0.91
L2-4 BMD (g/cm²)	0.922±0.13	0.869±0.13	0.945±0.13	0.930±0.11	0.961±0.14
L2-4 T-score	-1.13±1.24	-1.63±1.32	-0.92±1.15	-1.06±1.04	-0.78±1.27
Femoral neck BMD (g/cm²)	0.698±0.12	0.703±0.17	0.696±0.09	0.684±0.09	0.709±0.09
Femoral neck T-score	-1.41±0.94	-1.51±1.23	-1.37±0.79	-1.48±0.74	-1.26±0.84
Total hip BMD (g/cm²)	0.825±0.14	0.782±0.15	0.843±0.13	0.838±0.13	0.849±0.14
Total hip T-score	-0.92±1.22	-1.16±1.52	-0.81±1.07	-0.86±1.03	-0.77±1.14
OC (µg/l)	18.53±6.57	17.79±8.26	18.79±5.96	18.5±6.6	19.1±5.5
β-CTX (µg/l)	0.302±0.140	0.303±0.145	0.302±0.140	0.32±0.12	0.28±0.16
P1NP (µg/l)	50.74±18.70	49.72±22.08	51.11±17.66	50.3±16.5	52.0±19.2
25-OH-vitD_3 (nmol/l)	58.20±28.16	56.19±27.66	58.92±28.69	65.1±31.9	52.8±24.4
PTH-I (pmol/l)	4.14±1.58	4.26±1.49	4.09±1.64	3.93±1.07	4.25±2.08
SOST (pmol/l)	31.99±10.52	30.08±13.64	32.65±9.39	32.9±6.7	32.4±11.7
OPG (pmol/l)	7.49±3.35	7.89±3.56	7.35±3.32	7.18±3.33	7.51±3.41
sRANKL (pmol/l)	0.127±0.10	0.147±0.15	0.120±0.08	0.13±0.08	0.11±0.09
OPG/RANKL ratio	214.16±620.8	150.69±149.8	235.97±71.6	115.7±201.5	109.3±119.6

See the list of abbreviations.

4.1.2 Determination of temporal windows

Before the TCD assessment, always the same ultrasonographer (Sepsi M) determined whether the right and left TAW were detectable and available for TCD measurements or not. The existence of TAW was identified in 60 control individuals and in 62 RA patients. The TAW score shows that the examined person has TAWF:

- 0 – on both sides,
- 1 – only on either side (left or right),
- 2 – on neither side.

In order to better understand the structural and pathophysiological basis of TAWF, we assessed the temporal bone by CT imaging (Somatom Definition AS, 64-slice, Siemens, Germany) in a subset of RA patients (n=43) and control subjects (n=35). Among the 43 patients, 11 received MTX only, while 32 were treated by biologics. For an enhanced view of the temporal bone around TAW, we examined the subjects using the "bone window" CT setting without the administration of contrast material. Thus, native images using 2 mm thick slices were captured.

Always the same radiologist (Kostyal L) and then a neuroradiologist (Olah C) also measured the thickness of the temporal bone in mm at a point 3 cm anterior from the earlobe and determined the structure of TAW. None of them knew whether an RA or a control subject's image was analyzed.

Either a thin, homogeneous, compact, "condense" cortical bone or a thicker, heterogeneous, "sandwich"-like trilayer (cortical-diploe-cortical) structure were detected around the TAW [58, 68]. In this region we could not measure local bone density, however, it is always lower in the diploe and higher in the compact cortical bone [71].

4.1.3 Bone densitometry and bone markers

BMD, T- and Z-scores of the lumbar L1 and L2 vertebrae, total L2-4 vertebrae, femoral neck and total hip were assessed by dual emission x-ray absorptiometry (DEXA) (Hologic 010-0575, Bedford, MA, USA).

Among bone biomarkers, serum calcium and phosphate were determined by routine laboratory methods. Serum osteocalcin (OC; normal: <41 μg/l), β-CrossLaps (CTX; normal: <0.57 μg/l), procollagen type 1 amino-terminal propeptide (P1NP; normal: <75 μg/l), 25-hydroxy-vitamin D_3 (25-OH-vitD_3; normal: >75 nmol/l),

parathormone (PTH; normal: 1.6-6.9 pmol/l), osteoprotegerin (OPG; median: 2.7 pmol/l, normal: to be determined [TBD] locally in the lab), sclerostin (SOST; median: 24.1 pmol/l, normal: TBD), free soluble receptor activator of nuclear factor kappa-B ligand (sRANKL; median: 0.37 pmol/l in females, normal: TBD) were determined by enzyme-linked immunosorbent assay (ELISA) (Biomedica, Vienna, Austria). OPG/sRANKL ratios were also calculated. The laboratory examinations were performed under Batthoa HP's supervision.

4.1.4 Statistical analysis

The SPSS 22.0 software (IBM, Atmonk, NY, USA) was used for statistical analysis. Data are expressed as the mean ± SD and frequencies and percentages.

The statistical analysis was made in cooperation Hodosi K and Szekanecz Z.

Continuous variables were evaluated by independent samples t-test and Mann-Whitney test. Nominal variables were compared between groups using the chi-squared or Fisher's exact test, as appropriate. Simple correlations were determined by Spearman's analysis. To compare the right and left values of the same population were used the paired two-tailed t-test and the Wilcoxon test.

Multiple linear regression using the stepwise method was used to determine correlations and independent associations between parameters. TAW parameters were the dependent variables and several other clinical and laboratory parameters were independent variables. The B (+95% CI) regression coefficient indicated independent association between the dependent and independent variable during changes. P values < 0.05 were considered significant.

4.2 Study 2

4.2.1 Patients and controls

As described above, the prerequisite for successful TCD assessment is adequate TAW. Forty-one out of 62 RA patients had accessible TAW, while 21 subjects had TAWF. Only the 41 patients with adequate TAW were carried forward to Study 2.

From the 60 control subjects of Study 1 fifty-two persons had intact TAW at least on one side. They were all included to Study 2 and 8 another healthy persons were added to the control group.

The main characteristics of these 41 RA patients and the 60 control subjects did not differ significantly (Table 3). All participants were females, the mean age was 58.2±9.7 (range: 27-78) years in the RA group and 58.4±4.4 (range: 46-79) years in the control group. The mean disease duration of RA patients was 12.2±8.0 (range: 2-36) years. Altogether 70% of RA individuals were IgM type RF positive, and 65% were ACPA positive. Twelve RA patients received MTX monotherapy. The mean MTX treatment duration was 6.1±3.8 (range: 2-15) years and the mean dose was 14.6±6.1 (range: 5-25) mg per week. Among the other 29 patients, 15 patients had been receiving IFX and the other 14 TCZ as biologic treatment with a mean duration of 5.3±2.0 (range: 1-10) years. Biologic-treated RA patients also received MTX for a mean duration of 6.3±3.5 (range: 1-15) years.

IRB approval and consent issues are described above in Study 1.

Table 3 General characteristics and laboratory markers of assessed RA patients and control subjects in Study 2

	RA total	RA MTX	RA biol	Controls
n	41	12	29	60
age (years)	58.24±9.72	58.67±9.88	58.07±9.88	58.42±4.41
disease duration (years)	12.15±8.03	11.00±8.63	12.62±7.87	-
RF positivity (%)	70%	82%	66%	-
anti-CCP positivity (%)	65%	75%	64%	-
MTX duration (years)	6.15±5.82	5.83±4.57	6.28±3.54	-
MTX dose (mg/week)	14.63±6.11	14.17±5.15	14.83±6.54	-
DAS28	2.44±0.87	2.88±0.75	2.26±0.86	-
ESR (mm/h)	17.07±15.09	17.00±9.16	17.10±17.10	5.30±2.40
hsCRP (mg/l)	3.93±4.53	5.31±3.90	3.36±4.70	0.30±0.30
BMI	28.38±5.51	28.08±6.77	28.51±5.02	30.20±8.70
TC (mmol/l)	5.34±1.08	5.63±1.00	5.23±1.10	5.27±1.15
HDL-C (mmol/l)	1.47±0.43	1.38±0.53	1.49±0.40	1.70±0.42
TC/HDL-C ratio	3.94±1.36	4.70±3.73	3.73±1.20	3.62±0.95
LDL-C (mmol/l)	3.17±0.82	3.36±0.90	3.12±0.81	3.40±0.94
TG (mmol/l)	1.46±0.68	1.53±0.76	1.43±0.66	1.49±0.61
Lp(a) (ng/l)	260.14±317.20	345.29±426.55	239.59±290.74	-
apoA/apoB ratio	1.81±0.51	1.71±0.49	1.83±0.53	-

See the list of abbreviations.

4.2.2 Transcranial Doppler assessment

TCD examinations were performed by using a Multi-Dop T Digital (DWL Compumedics GmbH, Singen, Germany) device. Among TCD results, MFV (cm/sec), PSFV and EDFV are the most valuable parameters. We determined MFV, PI and RI first at rest (r) [MFV(r), PI(r), RI(r)].

There are many mechanical, pharmacological, static or dynamic methods for studying cerebral autoregulation but there is no gold standard technique and there are no standardized values [47, 72]. Breath-holding and hyperventilation tests seem to be a practical alternative to acetazolamide and the CO2 inhalation method in the assessment of cerebral hemodynamics [73]. Therefore we (Kardos Z, Olah C) chosen noninvasive, nonpharmacological maneuvers, such as 30 seconds lasting hyperventilation [MFV(h), PI(h), RI(h)] and then, after normal breathing, 30 seconds lasting breath-holding [apnea (a)] [MFV(a), PI(a), RI(a)] [73, 74].

By applying these maneuvers, we could assess cerebral vasoreactivity, which refer to autoregulatory function of the intracranial vessels. The cerebrovascular system is highly sensitive for changes in arterial partial carbon dioxide pressure (PaCO2). Hyperventilation due to hypocapnia results in vasoconstriction of cerebral resistance arteries. Consequently, blood flow velocity (BFV) decreases in upstream vessels. In contrast, breath-holding leads to hypercapnia and vasodilation resulting in increased BFV in the upstream vessels [57, 73]. The normal response to these manoeuvers is and at least 15% change of flow velocity in MCA after hyperventilation and 30% after breath holding. Changes below these values suggest impaired CRC [73, 74].

The TCD technique is highly operator dependent. Therefore, in this study every measurement was performed by a single technician (Sepsi M) and data were validated by the same neuroradiologist (Olah C). We examined MCA and BA and determined all TCD parameters described above in RA patients and control subjects.

4.2.3 Carotid and vertebral artery ultrasound examination

US examination of carotid arteries is widely used for detecting atherosclerosis including cIMT and plaques. Sonographic cIMT measurement is a surrogate marker for detecting subclinical atherosclerosis and for CV disease risk assessment. It has been recommended by the American Heart Association (AHA), American Society of Echocardiography (ASE) and Society for Vascular Medicine (SVM) as a screening test

for heart disease in apparently healthy individuals [75]. Increasing body of evidence shows that cIMT measurement supplemented with plaque scan consistently improves the prediction CV disease [76]. This technique is also ideal for screening high CV or cerebrovascular risk RA patients in preclinical state [56, 77].

We assessed our RA patients (n=41) and controls (n=60) using a duplex US system (Vivid E, GE Healthcare, Wauwatosa, WI, USA) with a 8 MHz linear array transducer (GE Probe 8L-RS). However, there is no standardized cIMT assessment methodology we carried out five measurements and calculated an average value on both sides. The examinations were performed by the same observer (Sas A) for each participant.

Longitudinal high-resolution B-mode US scans were carried out on the posterior wall of the distal part of common carotid artery (CCA), proximal to the carotid bulbs and were R-synchronized and recorded. On US, cIMT was defined as the distance between two echogenic lines representing the lumen-intima interface and the media-adventitia interface of the carotid arterial wall. These data are expressed in mm. I have averaged five measurements on both sides.

Carotid plaque presence also improves prediction of stroke or transient ischemic attacks [76]. Quantification and qualification of plaques seems to be more important to predict CV events and to identify high risk patients.

In our study, all visible plaques were detected, measured and classified as fibrous, calcified or soft, whether cause stenosis or not. This last subgroup, the soft plaques represents the most dangerous, unstable form of plaques [7, 78].

I created a "plaque score" on a 0 to 5 semiquantitative scale:

0- no plaque,
1- fibrous,
2- non-stenotic calcified,
3- stenotic calcified,
4- soft,
5- stenotic soft.

Each patient received their score for both sides.

Vertebral arteries were also visualized and normal circulation versus no circulation due to thin arteries was noted.

4.2.4 Brain MRI investigations

Intracranial atherosclerosis can be responsible for ischemic stroke, focal vascular lesions, emollition and atrophy. MRI imaging can detect even those small ischemic lesions which cause not clinical symptoms. That is why a brain MRI investigation was performed for all 41 RA patients to explore these features.

A Siemens Magnetom Verio 3 Tesla MRI instrument (Siemens, Munich, Germany) was used for these examinations. All MRI scans were first performed by a single radiologist (Kostyal L) and also revised by a neuroradiologist (Olah C). The presence or absence of vascular lesions, emollition and/or atrophy was determined.

4.2.5 Laboratory assessments

Serum IgM RF and high sensitivity CRP (hsCRP) were assessed by quantitative nephelometry (COBAS MIRA Plus, Roche Diagnostics, Indianapolis, IN, USA), using RF and CRP reagents, respectively (both from DIALAB, Wiener Neudorf, Austria). RF levels >50 IU/ml indicated seropositivity and hsCRP levels >5 mg/l were considered elevated. Anti-CCP/ACPA autoantibodies were detected in serum samples using the second-generation Immunoscan-RA CCP2 enzyme-linked immunosorbent assay (Euro-Diagnostica, Arnhem, The Netherlands). The assay was performed according to the instructions of the manufacturer. A concentration >25 IU/ml indicated seropositivity.

After overnight fasting, blood samples were taken from the patients and control subjects for TC, LDL-C, HDL-C and triglyceride (TG). Lipids were determined using routine laboratory methods. Serum lipoprotein a [Lp(a)] was assessed by latex-sensitized immunoturbidimetry (Roche Diagnostics). Serum ApoA and ApoB levels were measured by immunoturbidimetry using Tina-Quant ApoA and ApoB reagents (Roche Diagnostics) and a Cobas Integra 700 analyzer (Roche Diagnostics). The laboratory examinations were performed under Batthoa HP's supervision. ApoB/ApoA ratio, a good marker for atherosclerotic risk [82, 84] was also calculated (Table 3).

4.2.6 Statistical analysis

In general, statistical analysis was performed as described in Study 1 by using the SPSS 22.0 software, in cooperation Hodosi K and Szekanecz Z.

In multiple regression analysis, TCD/carotid parameters were the dependent variables, and other clinical and laboratory parameters such as age, disease duration, RF and anti-CCP positivity, MTX dose and duration, biologic therapy duration, DAS 28, ESR, hsCRP, BMI, lipid levels were independent variables.

5 RESULTS

5.1 Study 1

5.1.1 Detectability of TAW in RA versus controls

As described above, the presence of TAW is essential for TCD assessments. In RA (n=62), the right and left TAW were inadequate in 35% and 53% of patients, respectively. In addition, there was TAWF on both sides in 34% of RA subjects. In 34 out of 62 patients (54.8%) TAW could not be identified at least one side. In contrast, TAWF was found on the right, left or both sides in 20%, 20% and 13% of control subjects (n=60) (p<0.001) (Table 4).

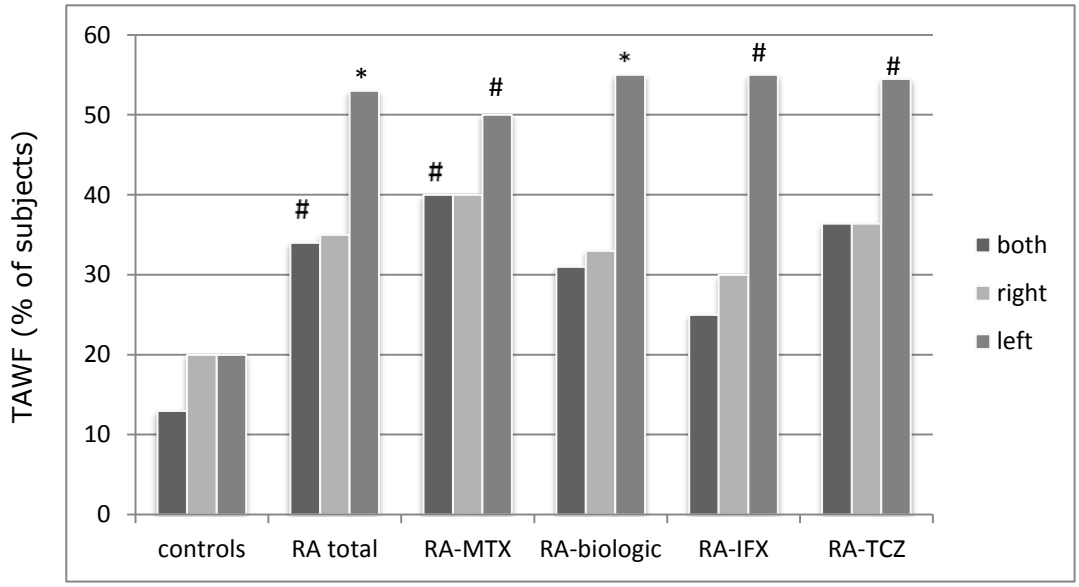
Table 4 TAW features in RA patients and controls

	Controls (n=60)	**RA total (n=62)**	**RA MTX (n=20)**	**RA biol (n=42)**	**p**		
					RA total vs. controls	**RA MTX vs. controls**	**RA biol vs. controls**
TAW failure							
right	12 (20%)	22 (35%)	8 (40%)	14 (33%)			
left	12 (20%)	33 (53%)	10 (50%)	23 (55%)	<0.001	0.021	<0.001
both	8 (13%)	21 (34%)	8 (40%)	13 (31%)	0.014	0.006	
TAW score	1.66±0.69	1.11±0.89	1.1±0.97	1.12±0.86	0.005	0.036	0.008
0	8 (13%)	21 (34%)	8 (40%)	13 (31%)			
1	8 (13%)	13 (21%)	2 (10%)	11 (26%)			
2	44 (74%)	28 (45%)	10 (50%)	18 (43%)			
Sandwich-like texture	(n=35)	(n=43)	(n=11)	(n=32)			
right	1	26 (60%)	6 (55%)	20 (62%)	<0.001	<0.001	<0.001
left	1	23 (53%)	4 (36%)	19 (59%)	<0.001	0.009	0.018
Bone thickness around TAW (mm)							
right	2.92±1.22	3.58±1.43	3.77±1.34	3.52±1.48		0.041	
left	2.90±1.16	1.56±1.16	4.62±1.42	4.00±1.59	0.001	0.001	0.007
p (right vs. left)		0.001	0.007	0.020			

See the list of abbreviations.

When the various RA subpopulations were analyzed, TAWF could be detected on the right, left or both sides in 40%, 50% and 40% of MTX-treated, 33%, 55% and 31% of any biologic-treated, 30%, 55% or 25% of IFX-treated and 36.4%, 54.5% and 36.4% of TCZ-treated RA patients (Figure 7).

Figure 7 TAWF in RA patients and controls



*p<0.001; #p<0.05 vs controls

Significant differences were found in the detectability of the left TAWs between the total RA cohort vs controls (p<0.001), as well as the biologic-treated (p<0.001), IFX-treated (p=0.015) and TCZ-treated RA patients vs controls (p=0.045).

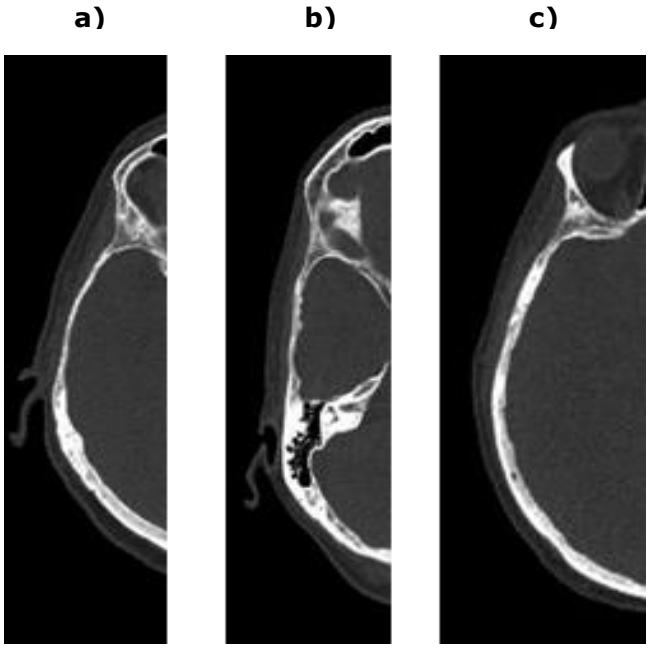
With respect to differences between left and right TAWs, TAWF was determined significantly more commonly on the left side compared to the right side in the full RA cohort (53% vs 35%; p=0.002), as well as in biologic-treated (55% vs 33%, p=0.005), IFX-treated (55.0% vs 30.0%, p=0.046) and TCZ-treated RA patients (54.5% vs 36.4%, p=0.042). In contrast, healthy controls exerted similar detectability (20% vs 20%, p=NS) (Figure 7).

The TAW detectability scores were calculated by assigning scores of 0, 1 or 2 to patients with TAWF on both sides, either or neither side. These scores were 1.11±0.89, 1.10±0.97, 1.12±0.86, 1.15±0.81, 1.09±0.92 and 1.66±0.69 in the total RA, MTX-, biologic-, IFX- and TCZ-treated RA patient subsets and control subjects, respectively. Significant differences were found in TAW scores between the total RA cohort (p=0.005), MTX-treated (p=0.036) and biologic-treated (p=0.008) vs controls (Table 4). No differences between MTX-treated RA patients vs biologic-treated patients were determined (data not shown).

5.1.2 Texture of TAW in RA and controls

As described above, when assessed by CT, the temporal bone around the TAW is normally composed of condense, homogeneous, monolayer cortical bone (condense structure). On the other hand, it may also show a cortical-diploe-cortical "sandwich"-like, heterogeneous trilayer pattern (Figure 8).

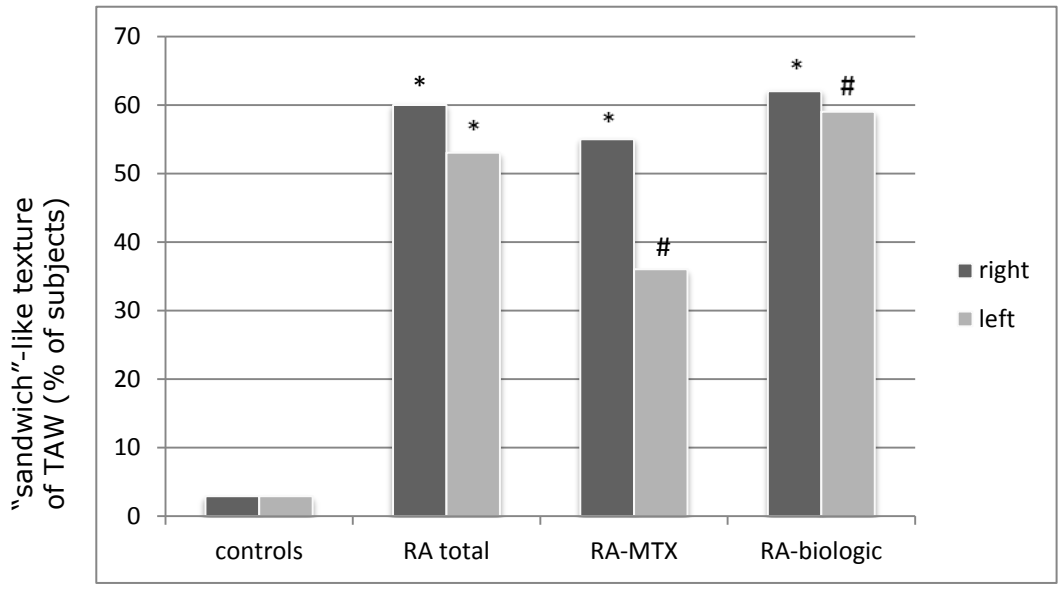
Figure 8 Thickness and structure of TAWs determined by CT: a) normal thin cortical; b) "condense" cortical bone; c) "sandwich"-like trilayer



We examined the bone structure around TAW in 43 RA patients and 35 control subjects. Only two out of 35 healthy control individuals had heterogeneous "sandwich"-like structure on one side (2.9%). In contrast, among RA patients, more than half (53%) had this cortical-diploe-cortical trilayer at least on one side ($p<0.001$) (Table 4).

The right TAWs exerted this trilayer texture in 60%, 55%, 62% of total RA, MTX-, biologics-treated RA patients, respectively. With respect to the left TAWs, these values were 53%, 36%, 59%. Significant differences were found in TAW texture (condense or sandwich) between the total RA cohort vs controls (right: $p<0.001$, left: $p<0.001$), MTX-treated patients vs controls (right: $p<0.001$, left: $p=0.009$) and biologic-treated RA patients vs controls (right: $p<0.001$, left: $p=0.018$) (Figure 9). No differences between MTX- vs biologic-treated RA patients, or IFX- vs TCZ-treated patients could be observed (data not shown).

Figure 9 Cortical-diploe-cortical, "sandwich"-like texture of TAW (% of subjects)



*p<0.001;#p<0.02 vs controls

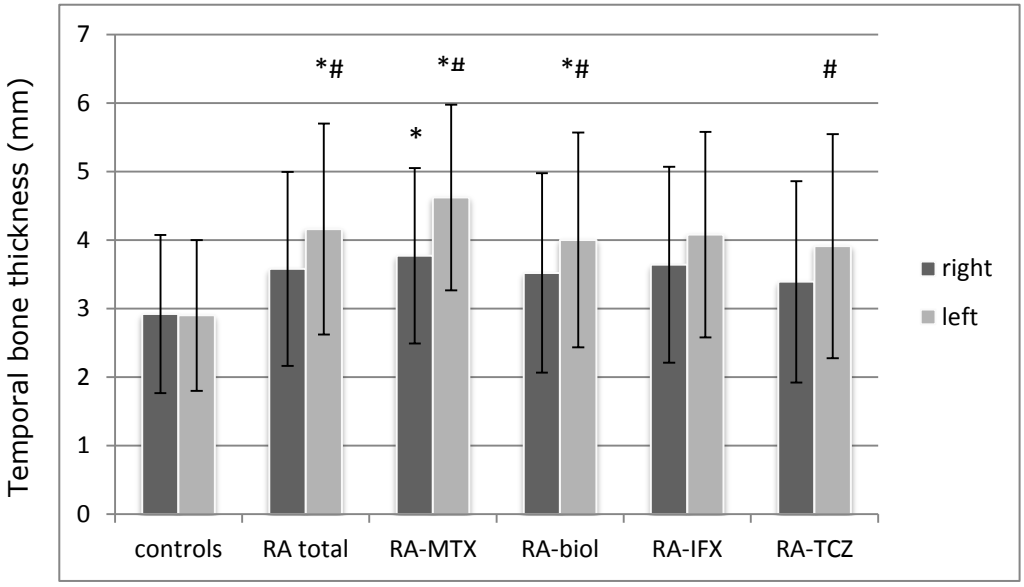
There were no differences between left and right TAW with respect to temporal bone texture in RA patients or in control subjects whatsoever (Figure 9).

5.1.3 Thickness of TAW in RA compared to controls

The thickness of temporal bone around TAW was determined by CT in 43 RA patients and 35 controls. These values were presented in mm. The mean temporal bone thickness values of the right TAWs were 3.58±1.43, 3.77±1.34, 3.52±1.48, 3.64±1.47, 3.39±1.52 and 2.92±1.22 mm in the total RA, MTX-, biologic-, IFX-, TCZ-treated RA populations and controls, respectively. With respect to the left side, these values were 4.16±1.56, 4.62±1.42, 4.00±1.59, 4.08±1.54, 3.91±1.69 and 2.90±1.16 mm (Figure 10).

Significant differences were found in left temporal bone thickness between total RA patients (p=0.047), MTX- (p=0.014) or biologic-treated RA patients (p=0.044) compared to controls and also in the right temporal bone thickness between MTX-treated RA patients vs controls (p=0.041). There was no difference in bone thickness on either side between IFX- or TCZ-treated RA patients vs controls, between total and biologic-treated patients in the thickness of right TAW bone whatsoever (Figure 10).

Figure 10 Bone thickness around TAW



*p<0.05 vs controls; #p<0.05 vs right side

The left temporal bone was significantly thicker than the right in the total RA population (p=0.001), as well as in the MTX-treated (p=0.006), biologic-treated (p=0.021), and TCZ-treated RA subset (p=0.039). In contrast, healthy controls exerted similar temporal bone thickness (p=NS) (Figure 10).

5.1.4 Correlations between TAW features and other disease markers in the RA population

We correlated the various TAW parameters with each other in the RA population (Table 5). There have been significant associations between TAW detectability, thickness and structure. In the total RA cohort (n=62), the absence of right TAW was significantly associated with increased thickness (R=0.811, p<0.001) and the sandwich-like texture of the bone (R=0.712, p<0.001). Similar observations were made with respect to the left TAWs (thickness: R=0.768, p<0.01, texture: R=0.791, p<0.01). The thickness of right and left TAWs also correlated with the heterogeneous texture on the corresponding sides (right: R=0.569, p<0.01, left: R=0.457, p=0.02) (Table 5).

Table 5 Correlations between various TAW features in RA patients (n=62)

Parameter 1	Parameter 2	R value	p value
Right TAW			
TAWF	thickness	0.811	<0.001
TAWF	heterogeneous texture	0.712	<0.001
thickness	heterogeneous texture	0.569	<0.001
Left TAW			
TAWF	thickness	0.768	<0.001
TAWF	heterogeneous texture	0.791	<0.001
thickness	heterogeneous texture	0.457	0.002
Right vs Left TAW			
TAWF (R)	TAWF (L)	0.634	<0.001
thickness (R)	thickness (L)	0.754	<0.001
texture (R)	texture (L)	0.721	<0.001

See the list of abbreviations.

BMD and T-score values of the L1 and L2-4 vertebrae, those of the femoral neck and the total hip were determined in all RA patients (Table 2). RA patients on biologics exerted higher L1, L2-4, femoral neck and total hip BMD values than MTX-treated patients, but the differences were not statistically significant. Bone biomarkers did not show any notable differences between the biologic- and MTX-treated RA subset (Table 2).

Binary logistic regression analysis was performed in order to determine correlations between TAW characteristics (dependent variables) and other clinical and laboratory parameters (independent variables) (Table 6 and 7). In the linear regression analysis, TAWF (lower TAW scores) on both sides (p=0,007) significantly correlated with age. Interestingly, left TAW was associated with OPG levels (p=0,012) (Table 6). In addition, this texture on right (p=0,045) or left (p=0,016) side exerted significant correlation with OPG. Right (p=0,025) or left (p=0,007) TAW bone thickness correlated with age. Finally, TAW thickness on the left side correlated with femoral neck and total hip bone loss (Table 6). However, neither TAWF, nor temporal bone texture, nor thickness showed any association with the duration of RA or disease activity (data not shown).

Table 6 Correlations between TAW features and other characteristics in RA patients as determined by linear binary logistic regression (n=62)

	Odds [Exp(B)] (CI 95%), p value		
side	**right**	**left**	**both**
TAW failure/score			
age (years)	1.1 (1.025-1.181), p=0.008	1.190 (1.079-1.311), p<0.001	1.106 (1.028-1.189) p=0.007
OPG (pM)		1.690 (1.123-2.542), p=0.012	
TAW texture (sandwich-like)			
age		0.081 (0.023-0.140), p=0.007	-
OPG (pM)	0.807 (0.647-1.005), p=0.045	0.653 (0.462-0.922), p=0.016	-
TAW thickness			
age	0.063 (0.008-0.118), p=0.025	0.081 (0.023-0.140), p=0.007	-
femoral neck BMD (g/cm²)		-5.708 (-9.995- -1.420), p=0.010	-
femoral neck T-score		-0.633 (-1.109- -0.157), p=0.010	-
total hip BMD (g/cm²)		-4.037 (-7.110- -0.963), p=0.011	-
total hip T-score		-0.493 (-0.871- -0.116), p=0.012	-

Table only shows statistically significant correlations. See the list of abbreviations.

Multiple regression analysis was performed to determine independent prognostic factors for TAWF in RA. Age (p=0,026) and serum OPG levels (p=0,012) were independent predictors of left TAWF. Age was also a predictor of sandwich-like texture of the left TAW (p=0,007) (Table 7). There were no correlations between other biomarkers including calcium, phosphate, osteocalcin, CTX, P1NP, PTH, 25-OH-D_3, SOST and RANKL (data not shown).

Table 7 Independent prognostic factors for TAW failure in RA as determined by multivariate binary logistic regression (n=62).

	Odds [Exp(B)] (CI 95%), p value		
side	**right**	**left**	**both**
TAW failure/score			
age (years)		1.531 (1.051-2.231), p=0.026	
OPG(pM)		1.154 (1.005-1.325), p=0.012	
TAW texture (sandwich-like)			
age		0.081 (0.023-0.140), p=0.007	-

Table only shows statistically significant correlations. See the list of abbreviations.

In control subjects, representing the general population, TAWF on the right ($R=0.393$, $p<0.001$) and left side ($R=0.407$, $p<0.001$) positively correlated with age. However in control subjects, the thickness and texture of TAW did not show any association with age.

5.2 Study 2

5.2.1 Comparative description of RA patient subsets

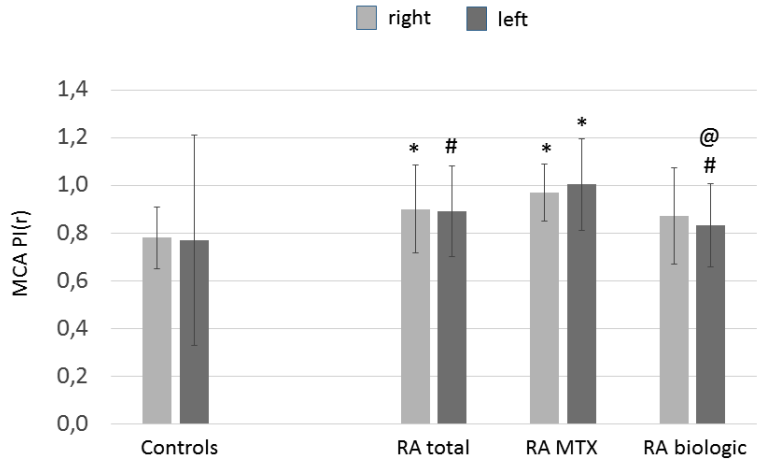
RA patients, the MTX-treated subgroup and the biologic-treated patients had corresponding characteristics in the main clinically and laboratory findings (Table 3). Except for that the MTX-treated patients had significantly higher CRP level (5.3±3.9 mg/l vs 3.4±4.7 mg/l; $p<0.05$) and mean DAS 28 scores (2.88±0.75 vs 2.26±0.86; $p=0.006$) than the biologic-treated subjects (Table 3). However all above described lipid parameters have been investigated we could not find any major differences between RA subsets. Results are seen in Table 3.

5.2.2 TCD assessments in RA patients, RA subsets and control subjects

Forty-one RA patients and 60 control subjects were investigated by TCD US technique. The main TCD parameters, PI, RI and MFV of MCA were determined at rest (r), after hyperventilation (h) and 30 seconds lasting breath holding [apnea (a)] on the left and the right sides. MCA CRC values were also assessed on both sides. Furthermore, BA PI (r) and MFV (r) were investigated in all individuals.

The MCA PI values are shown in Figure 10A. Both the total RA group (right: 0.90±0.18, $p<0.001$; left: 0.89±0.19, $p=0.003$) and the MTX-treated subgroup (right: 0.97±0.12, $p<0.001$; left: 1.00±0.19, $p<0.001$) had significantly higher PI at rest than controls (right: 0.78±0.13; left: 0.77±0.44) (Figure 11A). When biologic-treated RA cohort was compared to controls, significantly higher PI(r) was found on the left MCA (0.83±0.17 vs 0.77±0.44, $p=0.021$) but significantly lower MCA PI(r) was measured compared to MTX-treated subgroup (0.83±0.17 vs 1.00±0.19, $p=0.021$) (Figure 11A).

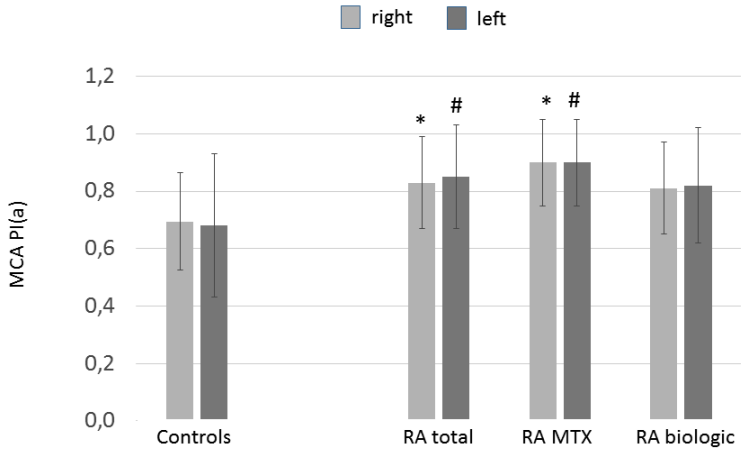
Figure 11A MCA PI values at rest in controls, in patients with RA, in MTX- and



*p<0.001 vs controls; #p<0.05 vs controls;
@p<0.05 vs MTX

After breath holding [apnea (a)], we measured significantly higher PI in the total RA group (right: 0.83±0.16, p<0.001; left: 0.85±0.03, p=0.002) and also in the MTX-treated subgroup (right: 0.90±0.15, p<0.001; left: 0.90±0.15, p=0.009) on both sides compared to the control population (right: 0.69±0.17; left: 0.68±0.25) (Figure 11B).

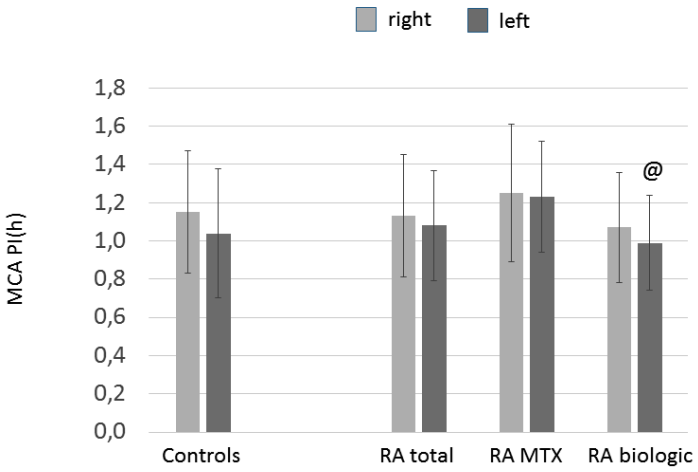
Figure 11B MCA PI values after breath holding in controls, in patients with RA, in MTX- and biologic-treated subgroups



*p<0.001 vs controls; #p<0.05 vs controls

After hyperventilation, the PI(h) values did not differ significantly in the RA population compared to controls. The only significant difference was found between the PI(h) value of the MTX- and biologic-treated subgroups on the left side. MTX-treated subjects had significantly higher MCA PI(h) than biologic-treated ones (1.23±0.29 vs. 0.99±0.25, p=0.029) (Figure 11C).

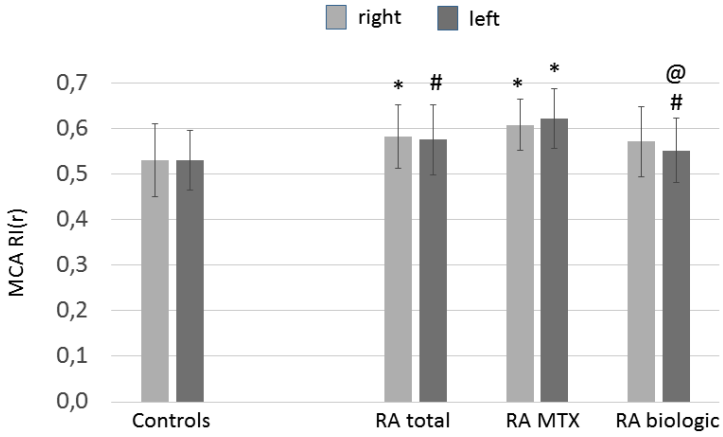
Figure 11C MCA PI values after hyperventilation in controls, in patients with RA, in MTX- and biologic-treated subgroups



@p<0.05 vs MTX

With regards to MCA RI values at rest the total (right: 0.58±0.07, p<0.001; left: 0.58±0.08, p=0.004) and MTX-treated RA patients (right: 0.61±0.06, p<0.001; left: 0.62±0.07, p<0.001) had significantly higher RI(r) values compared to controls (right: 0.53±0.08; left: 0.53±0.07) (Figure 12A). Interestingly, again, biologic-treated RA patients had significantly higher RI(r) on the left side compared to controls (0.55±0.07 vs 0.53±0.07, p=0.018) but significantly lower than the MTX-treated patients (0.55±0.07 vs 0.62±0.07, p=0.018) (Figure 12A).

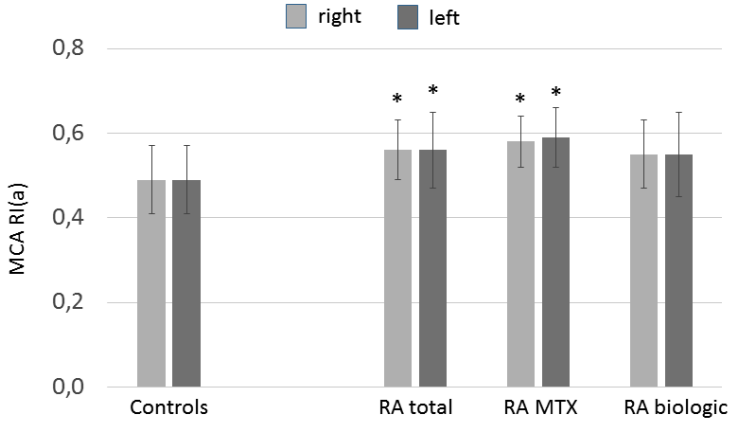
Figure 12A MCA RI values at rest in controls, in patients with RA, in MTX- and biologic-treated subgroups



*p<0.001 vs controls; #p<0.05 vs controls;
@p<0.05 vs MTX

After breath holding (apnea), the total RA cohort (right: 0.56±0.07, p<0.001; left: 0.56±0.02, p<0.001) and also the MTX-treated subgroup (right: 0.58±0.06, p<0.001; left: 0.59±0.07, p<0.001) had significantly higher RI(a) values compared to controls (right: 0.49±0.08; left: 0.49±0.07) on both sides, respectively (Figure 12B).

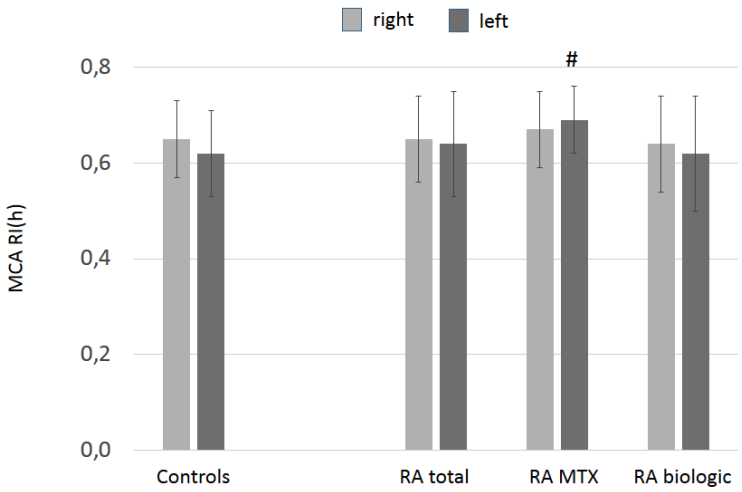
Figure 12B MCA RI values after breath holding in controls, in patients with RA, in MTX- and biologic-treated subgroups



*p<0.001 vs controls

After hyperventilation, RI(h) differences seemed to be eliminated between the RA and control population. The only significant difference was that the MTX-treated patients with RA had higher MCA RI(h) compared to controls (0.69±0.07 vs 0.62±0.29, p=0.09) on the left side (Figure 12C).

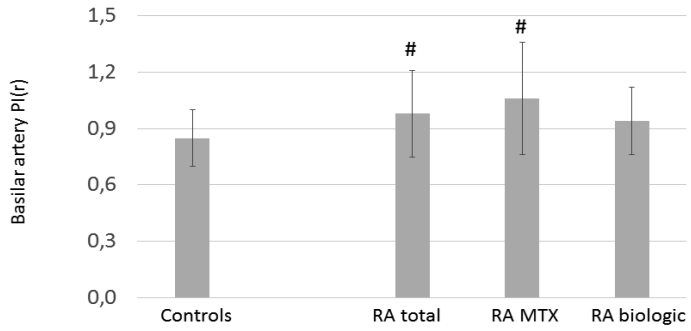
Figure 12C MCA RI values after hyperventilation in controls, in patients with RA, in MTX- and biologic-treated subgroups



#p<0.05 vs controls

With regards to the BA, the total RA population (0.98±0.26, p=0.005) and the MTX-treated subset (1.05±0.38, p=0.001) also had significantly higher PI(r) than controls (0.85±0.15) (Figure 13).

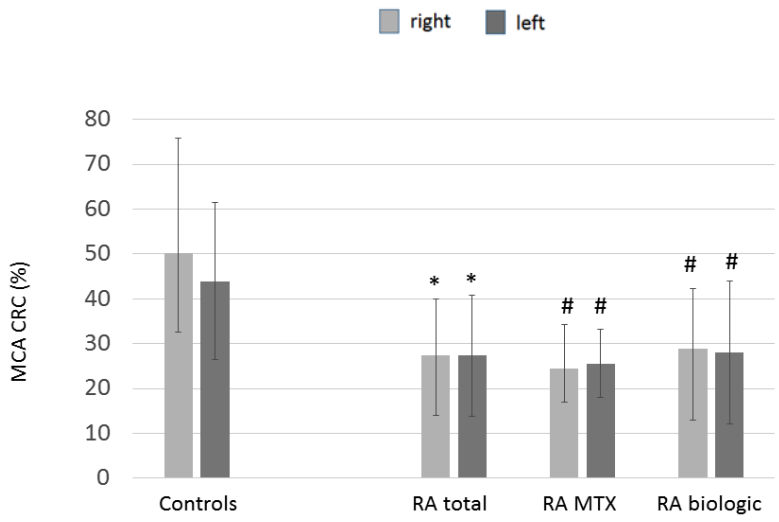
Figure 13 Basilar artery PI(r) values in controls, in patients with RA, in MTX- and biologic-treated subgroups



#p<0.05 vs controls

In the total RA cohort, right and left MCA CRC values were 27.5±12.5% and 27.3±13.5%, in the MTX-treated subgroup these values were 24.5±9.8 on the right side and 25.6±7.6 on the left side, by biologic-treated patients 28.8±13.5 on the right and 28.2±15.9 on the left side, respectively. In controls, the right and left mean CRC values were significantly higher: on the right: 50.2±30.6 ($p<0.001$, $p=0.005$, $p<0.001$); on the left: 44.0±17.6 ($p<0.001$, $p=0.002$ $p<0.001$) (Figure 14).

Figure 14 MCA CRC values in controls, in patients with RA, in MTX- and biologic-treated subgroups



*p<0.001 vs controls; #p<0.05 vs controls

Within the RA patient cohort, MTX- versus biologic-treated patients significantly differed in left MCA PI(r) (1.00±0.17 vs 0.83±0.17, $p=0.021$), PI(h) (1.23±0.29 vs 0.99±0.25, $p=0.029$) and RI(r) (0.62±0.07 vs 0.55±0.07, $p=0.018$), respectively (Figure 11A, 11C, 12A). There were also a non-significant tendency in difference of right MCA PI(r) (0.97±0.12 vs 0.88±0.20), PI(a) (0.90±0.15 vs 0.82±0.17), PI(h) (1.25±0.35 vs 1.07±0.27), RI(r) (0.61±0.05 vs 0.57±0.07), RI(a) (0.58±0.06 vs 0.55±0.07), RI(h) (0.67±0.08 vs 0.64±0.09), left MCA PI(a) (0.9±0.12 vs 0.82±0.2), RI(a) (0.59±0.08 vs 0.55±0.1), RI(h) 0.69±0.08 vs 0.62±0.1), basilar artery PI(r) (1.05±0.38 vs 0.94±0.16) values between MTX- versus biologic-treated patients (Figure 11A, 11B, 11C, 12A, 12B, 12C, 13).

CRC on both sides were also non-significantly higher in biologic-treated compared to MTX-treated patients. On the right side CRC was 28.8±13.5 in the biologic-treated vs 24.5±9.8 in the MTX-treated RA population and on the left side these values were 28.2±15.9 vs 25.6±7.6 (Figure 14).

5.2.3 Carotid and vertebral artery assessments

All RA subjects (n=41) and control individuals were investigated by carotid ultrasonography on both sides. Significantly more RA patients from the total RA population (n=21, 51%; p=0.021) and MTX-treated patients (n=8, 67%; p=0.016) had at least one carotid plaque compared to controls (n=16, 27%) on the right side. In contrast on the left side there were no significant differences between the examined subgroups (Table 8).

Table 8 Carotid IMT assessments

	RA total (n=41)	**RA MTX (n=12)**	**RA biol (n=29)**	**Controls (n=60)**	**p**			
					RA total vs. controls	**RA MTX vs. controls**	**RA biol. vs. controls**	**RA MTX vs RA biol.**
Carotid ultrasound studies								
Right cIMT (mm)	0.72±0.24	0.72±0.25	0.72±0.23	0.40±0.28	***<0.001***	***<0.001***	***<0.001***	
Left cIMT (mm)	0.73±0.23	0.69±0.22	0.75±0.24	0.40±0.28	***<0.001***	***<0.001***	***<0.001***	
Right carotid plaques (%)	21 (51%)	8 (67%)	13 (45%)	16 (27%)	***0.021***	***0.016***		
Left carotid plaques (%)	18 (44%)	6 (50%)	12 (41%)	21 (35%)				
Right carotid plaque score	1.22±1.35	1.58±1.31	1.07±1.36	0.60±0.99	***0.016***	***0.008***		
Left carotid plaque score	1.00±1.16	1.25±1.55	0.90±0.98	0.78±1.12				

As described above, carotid plaques were also scored (from 0 to 5) with respect to absence, fibrous, calcified phenotype and stenotic or non-stenotic nature. We also calculated plaque-score by the controls and patients on both side. Regarding to previous findings, not surprisingly, the right but not the left carotid plaque score was significantly higher in the total (1.22±1.35; p=0.016) and MTX-treated RA subset (1.58±1.31; p=0.08) vs controls (0.60± 0.99) (Table 8).

There were no statistical differences with regards to presence or absence carotid plaques and plaque scores among the MTX- (right: 1.58±1.31, left: 1.25±1.55) and biologic-treated patients (right: 1.07±1.36, left: 0.90±0.98) (Table 8).

Carotid stenosis was also determined in all patients and controls. However, only two patients (one MTX- and one TCZ-treated ones) had 30% and 60% lumen stenosis in the right, and only one patient (MTX-treated) had a 60% stenosis in the left carotid artery. No stenosis was observed in any control subject. Therefore no statistical analysis could be performed (data not shown).

The cIMT were also measured on both sides. Patients with RA irrespective of the treatment in each subgroup had significantly higher values than controls. On the right side cIMT of the carotid artery was 0.72±0.24 mm in the total RA group ($p<0.001$), 0.72±0.25 mm in the MTX-treated subgroup ($p<0.001$) and 0.72±0.23 mm in the biologic-treated population ($p<0.001$) versus 0.40±0.28 mm in controls. On the left side these values were 0.73±0.23 mm ($p<0.001$), 0.69±0.22 mm ($p<0.001$) and 0.75±0.24 mm ($p<0.001$) compared to controls 0.40±0.28 mm. There were no statistical differences with regards to cIMT among the two treatment RA subsets (Table 8).

Vertebral arteries were also assessed in all patients and controls. All 41 RA patients and 60 control subjects had normal vertebral circulation in the right artery. Circulation in the left vertebral artery was undetectable in two RA patients and one control. No statistical analysis could be performed (data not shown).

5.2.4 Brain MRI investigations

All 41 patients with RA were investigated by 3T brain MRI. We were looking for minor vascular lesions in both hemispheres. As much as 39% of the total RA patients (n=16), 42% of MTX- (n=5) and 38% of biologic-treated RA patients (n=11) had at least one vascular lesion on both side. Signs of cerebral emollition were detected in 2 RA patients (5%), all of them were biologic-treated (Table 9). Cerebral atrophy could be detected in 8 RA patients (20%). Among these subjects, only one received MTX (8% of MTX-treated patients) and 7 biologics (24%). With respect to emollition and atrophy, no statistically significant differences could be determined among the RA subsets (Table 9).

Table 9 Results of brain MRI assessments

	RA total (n=41)	**RA MTX (n=12)**	**RA biol (n=29)**
Right vascular lesions (%)	16 (39%)	5 (42%)	11 (38%)
Left vascular lesions (%)	16 (39%)	5 (42%)	11 (38%)
Emollition	2 (5%)	0	2 (7%)
Atrophy	8 (20%)	1 (8%)	7 (24%)

There were no statistically significant differences between vascular lesions, emollition or atrophy among MTX- and biologic treated RA subset.

5.2.5 Correlations between TCD, carotid ultrasound features and other parameters in RA patients

As described above, multiple linear regression analysis was performed in order to determine associations between TCD/carotid parameters as dependent variables and other independent clinical and laboratory variables. As seen in Table 10, numerous TCD variables correlated with age. When assessing disease-related factors, left MCA PI(r) and PI(h) positively, while right MCA CRC inversely correlated with disease activity. With respect to metabolic factors, right PI(h) and right cIMT correlated with BMI. Left RI(h) inversely correlated with HDL-C.

Table 10 Associations between TCD/carotid parameters and other variables - multiple linear regression analysis.

Parameter 1	Parameter 2	B (CI 95%)	β	p
right PI(r)	**age**	0.007 (0.002-0.013)	0.384	0.011
right RI(r)	**age**	0.004 (0.002-0.006)	0.508	0.001
right PI(h)	**BMI**	0.024 (0.006-0.042)	0.410	0.011
right RI(h)	**age**	0.004 (0.002-0.007)	0.456	0.002
left PI(r)	**age**	0.009 (0.002-0.017)	0.447	0.012
left RI(r)	**age**	0.004 (0.001-0.007)	0.459	0.011
left PI(r)	**DAS28**	0.086 (0.013-0.159)	0.368	0.023
left PI(h)	**CRP**	0.028 (0.005-0.050)	0.442	0.016
	DAS28	0.087 (0.008-0.166)	0.372	0.032
left RI(h)	**HDL-C**	-0.104 (-0.203--0.006)	-0.415	0.039
basilar PI	**age**	0.008 (0.002-0.014)	0.325	0.010
right CRC	**DAS28**	-4.633 (-9.047--0.219)	-0.326	0.040
right cIMT	**BMI**	0.002 (0-00.3)	0.306	0.015

β: standardized linear coefficient; B (+95% CI): regression coefficient

We also correlated TCD variables with carotid ultrasound and brain MRI results. When comparing RA patients with (n=18) and without left carotid plaques (n=23), right MCA PI(a), RI(r), RI(a), as well as left MCA PI(a), PI(h) and RI(a) values were significantly higher in patients with carotid plaques (Table 11). In addition, right MCA PI(h) significantly correlated with right cIMT (R=0.377, p=0.018) (data not shown).

Table 11 Associations between TCD and left carotid artery plaques

TCD variable	Presence of plaque (n=18)	Absence of plaque (n=23)	p
right PI(a)	0.92±0.17	0.77±0.12	0.002
right RI(r)	0.61±0.07	0.56±0.07	0.021
right RI(a)	0.59±0.09	0.53±0.05	0.007
left PI(a)	0.94±0.18	0.79±0.17	0.032
left PI(h)	1.23±0.29	0.94±0.25	0.021
left RI(a)	0.61±0.10	0.53±0.07	0.022

There were no significant correlation between TCD, carotid parameters and any brain MRI findings (data not shown).

6 DISCUSSION

6.1 Study 1

Accelerated atherosclerosis among patients with RA is well-known such as increased risk of CV diseases. The same mechanism can also lead to cerebrovascular morbidity and mortality [31, 33, 79-80]. Uncontrolled RA patients with higher disease activity and higher inflammatory parameters are the most affected by cerebrovascular morbidity such as stroke [33, 79-81].

TCD technique is essential to exam the function of intracranial vessels, arterial circulation and pressure characteristics [44, 47]. Without the presence of an intact TAW, the examination cannot be carried out. Nevertheless, even in the general population, TAW may be inadequate or undetectable (TAWF) in about 8-20% of individuals [45, 60-61]. This phenomenon can be more common (34%) among stroke patients [58, 63]. TAWF may be a developmental issue, however, TAWF has been correlated with age in the general population [45, 60], as well as in stroke patients [63].

The thickening and the changed structure of the temporal bone around the TAW may be responsible for TAW failure [58, 68]. Our best knowledge, the texture and other characteristics of TAW have not yet been studied in RA patients so we could not compare our results with any reported data.

We assessed 62 RA patients and 60 non-RA, healthy controls with TCD examination. In much more RA patients than controls was the TAW inadequate. In RA, on the right side 35%, on the left side 53%, on both side 34% TAWF was present, in contrast in controls in 20-20% of subject on the right and left side too, while only 13% on both sides. Our results and other data that was reported in the literature from the general population shows that in RA patients vs controls the presence of TAWF was significantly more common [45].

With respect to treatment, TAWF was more frequent and the TAW score was lower in biologic-treated, but not in MTX-treated RA patients compared to controls. Although TAWF showed no association with disease duration, biologic-treated patients may represent a more severe and progressive RA subset compared to those treated

with MTX only. Thus, disease severity and the need for biologics may correlate with TAWF.

TAWF, as well as thicker temporal bone was more common on the left side in RA patients but not in controls representing the general population. This phenomenon, which was also more pronounced in biologic-treated compared to MTX-treated patients, requires further studies and explanation.

With respect to temporal bone texture, we found by only one control subject (3%) trilayer "sandwich"-like structure compared in RA patients this texture was more common, 53% on the left and 60% on the right side. This heterogeneous bone alteration also been described among stroke patients [68]. Here both MTX- and biologic-treated patients had this "sandwich"-like texture more commonly than controls. Furthermore, this bone structure was equally observed on both left and right sides.

RA patients had thicker (3.6-4.6 mm) temporal bones than controls (2.9 mm). The temporal bone was also found thicker in stroke patients [58, 68].

By applying linear logistic regression analysis TAWF, TAW score and temporal bone thickness and heterogeneous texture also correlated with age in RA patients, primarily on the left side indicating that with age the temporal bone gets thicker leading to reduced detectability of the foramen. However, in our control population only TAW detectability and TAW score showed associations with age, texture and thickness did not.

We also correlated TAW feature with bone density, as well as bone biomarkers. Serum OPG levels correlated with TAWF and "sandwich"-like bone structure, interestingly. We could not find any other bone biomarkers expect for OPG that correlated with either TAW phenomena. The thickness of the bone around TAW on the left side inversely correlated with femoral neck and total hip BMD and T-scores. These data show that there is locally bone thickening around TAW even if patient has pronounced generalized osteopenia or osteoporosis.

By performing multivariable logistic regression analysis age was independent prognostic factor for heterogeneous trilayer TAW structure and TAW failure, while serum OPG was an independent predictor of TAWF in RA patients. Others also found similar correlations in stroke patients [68] and in the general population [63].

Bone loss is common among RA patients, more pronounced in highly active, progressive RA disease, mainly caused by inflammatory bone resorption [82-83]. TAW thickness correlated inversely with femoral neck and total hip BMD. These correlations were only present in biologic-, but not in MTX-treated patients indicating the interplay

between generalized bone loss and TAW abnormalities in more severe RA patients. As discussed above, no data has become available in RA. Other studies carried out in stroke patients found no correlation between TAWF and BMD. However similarly to our results, the heterogeneous texture of the temporal bone was inversely associated with BMD [68].

OPG is a cytokine receptor and member of the TNF receptor superfamily. It is a decoy receptor for RANKL and inhibitor of inflammatory bone loss through the RANK/RANKL system [84]. Thus, OPG has been associated with TNF-α-mediated bone loss too [82]. Biologics, but not MTX itself, both anti-TNF-α [85] and anti-IL-6 [86] may further increase the level of circulating OPG. OPG production may be a counter-regulator of bone resorption [84, 87] in RA. By our studied RA patients TAWF on both sides and temporal bone "sandwich"-like texture were correlated with higher circulating OPG levels. The features of TAWF with thick and heterogeneous temporal bone may be characteristic for the more severe RA population in need for biologics, also with more pronounced generalized bone loss and increased production of OPG. Further studies in large number of patients are required to find more relationships between TAWF, features of temporal bone and bone biology.

6.2 Study 2

The carotid arteries by US and the intracranial vessels by TCD technique can be examined. Before the appearance of clinical symptoms with these investigations can be detectable the cerebrovascular risk and the asymptomatic morbidity of the patients [12, 78, 81].

To our best knowledge, this is the very first study using functional TCD to assess the intracranial vessels among RA patients, moreover in combination with carotid and vertebral arteries ultrasonography and brain MRI, in addition by the same RA population. These values were compared MTX- to biologic subset, the total RA population to healthy individuals. Because of there is no comparable reported study in the literature, we could not compare our results with others findings.

With TCD technique we could exam the intracranial vessels dynamically [44, 47]. TCD is a non-invasive, cost-effective and bedside tool for provides physiological information on the cerebral hemodynamic which is often complementary to structural imaging [41, 46].

The blood supply territory of the MCA is the most frequently affected in a cerebral infarction because of the direct flow from internal carotid artery into the MCA and because of the large size of the supplied territory. Therefore the MCA is the most important intracranial vessel to assess by TCD. Impaired values of PI, RI, CRC, MFV of MCA may predict future cerebrovascular morbidity [36, 60-61].

The main limitation of the TCD is that, as described above in Study 1, TAW may be inadequate even in the general population, more often among RA patients [13]. On the other hand, all patients were suitable for carotid and vertebral artery studies and many patients also for brain MRI.

We carried out detailed functional TCD investigation using different manoeuvres in 41 patients with RA and 60 age- and sex-matched healthy individuals as controls. Furthermore we differentiated the total RA group to two subsets based on the treatment with MTX or biologics (IFX or TCZ). There was no statistically significant difference between the main characteristics of these groups expect that the disease activity was higher among the MTX-treated patients than the biologic-treated ones.

The right and left MCA PI and RI were significantly higher among the examined patients with RA at rest and after breath holding (apnea) too compared to the control population. These findings indicate increased distal resistance or stenosis. The lower CRC indicate impaired function of the resistance arteries. CRC is an independent risk

factor for stroke [56]. RA patients had lower CRC values compared to controls. With respect to BA PI was also significantly higher in the RA population than controls. These data suggest that RA may have associated with impaired function of intracranial vessels compared to healthy individuals.

Our results show that patients treated with MTX had higher MCA PI(r), PI(h) and RI(r) on the left side than those who received biologics. Every other measured TCD parameters showed a non-significant tendency of being increased and were unfavorable in the MTX-treated subgroup than biologic treated one. It seems that biologics may beneficially influence the function and some measurable parameters of the intracranial vessels.

The traditional investigation method to determine atherosclerosis is the carotid artery US examination. We also completed our study with this method. To our knowledge, to date, carotid arteries US results has been not compared to TCD assessment in patients with RA.

Increased cIMT and more frequent presence of carotid plaque among RA patients compared to the general population have been reported by a number of investigators [7, 12, 88-92]. Not surprisingly, in our study, more RA patients had carotid plaques and higher plaque score compared to controls. We found significant difference in both values on the right side. Carotid arteries cIMT was also higher in the total RA patients and in the two subsets treated with MTX and biologics on both sides, respectively. With regard to different treatment RA subgroups we could not find any significant differences in the presence of plaques or plaque scores.

Asymptomatic atherosclerosis of intracranial vessels may lead to minor cerebral vascular lesions. Therefore we carried out brain MR investigation to determine these phenomena. Thirty-nine percent of RA patients had at least one vascular lesion in either hemisphere. MTX-treated patients were slightly more affected than patients on biologics but the difference was not significant. Cerebral emollition or atrophy was also determined. In this regard there was no significant difference between the MTX- and biologic-treated patients. Cerebral emollition in RA has been not yet reported in the literature. Wartolowska et al [93] found association between cerebral atrophy and RA. In contrast Bekkelund et al [94] could not.

Multiple linear regression analysis showed multiple associations between right and left TCD parameters and age and some parameters with disease activity. We also correlated TCD and carotid ultrasonography results. In patients who had carotid plaque on the left side more TCD values indicating higher cerebrovascular resistance were increased. PI (h) also correlated with carotid cIMT on the right. Detailed future studies are needed to confirm these findings.

7 CONCLUSIONS

The prevalence of stroke is increased among RA patients. Vascular physiology should be assessed in the preclinical vascular state to determine stroke risk by patients. Assessment of intracranial vessels includes transcranial Doppler ultrasonography (TCD). TCD performance requires intact TAW on both sides to investigate right and left intracranial arteries. TAWF is more common in RA patients than in healthy individuals. Failure of TAW may be due to pathological bone metabolism.

We assessed RA patients undergoing various treatments in comparison to a control non-RA, healthy control group by TCD investigation, carotid ultrasonography and brain MRI too. We wished to examine the cerebral vasculature as complex as possible and to determine preclinical pathophysiological changes of them.

TAWF, thicker and heterogeneous temporal bones were associated with RA. RA has been associated with increased bone turnover and generalized bone loss [95-96], especially in those with highly active, more severe disease. We also studied the features of TAW in relation to BMD and bone biomarkers. Bone loss seen in RA may result in OPG release [85-86]. We can hypothesize that OPG, in turn, may also stimulate bone apposition around TAW leading to TAWF, increased thickness and because of more rapid burn turnover, heterogeneous bone texture. Further studies in large number of patients are needed to explore the relationships between TAWF, features of the temporal bone and their relation to bone biology.

We measured less favorable parameters by TCD in RA population than controls, MTX-treated subgroup than biologic treated one. We found increased distal MCA and BA resistance in RA. RA patients also exert CRC defect, which indicate impaired function of resistance arteries. We also confirmed increased carotid plaque formation, increased cIMT. Accordingly we found cerebral vascular lesions, emollition and atrophy in a proportion of RA patients. These data suggest that RA may have associated with impaired function of intracranial vessels compared to healthy individuals. Biologics, in comparison to MTX, may beneficially influence the function and some TCD parameters in the intracranial vessels.

8 RESEARCH LIMITATIONS

To our best knowledge this is the very first study to exam the intracranial vessels of RA patients by TCD investigation. The main limitation of this work is that there are no data in the literature for comparison. Data are only available on the general population and stroke patients.

Also, our cohort size is relatively small. Further, larger studies carried out in more RA patients are needed to explore the relationships between TAWF, features of the temporal bone and their relation to bone biology.

We intended to form as homogenous patient subsets as possible, therefore only female patients were included. Thus, we do not have any information about male subjects.

Because of the large data setting, we did not correlate our findings with traditional CV risk factors such as smoking habits, overweight etc.

Only a part of the RA subjects participated on the MRI examination, therefore result are limited due to the relatively small number of patients. Because of that, no statistically significant differences could be determined among the RA subsets. The high costs of MRI imaging did not allow us to examine healthy controls.

9 REFERENCES

1. Symmons DP: **Epidemiology of rheumatoid arthritis: determinants of onset, persistence and outcome.** *Best Pract Res Clin Rheumatol.* 2002; 16(5):707-22.

2. Van Doornum S, Mc Coll G, Wicks IP. **Accelerated atherosclerosis: an extraarticular feature of rheumatoid arthritis?** *Arthritis Rheum*. 2002; 46: 862–873.

3. Dougados M, Soubrier M, Antunez A, Balint P, Balsa A et al. **Prevalence of comorbidities in rheumatoid arthritis and evaluation of their monitoring: results of an international, cross-sectional study (COMORA).** *Ann Rheum Dis.* 2014; 73(1):62-8.

4. Hollan I, Meroni PL, Ahearn JM, Cohen Tervaert JW, Curran S et al. **Cardiovascular disease in autoimmune rheumatic diseases.** *Autoimmun Rev.* 2013; 12(10):1004–1015.

5. Sherer Y, Shoenfeld Y. **Mechanisms of disease: atherosclerosis in autoimmune diseases***. Nat Clin Pract Rheumatol.* 2006; 2(2): 99-106.

6. Skoog T, Dichtl W, Boquist S, Skoglund-Andersson C, Karpe F et al. **Plasma tumour necrosis factor-alpha and early carotid atherosclerosis in healthy middle-aged men.** *Eur Heart J*. 2002; 23(5): 376-83.

7. Kerekes G, Szekanecz Z, Der H, Sándor Z, Lakos G et al. **Endothelial dysfunction and atherosclerosis in rheumatoid arthritis: a multiparametric analysis using imaging techniques and laboratory markers of inflammation and autoimmunity.** *J Rheumatol.* 2008; 35(3): 398-406.

8. Sokolove J, Brennan MJ, Sharpe O, Lahey LJ, Kao AH et al. **Brief report: citrullination within the atherosclerotic plaque: a potential target for the anti-citrullinated protein antibody response in rheumatoid arthritis.** *Arthritis Rheum.* 2013; 65(7): 1719-24.

9. Montecucco F, Steffens S, Mach F. **The immune response is involved in atherosclerotic plaque calcification: could the RANKL/RANK/OPG system be a marker of plaque instability?** *Clin Dev Immunol.* 2007; 2007: 75805.

10. Szekanecz Z, Kerekes G, Der H, Sándor Z, Szabó Z et al. **Accelerated atherosclerosis in rheumatoid arthritis.** *Ann N Y Acad Sci.* 2007; 1108: 349-58.

11. Nurmohamed MT, Heslinga M, Kitas GD. **Cardiovascular comorbidity in rheumatic diseases.** *Nat Rev Rheumatol.* 2015; 11(12): 693–704.

12. Peters MJ, Symmons DP, McCarey D, Dijkmans BA, Nicola P *et al*. **EULAR evidence based recommendations for cardiovascular risk management in patients with rheumatoid arthritis and other forms of inflammatory arthritis.** *Ann Rheum Dis.* 2010; 69(2): 325-31.

13. del Rincon I, Williams K, Stern MP, Freeman GL, O'Leary DH et al. **Association between carotid atherosclerosis and markers of inflammation in rheumatoid arthritis patients and healthy subjects.** Arthritis Rheum. 2003; 48: 1833–1840.

14. Gualtierotti R, Ughi N, Marfia G, Ingegnoli F. **Practical Management of Cardiovascular Comorbidities in Rheumatoid Arthritis.** *Rheumatol Ther.* 2017; 4(2): 293-308.

15. Charles-Schoeman C, Watanabe J, Lee YY, Furst DE, Amjadi S et al. **Abnormal function of high-density lipoprotein is associated with poor disease control and an altered protein cargo in rheumatoid arthritis.** *Arthritis Rheum.* 2009; 60: 2870-9.

16. Szekanecz Z, Kerekes G, Soltesz P. **Vascular effects of biologic agents in RA and spondyloarthropathies.** *Nat Rev Rheumatol.* 2009; 5(12): 677-84.

17. Maxwell SR, Moots RJ, Kendall MJ. **Corticosteroids: do they damage the cardiovascular system?** Postgrad Med J. 1994; 70(830): 863-70.

18. Suissa S, Bernatsky S, Hudson M. **Antirheumatic drug use and the risk of acute myocardial infarction.** *Arthritis Rheum.* 2006; 55(4): 531-6.

19. Shea B, Swinden MV, Ghogomu ET, Ortiz Z, Katchamart W et al. **Folic acid and folinic acid for reducing side effects in patients receiving methotrexate for rheumatoid arthritis.** *J Rheumatol.* 2014; 41(6): 1049-60.

20. Avouac J, Allanore Y. **Cardiovascular risk in rheumatoid arthritis: effects of anti-TNF drugs.** *Expert Opin Pharmacother,* 2008; 9: 1121-1128.

21. Jacobsson LT, Turesson C, Gülfe A, Kapetanovic MC, Petersson IF et al. **Treatment with tumor necrosis factor blockers is associated with a lower incidence of first cardiovascular events in patients with rheumatoid arthritis.** *J Rheumatol*. 2005; 1213-1218.

22. Dixon WG, Watson KD, Lunt M, Hyrich KL, British Society for Rheumatology Biologics Register Control Centre Consortium et al. **Reduction in the incidence of myocardial infarction in patients with rheumatoid arthritis who respond to anti-tumor necrosis factor alpha therapy: results from the British Society for Rheumatology Biologics Register.** *Arthritis Rheum.* 2007; 56: 2905–12.

23. Bili A, Tang X, Pranesh S, Bozaite R, Morris SJ et al. **Tumor necrosis factor α inhibitor use and decreased risk for incident coronary events in rheumatoid arthritis.** *Arthritis Care Res.* 2014; 66: 355–63.

24. Myasoedova E, Crowson CS, Kremers HM, Roger VL, Fitz-Gibbon PD et al. **Lipid paradox in rheumatoid arthritis: the impact of serum lipid measures and systemic inflammation on the risk of cardiovascular disease.** *Ann Rheum Dis.* 2011; 70(3): 482-7.

25. Pollono EN, Lopez-Olivo MA, Lopez JA & Suarez-Almazor ME**. A systematic review of the effect of TNF-alpha antagonists on lipid profiles in patients with rheumatoid arthritis.** *Clin Rheumatol. 2010;* 29: 947-55.

26. Schultz O, Oberhauser F, Saech J, Rubbert-Roth A, Hahn M et al. **Effects of inhibition of interleukin-6 signalling on insulin sensitivity and lipoprotein (a) levels in human subjects with rheumatoid diseases.** *PLoS ONE.* 2010; 5(12).

27. Agca R, Heslinga SC, Rollefstad S, Heslinga M, McInnes IB et al. **EULAR recommendations for cardiovascular disease risk management in patients with rheumatoid arthritis and other forms of inflammatory joint disorders: 2015/2016 update.** *Ann Rheum Dis.* 2017; 76(1): 17-28.

28. Aviña-Zubieta JA, Choi HK, Sadatsafavi M, Etminan M, Esdaile JM et al. **Risk of cardiovascular mortality in patients with rheumatoid arthritis: a meta-analysis of observational studies.** *Arthritis Rheum*. 2008; 59: 1690-1697.

29. Meune C, Touzé E, Trinquart L, Allanore Y. **Trends in cardiovascular mortality in patients with rheumatoid arthritis over 50 years: a systematic review and meta-analysis of cohort studies.** *Rheumatology (Oxford).* 2009; 48: 1309-1313.

30. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C et al. **2016 European guidelines on cardiovascular disease prevention in clinical practice: the Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) Developed with the special contribution of the European Association for Cardiovascular Prevention and Rehabilitation (EACPR)** *Eur Heart J.* 2016; 37(29): 2315–2381.

31. Sodergren A, Stegmayr B, Ohman ML, Wallberg-Jonsson S. **Increased incidence of stroke and impaired prognosis after stroke among patients with seropositive rheumatoid arthritis.** *Clin Exp Rheumatol*. 2009; 27(4): 641–4.

32. Shoenfeld Y, Gerli R, Doria A, Matsuura E, Cerinic MM et al. **Accelerated atherosclerosis in autoimmune rheumatic diseases.** *Circulation.* 2005; 112(21): 3337–47.

33. Wiseman SJ, Ralston SH, Wardlaw JM. **Cerebrovascular disease in rheumatic diseases: a systematic review and meta-analysis.** *Stroke.* 2016; 47(4): 943–50.

34. Meune C, Touzé E, Trinquart L, Allanore Y**. High risk of clinical cardiovascular events in rheumatoid arthritis: Levels of associations of myocardial infarction and stroke through a systematic review and meta-analysis.** *Arch Cardiovasc Dis.* 2010; 103(4): 253-61.

35. Greenberg DA, Aminoff MJ (Michael Jeffrey), Simon RP. **Clinical neurology.** *New York: McGraw-Hill Education*. 2015; Ninth edition.

36. Hoksbergen AW, Legemate DA, Csiba L, Csáti G, Síró P et al. **Absent collateral function of the circle of Willis as risk factor for ischemic stroke.** *Cerebrovasc Dis.* 2003; 16(3): 191-8.

37. Zha AM, Di Napoli M, Behrouz R. **Prevention of Stroke in Rheumatoid Arthritis.** *Curr Neurol Neurosci Rep.* 2015; 15(12): 77.

38. Edelman SK. **Doppler in Understanding Ultrasound Physics.** *Texas: ESP, Inc.* 2007; Third edition.

39. Kremkau FW. **Part 1 Sonographic principles in Diagnostic Ultrasound: Principles and Instruments.** *Philadelphia: Saunders, Elsevier Publications. 2006;* Seventh edition.

40. Aaslid R, Markwalder TM, Nornes H. **Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries.** *J Neurosurg.* 1982; 57(6): 769-74.

41. Bathala L, Mehndiratta MM, Sharma VK. **Transcranial doppler**: **Technique and common findings** (**Part 1**). *Ann Indian Acad Neurol.* 2013; 16(2): 174-9.

42. Garami Z, Alexandrov AV. **Neurosonology.** *Neurol Clin.* 2009; 27(1): 89-108.

43. Yeo LL, Sharma VK. **Role of transcranial Doppler ultrasonography in cerebrovascular disease.** *Recent Pat CNS Drug Discov.* 2010; 5(1): 1-13.

44. Guan J, Zhang S, Zhou Q, Li C, Lu Z. **Usefulness of transcranial Doppler ultrasound in evaluating cervical-cranial collateral circulations.** *Interv Neuroradiol.* 2013; 2(1): 8–18.

45. Sharma VK, Paliwal PR, Teoh HL, Venketasubramanian N, Chan BP. **Role of diagnostic ultrasound in patient selection for stroke intervention.** *Recent Pat CNS Drug Discov.* 2011; 6(3): 181–95.

46. Fülesdi B, Réka Kovács K, Bereczki D, Bágyi P, Fekete I et al. **Computed tomography and transcranial Doppler findings in acute and subacute phases of intracerebral hemorrhagic stroke.** *J Neuroimaging.* 2014; 24(2): 124-30.

47. Alexandrov AV, Sloan MA, Tegeler CH, Newell DN, Lumsden A et al. **Practice standards for transcranial Doppler (TCD) ultrasound. Part II. Clinical indications and expected outcomes.** *J Neuroimaging.* 2012; 22(3): 215-24.

48. Leányvári Z, Vastagh I, Fülesdi B, Szirmai I, Lengyel A et al. **Computed tomographic and transcranial Doppler sonographic findings in acute and subacute phases of middle cerebral artery strokes.** *J Clin Ultrasound.* 2002; 30(1): 33-7.

49. Oláh L, Raiter Y, Candale C, Molnár S, Rosengarten B et al. **Visually evoked cerebral vasomotor response in smoking and nonsmoking young adults, investigated by functional transcranial Doppler.** *Nicotine Tob Res.* 2008; 10(2): 353-8.

50. Magyar MT, Valikovics A, Bereczki D, Ficzere A, Czuriga I et al. **Transcranial Doppler monitoring in hypertensive patients during physical exercise.** *Cerebrovasc Dis.* 2001; 12(3): 186-91.

51. Ficzere A, Valikovics A, Fülesdi B, Juhász A, Czuriga I et al. **Cerebrovascular reactivity in hypertensive patients: a transcranial Doppler study.** *J Clin Ultrasound.* 1997; 25(7): 383-9.

52. Settakis G, Páll D, Molnár C, Katona E, Bereczki D et al. **Hyperventilation-induced cerebrovascular reactivity among hypertensive and healthy adolescents.** *Kidney Blood Press Res.* 2006; 29(5): 306-11.

53. Settakis G, Páll D, Molnár C, Bereczki D, Csiba L et al. **Cerebrovascular reactivity in hypertensive and healthy adolescents: TCD with vasodilatory challenge.** *J Neuroimaging.* 2003; 13(2): 106-12.

54. Kerenyi LL, Fulesdi B, Ficzere A, Kaposzta Z, Valikovics A et al. **Cerebrovascular reserve capacity in patients with hyperlipidemia.** *J Clin Ultrasound.* 2000; 28(3): 115-21.

55. Naqvi J, Yap KH, Ahmad G, Ghosh J. **Transcranial Doppler ultrasound: a review of the physical principles and major applications in critical care.** *Int J Vasc Med*. 2013; 2013: 629378.

56. Orosz L, Fülesdi B, Hoksbergen A, Settakis G, Kollár J et al. **Assessment of cerebrovascular reserve capacity in asymptomatic and symptomatic hemodynamically significant carotid stenoses and occlusions.** *Surg Neurol.* 2002; 57(5): 333-9.

57. Purkayastha S, Sorond F. **Transcranial Doppler ultrasound: technique and application.** *Semin Neurol*. 2012; 32(4): 411-20.

58. Kollár J, Schulte-Altedorneburg G, Sikula J, Fülesdi B, Ringelstein EB et al. **Image quality of the temporal bone window examined by transcranial Doppler sonography and correlation with postmortem computed tomography measurements.** *Cerebrovasc Dis.* 2004; 17(1): 61-5.

59. Sobotta J, Putz R, Pabst R, Putz R. **Sobotta atlas of human anatomy: head, neck, upper limb, thorax, abdomen, pelvis, lower limb.** *Elsevier/Urban & Fischer.* 2008; 14th English edition.

60. Wijnhoud AD, Koudstaal PJ, Dippel DW. **The prognostic value of pulsatility index, flow velocity, and their ratio, measured with TCD ultrasound, in patients with a recent TIA or ischemic stroke.** *Acta Neurol Scand.* 2011; 124(4): 238–44.

61. Ni J, Yao M, Gao S, Cui LY. **Stroke risk and prognostic factors of asymptomatic middle cerebral artery atherosclerotic stenosis.** *J Neurol Sci.* 2011; 301(1-2): 63–5.

62. Bauer A1, Becker G, Henz P, Jachimczak P, Schwarz KQ et al. **Transcranial duplex ultrasound: experience with contrast enhancing agents.** *Int Angiol.* 1997; 16(4): 216-21.

63. Marinoni M, Ginanneschi A, Forleo P, Amaducci L. **Technical limits in transcranial Doppler recording: inadequate acoustic windows.** *Ultrasound Med Biol.* 1997; 23(8): 1275-7.

64. Bazan R, Braga GP, Luvizutto GJ, Hueb JC, Hokama NK et al. **Evaluation of the temporal acoustic window for transcranial Doppler in a multi-ethnic population in Brazil.** *Ultrasound Med Biol.* 2015; 41(8): 2131–2134.

65. Postert T, Federlein J, Przuntek H, Buttner T. **Insufficient and absent acoustic temporal bone window: potential and limitations of transcranial contrast-enhanced color-coded sonography and contrast-enhanced power-based sonography.** *Ultrasound Med Biol*. 1997; 23(6): 857–862.

66. Halsey JH. **Effect of emitted power on waveform intensity in transcranial Doppler.** *Stroke.* 1990; 21(11): 1573-8.

67. Oláh L, Valikovics A, Bereczki D, Fülesdi B, Munkácsy C et al. **Gender-related differences in acetazolamide-induced cerebral vasodilatory response: a transcranial Doppler study.** J Neuroimaging. 2000; 10(3): 151-6.

68. Kwon JH, Kim JS, Kang DW, Bae KS, Kwon SU. **The thickness and texture of temporal bone in brain CT predict acoustic window failure of transcranial Doppler.** *J Neuroimaging*. 2006; 16(4): 347–352.

69. Nicoletto HA, Burkman MH. **Transcranial Doppler series part II: performing a transcranial Doppler.** *Am J Electroneurodiagnostic Technol.* 2009; 49(1): 14-27.

70. Bathala L, Mehndiratta MM, Sharma VK. **Transcranial doppler: Technique and common findings (Part 1)** *Ann Indian Acad Neurol.* 2013; 16(2): 174-9.

71. Skrzat J, Brzegowy P, Walocha J, Wojciechowski W. **Age dependent changes of the diploe in the human skull.** *Folia Morphol (Warsz).* 2004; 63(1): 67-70.

72. Tiecks FP, Lam AM, Aaslid R, Newell DW. **Comparison of static and dynamic cerebral autoregulation measurements.** *Stroke*. 1995; 26(6): 1014-9.

73. Settakis G, Lengyel A, Molnár C, Bereczki D, Csiba L et al. **Transcranial Doppler study of the cerebral hemodynamic changes during breath-holding and hyperventilation tests.** *J Neuroimaging.* 2002; 12(3): 252-8.

74. Malatino LS, Bellofiore S, Costa MP, Lo Manto G, Finocchiaro F et al. **Cerebral blood flow velocity after hyperventilation-induced vasoconstriction in hypertensive patients.** *Stroke.* 1992; 23(12): 1728–32.

75. Greenland P, Alpert JS, Beller GA, Benjamin EJ, Budoff MJ et al. **American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines.** *Circulation.* 2010; 122(25): e584-636.

76. Ho SS. **Current status of carotid ultrasound in atherosclerosis**. *Quant Imaging Med Surg.* 2016; 6(3): 285-96.

77. Mohan A, Sada S, Kumar BS, Sarma KVS, Devi BV et al. **Subclinical atherosclerosis in patients with rheumatoid arthritis by utilizing carotid intima-media thickness as a surrogate marker.** *Indian J Med Res.* 2014; 140(3): 379–386.

78. Kerekes G, Soltesz P, Nurmohamed MT, Gonzalez-Gay MA, Turiel M et al. **Validated methods for assessment of subclinical atherosclerosis in rheumatology.** *Nat Rev Rheumatol.* 2012; 8(4): 224–34.

79. Wolfe F, Freundlich B, Straus WL**. Increase in cardiovascular and cerebrovascular disease prevalence in rheumatoid arthritis.** *J Rheumatol.* 2003; 30(1): 36–40.

80. Levy L, Fautrel B, Barnetche T, Schaeverbeke T. **Incidence and risk of fatal myocardial infarction and stroke events in rheumatoid arthritis patients: a systematic review of the literature.** *Clin Exp Rheumatol*. 2008; 26(4): 673–9.

81. Zha AM, Di Napoli M, Behrouz R. **Prevention of stroke in rheumatoid arthritis.** *Curr Neurol Neurosci Rep.* 2015; 15(12): 77.

82. Schett G, Gravallese E. **Bone erosion in rheumatoid arthritis: mechanisms, diagnosis and treatment.** *Nat Rev Rheumatol.* 2012; 8(11): 656–664.

83. Schett G. **Osteoimmunology in rheumatic diseases.** *Arthritis Res Ther.* 2009; 11(1): 210.

84. Schett G, Redlich K, Hayer S, Zwerina J, Bolon B et al. **Osteoprotegerin protects against generalized bone loss in tumor necrosis factor-transgenic mice.** *Arthritis Rheum.* 2003; 48(7): 2042–2051.

85. Wang SY, Liu YY, Ye H, Guo JP, Li R et al. **Circulating Dickkopf-1 is correlated with bone erosion and inflammation in rheumatoid arthritis.** *J Rheumatol.* 2011; 38(5): 821–827.

86. Terpos E, Fragiadaki K, Konsta M, Bratengeier C, Papatheodorou A et al. **Early effects of IL-6 receptor inhibition on bone homeostasis: a pilot study in women with rheumatoid arthritis.** *Clin Exp Rheumatol.* 2011; 29(6): 921–925.

87. Zwerina J, Hayer S, Tohidast-Akrad M, Bergmeister H, Redlich K et al. **Single and combined inhibition of tumor necrosis factor, interleukin-1, and RANKL pathways in tumor necrosis factorinduced arthritis: effects on synovial inflammation, bone erosion, and cartilage destruction.** *Arthritis Rheum.* 2004; 50(1): 277–290.

88. Gonzalez-Juanatey C, Llorca J, Martin J, Gonzalez-Gay MA. **Carotid intima-media thickness predicts the development of cardiovascular events in patients with rheumatoid arthritis.** *Semin Arthritis Rheum.* 2009; 38(5): 366–71.

89. van Sijl AM, Peters MJ, Knol DK, de Vet HC, Gonzalez-Gay MA et al. **Carotid intima media thickness in rheumatoid arthritis as compared to control subjects: a meta-analysis.** *Semin Arthritis Rheum*. 2010; 40(5): 389–97.

90. Rollefstad S, Ikdahl E, Hisdal J, Olsen IC, Holme I et al. **Rosuvastatin-induced carotid plaque regression in patients with inflammatory joint diseases: the Rosuvastatin in Rheumatoid Arthritis, Ankylosing Spondylitis and Other Inflammatory Joint Diseases Study.** *Arthritis Rhematol.* 2015; 67(7): 1718–28.

91. van den Oever IA, van Sijl AM, Nurmohamed MT. **Management of cardiovascular risk in patients with rheumatoid arthritis: evidence and expert opinion.** *Ther Adv Musculoskelet Dis.* 2013; 5(4): 166–81.

92. Gonzalez-Juanatey C, Llorca J, Testa A, Revuelta J, Garcia-Porrua C et al. **Increased prevalence of severe subclinical atherosclerotic findings in long-term treated rheumatoid arthritis patients without clinically evident atherosclerotic disease.** *Medicine (Baltimore).* 2003; 82(6): 407–13.

93. Wartolowska K, Hough MG, Jenkinson M, Andersson J, Wordsworth BP et al. **Structural changes of the brain in rheumatoid arthritis.** *Arthritis Rheum.* 2012; 64(2): 371–9.

94. Bekkelund SI, Pierre-Jerome C, Husby G, Mellgren SI. **Quantitative cerebral MR in rheumatoid arthritis.** *AJNR Am J Neuroradiol.* 1995; 16(4): 767–72.

95. Deal C. **Bone loss in rheumatoid arthritis: systemic, periarticular, and focal.** *Curr Rheumatol Rep.* 2012; 14(3): 231–237.

96. Schett G, Saag KG, Bijlsma JW. **From bone biology to clinical outcome: state of the art and future perspectives.** *Ann Rheum Dis.* 2010; 69(8): 1415–1419.

10 SUMMARY

Accelerated atherosclerosis and increased risk for CV morbidity of RA patients are well-known and confirmed by many studies. However increased CV risk is associated also with increased stroke occurrence, still we have only few information about the conditions of intracranial vessels.

For their investigation the carotid ultrasonography is the traditionally accepted method. However with this examination we can only infer the status of the cerebral arteries. With this work we aimed to explore the status and conditions of intracranial vessels of our RA patients, to assess the possible occurrence of cerebral vascular lesions even in clinically asymptomatic patients.

We performed a complex neurovascular examination in 62 female RA patients and healthy controls, which included conventional carotid US, functional TCD and brain MRI investigations.

The transcranial Doppler US technique is an excellent, non-invasive method for dynamically monitoring the cerebral blood flow and status of intracranial vessels. With TCD we could exam the middle cerebral- and basilar arteries throw the thinnest part of the temporal bone where is the so-called acoustic window which allows the ultrasound wave through the bone. The functional TCD was also performed after rest, breath holding and hyperventilation.

We wished to determine preclinical pathophysiological changes in the cerebral vasculature. But this window was not always open, RA subjects have so-called TAWF in 35% on the right side and 50% on the left side compared to controls by whom TAWF presented significantly lower, 20% on both sides. Behind this phenomenon we found that our RA patients' temporal bone was significantly thicker and texture was more heterogeneous with CT imaging. While control subject had a monolayer cortical in general, we found cortical-diploe-cortical trilayer by more than 50% of RA patients at least on either side.

TCD investigations were carried out by 41 RA patients, by whom we measured the mean flow velocity parameters, pulsatility- and resistance indices of ACM and basilar artery. The increase in MFV refers to stenosis, vasospasm and hyperdynamic circulation, while its increase may be due to hypotension, decreased cerebral blood flow or increased intracranial pressure.

The decrease of the pulsatility indices refers proximal, while its increase indicates distal stenosis. The RI shows the extent of distal resistance. MCA PI and RI at rest and after breath holding are significantly increased in the total RA population versus controls. Basilar artery pulsatile indices were also higher in total RA and also in the MTX-treated subgroup, while the values of the biologic-treated group did not differ significantly from the control controls.

CRC depends on the physiological vasoconstrictive and vasodilatory function of precapillary resistance arteries which is responsible for maintaining the proper cerebral blood flow under various pressure conditions. Value of CRC shows the difference in percentage between the resting blood flow and the maximum blood flow after vasoactive stimulation. Its decrease is an independent, hardcore stroke risk factor. CRC was impaired in all RA subgroups compared to controls both on the left and on the right side. Moreover, values of the MTX-treated group were unfavorable than the biologic-treated ones.

More RA patients had carotid plaques and increased cIMT than controls.

We correlated these values with age, disease duration, disease activity, several bone biomarkers and bone mineral density.

We also carried out MRI investigation to determine focal vascular lesions. Almost 40% of RA patients had at least one vascular lesion in either hemisphere. Cerebral emollition or atrophy was also determined. In this regard there was no significant difference between the MTX- and biologic-treated patients.

Biologics may beneficially influence some parameters in the intracranial vessels. With more effective disease control, the function of cerebral vessels can be better preserved.

11 ÖSSZEFOGLALÁS (SUMMARY IN HUNGARIAN)

A rheumatoid arthritises betegek akcelerált atherosclerosisa, fokozott cardiális kockázata jól ismert, számos vizsgálat által megerősített tény. A cardiális rizikó fokozódása azonban együtt jár a stroke kockázatának emelkedésével, az agyi erek állapotáról mégis keveset tudunk.

Ezek megítélésére hagyományosan a carotis ultrahang vizsgálat az elfogadott módszer. Ezen vizsgálattal azonban csak következtetni tudunk az agyi erek állapotára. Tanulmányunk során célul tűztük ki, hogy jobban megismerjünk rheumatoid arthritises betegeink intracraniális ereinek állapotát, felmérjük az agyi vascularis léziók esetleges előfordulását, még a tünetek megjelenése előtt.

62 rheumatoid arthritises nőbetegnél, valamint egészséges kontrollcsoportnál végeztünk komplex neurovascularis vizsgálatot, vagyis készült hagyományos carotis ultrahang-, funkcionális TCD vizsgálat, illetve agyi MRI felvétel.

A transcranialis Doppler (TCD) vizsgálat kiváló, nem invazív lehetőség az agyalapi keringés, az agyi erek állapotának dinamikus vizsgálatára. Az artéria cerebri mediát és az artéria basilarist vizsgáltuk a temporális csont legvékonyabb részén, az ún. akusztikus ablakon keresztül, ahol még képes az ultrahang áthatolni a csonton. A funkcionális TCD vizsgálatot nyugalomban, légzésvisszatartást és hyperventillációt követően is elvégeztünk.

Az agyi erek klinikai tünetekkel még nem járó pathofiziológiai elváltozásainak kimutatása volt a célunk. A vizsgálat azonban nem mindig volt sikeres, betegeinknél jobb oldalon 35%, baloldalon 53%-ban a TCD vizsgálat nem volt elvégezhető a csontablak elégtelensége miatt, mely szignifikánsan magasabb előfordulást jelent a kontroll populáció 20%-ához képest. Ennek hátterében koponya CT vizsgálattal megváltozott csontszerkezetet, illetve -vastagságot találtunk a csontablak területén. Míg az egészséges kontrolloknál általában egy egyrétegű kortikális alkotja a csont ezen részét, addig az RA-s betegek több mint 50%-ánál egy három rétegből álló, kortikális-diploe-kortikális szerkezetet találtunk legalább az egyik oldalon.

41 betegnél készült TCD vizsgálat, akiknél az artéria cerebri mediában és a basilarisban határoztuk meg az átlagos áramlási paramétereket, valamint a pulzatilitási- és a rezisztencia indexeket. Az átlagos áramlási sebesség növekedése stenosisra, vasospazmusra illetve hyperdinámiás keringésre utal, míg növekedését

hypotonia, csökkent cerebralis vérátáramlás, intracraniális nyomásfokozódás okozhatja.

A pulzatilitási index (PI) csökkenése proximális, míg növekedése disztális szűkületet jelez. A rezisztencia index (RI) a disztális ellenállás mértékét mutatja meg. Az artéria cerebri mediában mérhető PI mind a jobb, mind pedig a bal oldalon nyugalomban és légzésvisszatartás után is szignifikánsan magasabb volt az RA-s össz. populációban a kontroll csoporthoz képest. A PI növekedése az artéria basilarisokon is kimutatható volt az össz. RA-s és a MTX-kezelt csoportnál, míg a biológiai terápiát kapó betegek értékei nem különböztek szignifikánsan a kontrollokétól.

A cerebrovaszkuláris rezerv kapacitás az agyi rezisztencia erek vazodilatációs és vasokonstrikciós funkciójától függ, mely felelős a megfelelő agyi véráramlás fenntartásáért a változó nyomásviszonyok között. Vazoaktív inger hatására a nyugalmi véráramlási értékhez képest kialakuló maximális vérátáramlás változás %-os kifejezése. Csökkenése független, keménypontú stroke rizikófaktor. Értéke valamennyi RA-s alcsoportban csökkent a kontrollcsoporthoz viszonyítva mind a jobb, mind pedig a bal oldalon. A MTX kezelt csoport értékei kedvezőtlenebbek a biológiai terápiás alcsoporthoz képest.

Az egészséges kontrollpopulációhoz képest a rheumatoid arthritises betegeknél gyakoribbak az ateroszklerotikus plakkok, szignifikánsan emelkedett a carotis intima-media vastagság.

A mért adatokat korreláltuk az életkorral, a betegség fennállásának idejével, a betegség aktivitásával, számos csontmarkerrel és a csontdenzitás értékeivel.

Koponya MRI vizsgálatot is végeztünk a fokális vasculáris léziók kimutatására. Az RA-s betegek csaknem 40%-ánál találtunk legalább egy fokális vascularis léziót az egyik oldalon. Az emollitio és az agyi atrophia tekintetében nem volt szignifikáns különbség a MTX-ot és a biológikumot kapó betegcsoportok között.

A biológikumok alkalmazása kedvezően befolyásolhatja az intracraniális erek állapotának főbb mutatóit, a hatékonyabb betegségkontrollal az agyi erek funkciója is jobban megőrizhető.

12 KEYWORDS

12.1 Keywords

Rheumatoid arthritis, Accelerated atherosclerosis, Cerebrovascular disease, Transcranial Doppler ultrasonography, Temporal acoustic window failure, Carotid ultrasonography, Methotrexate, Biologic Therapy, Osteoprotegerin.

12.2 Tárgyszavak (Keywords in Hungarian)

Rheumatoid arthritis, akcelerált atherosclerosis, cerebrovaszkuláris betegség, transcraniális Doppler ultrahang, temporális csontablak elégtelenség, carotis ultrahang, methotrexate, biológiai terápia, osteoprotegerin.

13 PUBLICATIONS

13.1 Publications related to the thesis

1. **Kardos Z,** Oláh C, Sepsi M, Sas A, Kostyál L, Bóta T, Bhattoa HP, Hodosi K, Kerekes G, Tamási L, Bereczki D, Szekanecz Z. Increased frequency of temporal acoustic window failure in rheumatoid arthritis: a manifestation of altered bone metabolism? Clin Rheumatol. 2018 Jan 30.

2. Oláh C, **Kardos Z**, Sepsi M, Sas A, Kostyál L, Bhattoa HP, Hodosi K, Kerekes G, Tamási L, Valikovics A, Bereczki D, Szekanecz Z. Assessment of intracranial vessels in association with carotid atherosclerosis and brain vascular lesions in rheumatoid arthritis. Arthritis Res Ther. 2017 Sep 26;19(1):213.

3. Szekanecz Z, Kerekes G, Végh E, **Kardos Z**, Baráth Z, Tamási L, Shoenfeld Y. **Autoimmune atherosclerosis in 3D: How it develops, how to diagnose and what to do.** *Autoimmun Rev.* 2016 Jul;15(7):756-69.

4. Szekanecz Z, Kerekes G, **Kardos Z**, Baráth Z, Tamási L. **Mechanisms of inflammatory atherosclerosis in rheumatoid arthritis.** *Curr. Immunol. Rev.* 11, 1-12, 2015 Nov.

5. Kerekes G, Nurmohamed MT, González-Gay MA, Seres I, Paragh G, **Kardos Z**, Baráth Z, Tamási L, Soltész P, Szekanecz Z**. Rheumatoid arthritis and metabolic syndrome.** *Nat Rev Rheumatol.* 2014 Nov;10(11):691-6.

13.2 Other publications

1. Oláh C, Sandra S, Lajgut A, Demeter B, **Kardos Z**, Oláh M. **Balneológiai kezelések idegsebészeti kórképekben.** *Gerincgyógyászati Szemle* 2017. IV. évf.-1.szám: 38-42.

13.3 Conference abstracts

1. **Kardos Z**, Oláh C, Andrejkovics M, Bodnár E, Hodosi K, Fazekas K, Flórián Á, Lukács K, Miksi Á, Baráth Z, Tamási L, Szekanecz Z. **Assessment of cognitive function in rheumatoid arthritis.**
   EULAR Amsterdam, 2018.

2. **Kardos Z**, Oláh C, Sepsi M, Sas A, Kostyál L, Bhattoa HP, Hodosi K, Kerekes G, Tamási L, Valikovics A, Bereczki D, Szekanecz Z. **Stroke in autoimmune diseases: pathophysiology of cerebral vessels in rheumatoid arthritis.** 11th International Congress on Autoimmunitiy, Lisbon 2018.

3. **Kardos Z**, Oláh C, Sepsi M, Sas A, Kostyál L, Bhattoa HP, Hodosi K, Kerekes G, Tamási L, Valikovics A, Bereczki D, Szekanecz Z. **Assessment of intracranial vessels and vascular lesions in rheumatoid arthritis. A detailed transcranial Doppler, carotis ultrasound and brain MRI study.** EWRR, Genf, Annals of the Rheumatic Diseases 2018;77:A53-A54.

4. **Kardos Z**, Oláh C, Sas A, Kostyál L, Sepsi M, Tamási L, Szekanecz Z. **Rheumatoid arthritises betegek komplex vascularis állapotfelmérése.** MRE, Siófok. Magyar Reumatol 162, 2018.

5. Szamosi S, **Kardos Z**, Posta E, Nagy K. **A felnőttkori Still-betegség – regiszter indítása az északkelet-magyarországi régióban.** MRE, Siófok. Magyar Reumatol 176, 2018.

6. **Kardos Z**, Oláh C, Sepsi M, Sas A, Kostyál L, Bhattoa HP, Hodosi K, Kerekes G, Tamási L, Valikovics A, Bereczki D, Szekanecz Z. **Assessment of intracranial vessels and vascular lesions in rheumatoid arthritis. A detailed transcranial Doppler, carotid ultrasound and brain MRI study**. EULAR Madrid, *Annals of the Rheumatic Diseases* 2017;76:1027.

7. **Kardos Z,** Oláh C, Tamási L, Kostyál L, Sepsi M, Sas A, Bóta T, Bhattoa HP, Hodosi K, Román K, Szekanecz Z. **Nem látunk be rheumatoid arthritises betegeink fejébe.** MRE, Gyula. Magyar Reumatol 167, 2017.

8. Oláh C, **Kardos Z**, Sas A, Sepsi M, Valikovics A, Hodosi K, Tamási L, Szekanecz Z. **Rheumatoid arthritises betegek agyi keringésének vizsgálata transcraniális Dopplerultrahanggal.** MRE, Gyula. Magyar Reumatol 176, 2017.

9. **Kardos Z. A terápiás perzisztencia jelentősége reumatológiai betegek hosszú távú kezelésében**. MRE Ifjúsági Fórum, Vásárosnamény 2017.

10. Tamási L, Szabó T, **Kardos Z**, Fazekas K, Flórián Á, Lukács K, Spitzmüller N. **Dyslipidemia rheumatoid arthritisben.** MRE, Budapest. Magyar Reumatol 147, 2012.

11. Fazekas K, Lukács K, Flórián Á, Spitzmüller N, **Kardos Z**, Tamási L. **Biológiai terápiával kezelt spondylarthritis ankylopoeticás betegeinknél nyert tapasztalatok 5 év távlatában.** MRE, Eger. Magyar Reumatol 160, 2011.

12. **Kardos Z**, Szabó Z, Tamási L. **Spondylitis ankylopoeticában szenvedő nőbeteg TNF-α gátló kezelés alatt jelentkező terhessége.** MRE, Eger. Magyar Reumatol 161, 2011.

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List of publications related to the dissertation

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Clin. Rheumatol. 37 (5), 1183-1188, 2018.
DOI: http://dx.doi.org/10.1007/s10067-018-4003-8
IF: 2.141 (2017)

2. Oláh, C., **Kardos, Z.**, Sepsi, M., Sas, A., Kostyál, L., Bhattoa, H. P., Hódosi, K., Kerekes, G., Tamási, L., Valikovics, A., Bereczki, D., Szekanecz, Z.: Assessment of intracranial vessels in association with carotid atherosclerosis and brain vascular lesions in rheumatoid arthritis.
Arthritis Res. Ther. 19 (1), 213, 2017.
DOI: http://dx.doi.org/10.1186/s13075-017-1422-x
IF: 4.269

3. Szekanecz, Z., Kerekes, G., Végh, E., **Kardos, Z.**, Baráth, Z., Tamási, L., Shoenfeld, Y.: Autoimmune atherosclerosis in 3D: how it develops, how to diagnose and what to do.
Autoimmun. Rev. 15 (7), 756-769, 2016.
DOI: http://dx.doi.org/10.1016/j.autrev.2016.03.014
IF: 8.961

4. Szekanecz, Z., Kerekes, G., **Kardos, Z.**, Baráth, Z., Tamási, L.: Mechanisms of Inflammatory Atherosclerosis in Rheumatoid Arthritis.
Curr. Immunol. Rev. 11, 1-12, 2015.

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5. Kerekes, G., Nurmohamed, M. T., Gonzalez-Gay, M. A., Seres, I., Paragh, G., **Kardos, Z.**, Baráth, Z., Tamási, L., Soltész, P., Szekanecz, Z.: Rheumatoid arthritis and metabolic syndrome. *Nat. Rev. Rheumatol. 10* (11), 691-696, 2014.
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