Normalised time-to-peak-distribution curves correlate with cerebral white matter hyperintensities – Could this improve early diagnosis?

Christian Nasel1,2,3, Roland Boubela2,3, Klaudius Kalcher2,3 and Ewald Moser2,3

Abstract
Parameter-free assessment of the time-to-peak (TTP) histogram, termed ‘TTP-distribution curve’ (TDC), of dynamic susceptibility contrast-enhanced magnetic resonance imaging (DSC-MRI) was introduced as a robust method to evaluate cerebral perfusion. TDC-assessment works fully automatically without the need of an arterial input function, thereby providing full comparability between different measurements. In the investigated sample of 106 patients, a strong dependency of TDC on the hemodynamic state of cerebral microvessels and the arterio-venous bolus-transit time \( T_{av} \) was demonstrated. Accordingly, TDC-derived \( T_{av} \) was 3.3–3.7 s for control patients and 4.4 s for cerebral small vessel disease patients. Measurements of associated bolus spread velocities \( v \) and accelerations \( a \) additionally revealed a direct effect from spin–spin relaxation time \( T_2 \)-weighted white matter hyperintensity volume, considered to indicate microangiopathy in cerebral small vessel disease, on the TDC-measurements. This strongly supports the prevailing hypothesis that cerebral small vessel disease directly influences DSC-measurements, where the degree could be estimated from an analysis of TDC. While this may be used to correct DSC-parameters for undesirable effects from cerebral small vessel disease, it could also serve to potentially identify patients at risk for cerebral small vessel disease at an early stage, since a subset of patients without yet significant WHM-volume, but clearly altered hemodynamics in TDC-measurements, was identified in this study.

Keywords
Brain perfusion, cerebral small vessel disease, leukoaraiosis, perfusion imaging, perfusion magnetic resonance imaging

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Introduction
Recently, the analysis of the time-to-peak (TTP) histogram of dynamic susceptibility contrast-enhanced magnetic resonance imaging (DSC-MRI), termed as ‘TTP-distribution curve’ (TDC), was reported to detect hemodynamically meaningful variances of the individual cerebral bolus transit time.1 Most of the routinely used DSC-MRI-derived hemodynamic parameters, e.g. mean transit time, TTP, relative TTP, standardised TTP and \( T_{max} \), depend on the cerebral bolus transit time, which is a quantity of the mean arterio-venous transit time.2,3 In comparison to these parameters, it seems noteworthy that for TDC-based evaluations, no specific assumptions have been used except few basic physiological facts, i.e. that the blood is entering the brain from the arterial side, passing the parenchyma, and leaving via the venous branches. Investigations of the TDC revealed considerable variations of the individual cerebral bolus transit time even in control patients, which are not yet fully understood.
understood. According to a previous report, most of the encountered variability of the cerebral hemodynamics seems to relate to alterations in cerebral microvessels, namely arterioles, capillaries and venules.\textsuperscript{1} As TDC appeared quite sensitive to hemodynamic alterations triggered within these vessel segments, this could lead to a novel approach to investigate the state of cerebral small vessels on the one hand, and to correct DSC-derived perfusion parameters for degenerative vascular adaptations of the arteriovenous transit time on the other hand. Therefore, we specifically investigated the relation between TDC-derived parameters (i.e. bolus transit time $T$, bolus spread velocity $v$ and bolus spread acceleration $a$) and the extent of correlated T$_2$-weighted white matter hyperintensities (WMH) primarily attributable to cerebral small vessel disease (cSVD).\textsuperscript{4}

Materials and methods

Patients

A random sample of 106 patients with DSC-MRI examinations of sufficient image quality showing various degrees of WMHs was drawn from our institutional database ($n=\text{app. 6862}$ DSC-MRI examinations).\textsuperscript{5} Only patients with various degrees of leukoaraiosis and/or lacunar infarcts < 20 mm were eligible, while subjects presenting with circumscribed acute or chronic lesions, such as tumor, hemorrhage, thromboembolic infarcts (non-subcortical, > 20 mm), vascular malformations, inflammations, etc. were excluded. Although within the spectrum of cSVD, patients depicting signs of small lobar hemorrhages were also excluded from further analysis in order to reduce the overlap with cerebral amyloid angiopathy. Subjects suspected to suffer rare hereditary forms of cSVD (e.g. CADASIL, COL4A1-mutations) as well as patients with known chronic heart failure according to New York Heart Association (NYHA) class IV were also not included in the study. Written informed consent was obtained from all patients. The study was approved by the Lower Austrian Ethics Commission (GS4-EK-4/20-2013). All patients and data were handled following the WMA-recommendations of ethical principles for medical research involving human subjects.\textsuperscript{6}

Magnetic resonance imaging and assessment environment

All examinations were performed on a clinical 1.5T MR-scanner (Avanto, Siemens Medical Systems, Erlangen, Germany) according to our standard perfusion-MRI protocol, which consisted of DSC-MRI, diffusion weighted MRI (dual-b SE-EPI: b1 = 0 s/mm\textsuperscript{2}, b2 = 1000 s/mm\textsuperscript{2}), MR-angiography and conventional MRI including enhanced proton density (PD)/T2w-IR-imaging.\textsuperscript{7} Specifically, DSC-MRI was performed using a short-TR dynamic contrast-enhanced T$_2$-weighted single shot GRE-EPI-sequence (TR = 689 ms/ TE = 17 ms/FOV = 240 mm/ slice thickness: 6 mm/ image matrix: 164 x 208 voxels) that allowed acquisition of 81 image-stacks, with 20 slices each, at a scan time of approx. 60 s. This MRI-sequence offers an acceptable trade-off between sufficient signal-to-noise and high temporal resolution, which in turn enables critical sampling of the first pass of the administered contrast bolus through the brain at a sampling rate of 1.45 Hz for an expected minimal arterio-venous contrast bolus-transit time of about 3.5 s for regular perfusion.\textsuperscript{8,9} During the perfusion sequence a bolus of 0.1 mmol/kg (body weight) of MR-contrast medium, either Gadoteridol or Gadoterate meglumine was injected into a cubital vein via a 20-gauge venous cannula. All injections were performed at a flow rate of 5 ml/s using an automatic contrast injector (Optistar\textsuperscript{TM} Elite mobile, Mallinckrodt), 10 s after the perfusion sequence was started. Additionally, 20 ml saline (flow: 3 ml/s) was injected immediately after the contrast bolus, in order to reach a sufficient venous wash out at the injection site.

Assessment of T$_2$-weighted white matter hyperintensities

WMH-volumes were assessed using in-house developed R-scripts together with SPM12 segmentation routines. The first echo images of the enhanced PD/T$_{2\text{w}}$-IR-sequence served to obtain masks for grey and white matter, which defined all voxels considered to belong to brain parenchyma. An automatically generated contrast threshold defined as: median $+2 \times$ MAD (median absolute deviation) was derived from this parenchyma-voxel fraction using the signal intensities from the second echo images of the enhanced PD/T$_{2\text{w}}$-IR-sequence. The latter images were also used to segment the WMHs. The second echo of this sequence is equivalent to extended fluid attenuated inversion recovery imaging. From this segmentation, an estimate of the absolute WMH volume was computed and subsequently normalised to the sample mean total brain volume (TBV) to avoid bias from differing absolute brain volumes. Attempting to separate presumably ‘age-related’ micro-vascular degeneration from advanced cSVD, the small variance of the rather low ‘age-related’ degeneration via the size of the nWMH-volumes alone was impossible in these patients.
Compared to a widely accepted visual rating scale often used in large cSVD trials, these patients resembled grade 1 at maximum of the visual scale, where a grade 1 in visual scoring is given for minimal white matter hyperintensities only. Therefore, their nWMH-findings were considered as the most acceptable base line for the whole sample, which then defined group 0 (young control patients: \( n = 18, \text{age} < 53 \) years) in our study. According to investigations of WMHs attributable to cSVD and their correlation to cerebral vessel density, an exponential instead of a linear regression model was applied to extrapolate this base-line to patients aged \( \geq 53 \) years. To this end, linear regression was applied to the logarithm of the data, and regression line subsequently transformed back into the original scale. Finally, the variance of the base line was considered by adding the MAD estimated from group 0 to the exponentially increasing regression line, which allowed definition of an age-dependent threshold to additionally separate groups 1 (elderly control patients: \( n = 38, \text{age} \geq 53 \) years, nWMH-volume \( \leq \) threshold) and 2 (cSVD-patients: \( n = 50, \text{age} \geq 53 \) years, nWMH-volume \( > \) threshold) (Figure 1), both covering the same age range. Consistent with our data driven analysis, groups 0 and 1 were comparable to Fazekas grades 1 and 2 (= controls) and group 2 corresponded to Fazekas grade 3 (= cSVD patients).

**Assessment of cerebral hemodynamics**

Image data were anonymised and reviewed on a PC workstation using freely available and in-house developed software packages (MRicore). Perfusion images were coregistered to morphological images of the enhanced PD/T2w-IR-sequence using SPM12 (Statistical Parameter Mapping 12, UCL-Wellcome Trust Centre for Neuroimaging). The time-to-peak histogram or TDC, respectively, of each DSC-examination was provided by full-automatic calculation of standardised time-to-peak (stdTTP) parameter maps using in-house developed software (jPerfusion Module, version 2.0.0; available from the corresponding author). R-implemented scripts (R – statistical package) automatically detected the TDC-main peak.
Comparisons of different examinations were possible, as each \( z_f \)-value represents a unique point in the fitted model distribution \( TDC_f \) and thus indicates the same quantile in every DSC-examination. On the other hand, measurements of individually different hemodynamic aspects of a given DSC-examination still could be performed via the correlated absolute individual \( ttp \)-values.

The TDC-main peak originates in the cascaded filling of the cerebral vessels during the first pass of the administered contrast bolus and is described by \( TDC_f \). By separation of certain \( z_f \)-intervals from \( TDC_f \) assumed to represent different vessel segments, the course of the hemodynamic characteristics of the bolus transit was investigated. According to a former study, the complete bolus transit includes all voxels defined in the interval

\[
I_{AV} = \{\text{vxl}| -3 \leq z_f(ttp_{vxl}) \leq +3\}
\]

(1)

where vxl indicates the respective voxel with the absolute peak enhancement time \( ttp_{vxl} \) that is correlated to the \( TDC_f \)-quantile \( z_f \). The inflection points \( (IP) \) of \( TDC_f \) were considered to mark the transitions from large to small arterial \( (I_{ar}) \) and from small to large venous vessels \( (I_{v}) \), \( I_{ar} \) and \( I_{v} \) were consequently introduced as limits for the intervals

\[
I_{ar} = \{\text{vxl}| I_{ar} \leq z_f(ttp_{vxl}) \leq \max TDC_f \}
\]

and

\[
I_{v} = \{\text{vxl}| I_{v} \leq z_f(ttp_{vxl}) \leq +3\}
\]

(2)

which include all voxels with a suitable \( ttp_{vxl} \) during the supposed arterial macro-vessel wash-in and the venous macro-vessel wash-out phase, respectively. The IPs were determined in \( TDC_f \), the first derivative of \( TDC_f \), by numeric differentiation. Finally, the maximum of \( TDC_f \), \( \max TDC_f \) needed to mark the transition from arterioles to venules in the parenchyma, was used to additionally differentiate an arterial and a venous micro-vessel wash-in and wash-out phase

\[
I_{art} = \{\text{vxl}| I_{art} \leq z_f(ttp_{vxl}) \leq \max TDC_f \}
\]

and

\[
I_{ven} = \{\text{vxl}| \max TDC_f < z_f(ttp_{vxl}) < I_{art}\}
\]

(3)

Note that \( \max TDC_f \) is also the inflection point of the antiderivate of \( TDC_f \) which represents the perfused total brain volume pTBV (unit: cm\(^3\)) over time during the first pass of the bolus (Figure 2). The sum of pTBV and the volume of flagged unperfused voxels lying inside the brain parenchyma, provided automatically by the stdTPP-calculation, was taken as TBV. Additionally, an analysis of the whole arterio-venous transit interval \( I_{av} \), with \( I_{av} = I_{art} + I_{ven} \), was performed. The duration \( T_f \) of each interval \( I = \{I_{ar}, I_{av}, I_{art}, I_{ven}, I_{v}\} \) was then calculated as

\[
T_f = ttp(z_f = u_i) - ttp(z_f = l_i)
\]

(4)

where \( ttp \) is the absolute peak enhancement time at the time-point defined by the \( TDC_f \)-quantile \( z_f \), which corresponds either to the upper \( (u) \) or the lower \( (l) \) limit of the respective interval definition.

\( TDC_f \) showing the chronological change of pTBV per measured time step, where time step means the sequence repetition time \( TR \) was considered to model the velocity of the bolus spread or, simply, bolus spread velocity in the brain (unit: cm\(^3\)/s). Consequently, mean \( \overline{v}_I \) in each interval \( I \) was calculated as

\[
\overline{v}_I = T_I^{-1} \sum_{I_{v}} V_i \text{ with } V_i = |\{\text{vxl}| z_f(ttp_{vxl}) = i\}| \cdot V_{vxl}
\]

(5)

where \( V_{vxl} \) is the voxel-volume defined by the DSC-examination parameters, \( u \) and \( l \) are the upper and lower \( TDC_f \)-quantile limits and \( T_I \) is the duration of the respective interval \( I \). Furthermore, as the first derivative of \( TDC_f \) displays the change of \( \overline{v}_I \) over time, \( TDC_f \) was considered to give the bolus spread acceleration \( \overline{a}_I \) for each interval \( I \) was calculated as

\[
\overline{a}_I = T_I^{-1} \sum_{I_{v}} \frac{\Delta V_i}{TR}
\]

(6)

where \( T_I \) and \( V_i \) are the same as in formulation 5, TR is the sequence repetition time, and \( \Delta V_i \) is the change of \( V_i \) per TR. Note that \( \overline{a}_I \) typically shows negative values in the venous phases. The various interval durations as well as \( \overline{v}_I \) and \( \overline{a}_I \) for each interval \( I \) were calculated and compared between the three groups.

**Statistical analysis**

Quality of model fit was estimated by calculating \( R^2 \) and the Spearman rank correlation coefficient \( \rho \) between TDC and \( TDC_f \). Note that \( R^2 \) is not simply \( \rho^2 \), but was calculated as

\[
R^2 = 1 - \left( \sum_{z_f=3}^{+3} \left( TDC_{z_f} - TDC_f \right)^2 \right) - \left( \sum_{z_f=3}^{+3} \left( TDC_{z_f} - TDC_f \right)^2 \right)^{-1}
\]
where TDC refers to the TTP-histogram, TDC\textsubscript{f} to the fitted double Gaussian model, and \( z_f \) to the TDC\textsubscript{f}-quantiles.

Validity of nWMH-grouping and TCD-interval definition was visualised by creating cumulative maps showing the extent of nWMHs of the three groups and the location of the voxels included in the various TDC-intervals according to their \( z_f \)-value. Absolute measures of nWMHs and \( z_f \)-interval differences were further assessed statistically by group-level comparisons testing the whole microvascular arterio-venous transit, the micro-vessel arterial wash-in and the venous wash-out phase intervals \( I_{aw} \), \( I_{art} \) and \( I_{ven} \), since only these intervals represented the parenchymal microvessels targeted in the study. Group-level comparisons were performed using Kruskal–Wallis (K–W) tests with Dunnett’s Modified Tukey–Kramer Pairwise Multiple Comparison Test (DTK) as post hoc analysis to take potentially significant deviations from the normal distribution and inhomogeneity of variances, as well as the different size of the groups, into account.

Correction for multiple comparisons was performed conservatively using the Bonferroni-method. Step-wise regression analysis was performed to explain dependencies of nWMH-volume using least trimmed squares and MM-estimation to treat outliers in the explanatory and responsive variables. Descriptive statistics are given as mean, SD or/and confidence intervals. Otherwise, if applicable, data are quoted as median, MAD or/and 95% percentiles. All TDC calculations and statistical evaluations were performed in R (version 3.2.0) invoking packages FlexMix, robustbase, vcd and DTK.\textsuperscript{15–19}

Results

A summary of the demographic data of the three groups studied is given in Table 1. TBV was not significantly different between the three groups, and concerning age groups 1 and 2 were not significantly different as well (K-W test: TBV, age; \( p > 0.05 \)). Also, testing \( T_l \), \( V_l \), and \( \bar{a}_l \) for gender-related effects within and between
groups (including cross-over testing female vs. male) in the various intervals was not significant despite the obvious predominance of male patients in group 2 (K–W test: $T_i$, $V_i$, $A_i$) for $I = \{I_A, I_{av}, I_{art}, I_{ven}, I_V\}$ by gender; $p > 0.05$; post hoc: multiple t-tests; uncorrected). The odds to suffer from a certain risk factor were tested for groups 1 and 2, which were comparable concerning age. Here, only a significantly higher odds to suffer from hypertension was found in group 2 (odds-ratio test; group 1: $[25+, 13+]$ vs group 2: $[45+, 3+]$; $p = 0.0028$).

The group-classification concerning the extent of WMHs was reviewed using cumulative maps, where, expectedly, group 0 and group 1 were visually comparable, while both groups exhibited clearly smaller WMH-volumes than group 3 (Figure 3). Nevertheless, in the statistical analysis, a significant difference between all three groups was obtained.

**Table 1.** Demographic data.

|                      | Group 0 ($n = 18$) | Group 1 ($n = 38$) | Group 2 ($n = 50$) |
|----------------------|-------------------|-------------------|-------------------|
| Age (years)          | 42.3 ± 8.5$^*$    | 68.4 ± 9.0        | 75.7 ± 9.6        |
| Male/female$^\dagger$| 9/9               | 19/19             | 33/17             |
| Total brain volume (cm$^3$) | 1400.2 ± 104.6     | 1408.9 ± 101.6   | 1398.1 ± 138.6   |
| Normalised WMH-volume (cm$^3$) | 10.4 ± 2.3         | 17.1 ± 5.5$^*$   | 52.1 ± 17.7$^*$  |
| Hypertension         | 50%               | 65%               | 88%$^\dagger$    |
| Diabetes             | 22%               | 32%               | 38%              |
| Hyperlipidemia       | 50%               | 42%               | 58%              |
| Smoking              | 17%               | 13%               | 18%              |

Note: Demographic data of group 0 (young controls), group 1 (elderly controls) and group 2 (cSVD-patients).

$^*$Significantly different at $p < 0.01$.

$^\dagger$Significantly higher odds for risk factor at $p < 0.003$.

$^\ddagger$No significant effect from gender within and between groups.

**Figure 3.** Normalised cumulative maps showing the extent of nWMHs (depicted as black areas) in the three different groups. The nWMH-volume was significantly different between all three groups. Nevertheless visually, group 0 (top row; young controls, $n = 18$) was comparable to group 1 (middle row; elderly controls; $n = 38$), while in group 2 (bottom row; elderly cSVD-patients; $n = 50$) the cerebral white matter was severely affected by multiple confluent WMHs. Note that column-numbers given in the top row mark the same slices as in Figure 4.
(K–W test: nWMH-volume; \( p < 0.05 \); post hoc: DTK; correction: Bonferroni). In the same way, validity of vessel segment classification based on TDC\(_f\)-quantiles \( z_f = \{z(\text{IP}_a), z(\text{max}_\text{TDC}), z(\text{IP}_v)\} \) was verified by cumulative maps showing the location of all voxels belonging to the respective intervals \( I_a, I_{art}, I_{ven}, \) and \( I_v \), where the interval-definitions for \( I_{art} \) and \( I_{ven} \) effectively included the parenchymal microvessel segments (Figure 4). The quality of the model fit was excellent with \( \rho = 0.992 \) (Spearman; 95% interval: 0.965–0.997) and \( R^2 = 0.985 \) (95% interval: 0.882–0.996).

The evaluation of the bolus transit duration \( T_f \) in the intervals \( I_{art}, I_{art}, \) and \( I_{ven} \) showed a significant prolongation of \( T_{ven} \) for the whole transit period \( I_v \) as well as for the arterial microvessel wash-in phase \( I_{art} \) when comparing group 2 to the control-groups 0 and 1, while group 0 was not different to group 1 (K–W test: \( T_{art}, T_{art}, p < 0.05 \); post hoc: DTK; correction: Bonferroni). In the venous microvessel wash-out phase, a significant difference was detected only between group 0 and group 2 (K–W test: \( T_{ven}, p < 0.05 \); post hoc: DTK; correction: Bonferroni). The range of all durations in the control groups measured for the whole interval \( I_{art} \), which represents the global parenchymal arterio-venous bolus transit time, was \( 3.7 \pm 1.1 \) s (median ± MAD; range: 2.2–8.9 s). In the cSVD-group 2, this interval duration was \( 4.4 \pm 1.1 \) s (median ± MAD; range: 3.0–7.4 s). The transit duration \( T_A \) representing the wash-in phase of cerebral macrovessels was not significantly different between the various groups (K–W test: \( T_A; p > 0.05 \); post hoc: DTK; correction: Bonferroni).

For \( \bar{v}_A \) and \( \bar{a}_A \), significant differences between both control groups, 0 and 1, and group 2 were found for all intervals \( I_{art}, I_{art}, \) and \( I_{ven} \). Yet, in the specified intervals, no significant differences were found between the two control groups (K–W test: \( \bar{v}_A=I_{art}, I_{art}, I_{ven}, I_{ven}; p < 0.001; \) post hoc: DTK; correction: Bonferroni). A full summary of all group comparisons is given in Table 2.

A scatter analysis of \( \bar{v}_A \) in relation to \( T_{ar} \) using robust regression analysis suggests an exponential correlation between both variables. The regression lines of the control groups 0 and 1 show a broad overlap with cSVD-group 2 at the point \((282.4 \text{cm}^3/\text{tissue/s}, 3.4 \text{ s})\). Remarkably, below this intersection point with decreasing \( \bar{v}_A \), the duration \( T_{ar} \) increased clearly faster in group 2 than in both control groups, i.e. 0 and 1. Investigating the scatter and correlation of \( \bar{v}_A \), directly depicted by TDC, and nWMH volume also revealed an indirect exponential correlation between both variables in robust regression analysis. Median and MAD in group 2 were \( 212.2 \pm 48.7 \text{cm}^3/\text{tissue/s} \) for \( \bar{v}_A \) and \( 48.3 \pm 14.7 \text{cm}^3 \) for nWMH-volume, which enables to estimate a \( \bar{v}_A \)-range, where \( \bar{v}_A \)-values in the control-groups widely matched those of the cSVD-group. However, in this range in controls, at least in our sample, WMHs were not yet evident in T2-weighted MRI (Figure 5).

Finally, we performed robust regression analysis explaining the extent of nWMH-volume by \( \bar{v}_A \) with a step-wise inclusion of other relevant confounders of nWMH. Improvement of the model by each inclusion-step was tested using analysis of variance, where no significant improvement or effect was found.
Table 2. Results from TDC analysis.

Both groups 0 \((n = 18)\) and 1 \((n = 38)\) different from group 2 \((n = 50)\):
\(\ast p < 0.05; \ast\ast p < 0.01\)

| Group | Interval \(I_{\text{av}}\) \((I_{\text{art}} + I_{\text{ven}})\) | Interval \(I_{\text{art}}\) | Interval \(I_{\text{ven}}\) |
|-------|--------------------------------------------------|-----------------------------|-----------------------------|
| Group 0 controls young | 284.3 ± 52.6 | 302.3 ± 59.0 | 266.4 ± 52.9 |
| Group 1 controls elderly | 257.6 ± 53.3 | 267.8 ± 58.2 | 247.3 ± 51.7 |
| Group 2 cSVD elderly _ | 211.9 ± 44.0** | 215.2 ± 51.0*** | 208.6 ± 44.4*** |

**Bolus spread velocity \(v [\text{cm}^3\text{issue}/\text{s^2/s}]\)**

| Group | Interval \(I_{\text{art}}\) | Interval \(I_{\text{ven}}\) |
|-------|-----------------------------|-----------------------------|
| Group 0 controls young | 85.3 ± 25.5 | 105.6 ± 39.9 | 65.0 ± 26.7 |
| Group 1 controls elderly | 67.2 ± 32.7 | 82.6 ± 43.6 | 51.9 ± 26.9 |
| Group 2 cSVD elderly _ | 44.0 ± 24.3*** | 55.7 ± 30.2*** | 30.3 ± 27.3*** |

**Bolus spread acceleration \(\alpha [\text{cm}^3\text{issue}/\text{s^2}]\)**

| Group | Interval | Interval | Interval |
|-------|----------|----------|----------|
| Group 0 controls young | 3.3 ± 0.6 | 1.6 ± 0.4 | 1.6 ± 0.3 |
| Group 1 controls elderly | 3.7 ± 1.1 | 1.9 ± 0.6 | 1.8 ± 0.6 _ |
| Group 2 cSVD elderly _ | 4.4 ± 1.1 | 2.2 ± 0.6* | 2.1 ± 0.6* |

**Bolus transit time \(T_I [\text{s}]\)**

Note: \(T, v\) and \(\alpha\) derived from DSC measurements during the intervals \(I_{\text{av}}, I_{\text{art}}\) and \(I_{\text{ven}}\) \((\ast p < 0.05; \ast\ast p < 0.01)\). All analyses were corrected for different group sizes, heteroscedasticity and multiple comparisons. Note that \(\alpha_{\text{ven}}\) in \(I_{\text{ven}}\) (wash-out phase) is negative, however, the absolute values are given.

Figure 5. Bolus spread velocity \(v_{\text{av}}\) and normalised white matter hyperintensity volume \(n\text{WMH-V}\) showed an indirect exponential correlation (dashed grey regression line). The scattering of both variables in group 2 (red dots), when described by robust statistics (median [horizontal green and vertical red solid lines] ± MAD [horizontal green and vertical red dashed lines]), suggests a certain \(v_{\text{av}}\)-range where below cSVD is most likely but WMHs may not yet have developed (red shaded area). Note that 41% of the control patients (group 0: 6 cases, 33.3%; group 1: 17 cases, 44.7%) were within this range. Blue and green dots mark cases of young and elderly controls, represented in groups 0 and 1, respectively.
for: gender, diabetes, hyperlipidemia and smoking (ANOVA, \( p > 0.05 \) n.s.). The base model (nWMH by \( \tilde{v}_{av} \)) showed an adjusted-\( R^2 \) of 0.2696 (\( p < 0.001 \)). The strongest confounding effect was obtained for age (nWMH by \( \tilde{v}_{av} + \text{age} \); adjusted-\( R^2 = 0.5766; p < 0.001 \)), while hypertension exhibited a non-significant, weak effect on the model (nWMH by \( \tilde{v}_{av} + \text{age} + \text{hypertension} \); adjusted-\( R^2 = 0.5808; p = 0.1168 \) n.s.).

**Discussion**

Investigating TTP-histograms, dubbed TDC, derived from established DSC-MRI, we found striking evidence that a substantial part of the hemodynamic alterations detected by the TDC may directly arise from modifications in the cerebral microvascular bed. The evaluation of the bolus transit dynamics in cerebral parenchyma revealed an exponential decrease of the TDC-derived mean bolus spread velocity \( \tilde{v}_{av} \) in cerebral parenchyma, which was normalised volume of cerebral WMHs, primarily attributable to cSVD, increased. The concomitant delay in the filling of the various vessel segments, documented as a significant reduction of the bolus volume acceleration \( \tilde{a}_{j} \) during the parenchymal wash-in phase in patients with clear signs of cSVD, also suggests a microvascular modification that seems to restrict the general bolus spread in the brain over time. This finding matches recently discussed theories about cSVD, where diffuse endothelial failure in cerebral microvessels was postulated as an early stage of the disease. Not until microvascular degeneration causes critical ischemia by luminal narrowing or occlusion of severely affected vessels, however, focal infarctions and, consecutively, WMHs occur." Immediate effects from cSVD would therefore stay undetectable in conventional MRI pending an ischemic event triggered by the postulated endothelial failure. In this context, an animal experiment in rats prone to microangiopathy revealed that histologically proven microvessel wall alterations led to accumulations of erythrocytes with stasis of blood flow in capillaries in the early stage of disease, which were not detectable from conventional high-resolution 3T MRI." Though this remains to be proven in humans, at this stage, when temporary stasis of capillary flow is the major impairment caused by cSVD, the only alteration measurable in MRI would most probably be a prolongation of the arterio-venous transit time. Actually, this was found in TDC-measurements, which also complies with the known reduction of regional cerebral blood flow not only in WMHs, but also in unaffected white matter in patients with cSVD documented by DSC-MRI and pulsed arterial spin labelling measurements."

Prior to this, another study demonstrated histologically the reduction of the cerebral vessel density not only in WMHs but also in normal appearing white matter in cSVD-patients." Consequently, endothelial degeneration, eventually followed by loss of capillaries, may be understood as a chronic process and the vascular impairment itself seems to take place long before WMHs become detectable via MRI. This conforms to our finding that \( \tilde{v}_{av} \) values overlap between all groups in the range below app. 261–282 cm\(^3\)/s, where about 41% of controls exhibited nearly no WMHs and patients in group 2 show clearly visible WMHs (Figure 5). Thus, considering cases with a normal appearing white matter and a low \( \tilde{v}_{av} \) to be at risk for cSVD, estimation of \( \tilde{v}_{av} \) via the TDC could open a novel approach to investigate cSVD clearly before WMHs become manifest at a stage where microvascular degeneration is less progressed and probably more susceptible to medical treatment." Of course, this has to be investigated and proven in future prospective studies. On the other hand, the TDC-findings are in full agreement with earlier reports also using DSC-MRI on this topic, where a reduction of regional cerebral blood flow in normal appearing white matter was described."

Moreover, comparisons between positron emission tomography and DSC-MRI using an AIF for perfusion assessment showed a considerable dependency of calculated parameter values on the location of the AIF." In a conceivable cascade of endothelial failure, perfusion impairment and loss of capillaries, quasi as a subsequent feature in the course of cSVD, arteriolar tortuosity and venous collagenosis were also reported to deteriorate microvascular flow and, therefore, to reinforce the effect from initial endothelial failure. Despite the fact that the exact relationship between these findings and cSVD is discussed controversially, a histologically visible loss of white matter cellular components was proven in all patients in the respective study, where the authors themselves stated that: "... Only the very largest of these lesions might be detected on MRI scans. ..." This underlines our proposal to rather focus TDC- evaluations on patients of groups 0 and 1 who present with a drop of \( \tilde{v}_{av} \) and a prolongation of \( T_{av} \). In this setting, persons potentially suffering from cSVD would most probably remain undetected in any investigation relying on visual WMH-scoring only, but could be detected assessing the TDC.

Importantly, our results suggest that microangiopathic flow impairment is sensitively reflected by the increase in \( T_{av} \) and a decrease of \( \tilde{v}_{av} \), since both parameters would respond this way to a reduction of the active microvascular cross-section that is available for tissue perfusion. This also perfectly agrees with...
measurements comparable to the TDC-interval \( I_{av} \) performed using contrast-enhanced transcranial Doppler sonography, where the means for the transit times in patients with cSVD where reported ranging from 4.4 s to 6.7 s, and for controls or patients with neurodegenerative disease, a regular range of 1.3–3.7 s was measured.\(^{27,28}\) Similarly, TDC-derived transit times were 3.3–3.7 s for controls and 4.4 s for cSVD-patients in our study, limited of course by the lower temporal resolution of MRI. Therefore, the generic bolus passage interval classification based on \( TDC_f \)-quantiles \( z_f \) proves to be valid, which is also supported by the excellent correlations between the raw TDC and the fitted model \( TDC_f \). Hence, \( T_{av} \) directly calculated from \( TDC_f \) is a reliable measure of the arterio-venous transit time. Because all DSC-derived hemodynamic perfusion time parameters were shown to directly depend on the arterio-venous transit time, \( T_{av} \), that is directly available from the respective DSC-MRI examination, it could be useful to either correct the parameter or the associated critical perfusion-threshold for individually different degenerative microvascular adaptions in order to obtain more robust predictions of the infarct core in acute stroke.\(^1-3\) Given that our results strongly suggest microvascular flow impairment as the major source for variations of \( T_{av} \), the consideration to adapt measurements to an intrinsic threshold rather than to a global one gets even more important as the possibility was stressed that capillary flow itself may generally be rather heterogeneous.\(^{29}\) Moreover, besides gender, severe WMHs attributable to cSVD were also realised as an independent predictor for infarct size in acute ischemic stroke, where additionally a controversially discussed coincidence between a prolonged filling of collaterals, also important in acute stroke, and a larger extent of WMHs was reported.\(^{30-32}\) In any case, further prospective studies on this topic will be needed.

However, we also acknowledge certain limitations. Vascular risk factors and certain properties of those, such as duration and severity, could not be assessed specifically and we neither aimed to measure their effects on cerebral hemodynamics nor did we intend to relate hemodynamic findings with clinical manifestations caused by cSVD. Our investigation focused rather on the grade of hemodynamic alterations potentially caused by modifications in the microvascular bed due to any reason for cSVD in order to expand diagnostic capabilities of DSC-MRI. Despite this, the frequencies of the various risk factors in the respective groups were widely comparable, except for hypertension, which was more frequently found in group 2. Anyway, including hypertension into the model did not improve the model significantly, while age did. This poses the question whether patients at higher age often share a longer duration of cerebrovascular disease, which in turn increases the possibility for a higher nWMH-volume. On the other hand, relatively low nWMH-volumes in older patients could be very well explained by a late onset of cSVD. Interestingly, the only severe outlier from the model was an 80-year-old woman who presented with multiple recently discovered risk factors and a low nWMH-volume, but showed a clear decrease of \( v_{av} \). However, since our study lacks longitudinal time information, further studies on this topic will be needed.

Additionally, a considerable influence from reduced cardiac output on the shape of the TDC was not assessed explicitly, since patients with severe cardiac failure were not included in the study and a previous thorough analysis of major aspects of the central circulatory bolus spread in the heart and lungs showed only a weak influence from a reduced left ventricular ejection fraction on the bolus shape.\(^33\)

Also, we used an unbiased fully automatic segmentation algorithm that was designed to yield classifications comparable to the visual scores proposed by the LADIS study.\(^{10,11}\) Yet, patients of group 0 were to some extent assigned visually from the distribution of the nWMH-volumes to define the base-line. While this did not completely exclude an inclusion-bias in group 0, where bias should be lowest, this was quite avoided in groups 1 and 2. Though this has to be validated further in larger samples, differentiation of cSVD from controls seems acceptable in this study, which could be demonstrated by the cumulative maps depicting the extent of WMHs of the different groups. The automatic classification assigned grade 1 to group 0, group 1 resembled very much grade 2, and group 2 fitted grade 3, according to visual scoring as proposed in the literature.\(^{10,11}\) Note also that extracting patients from a hospital database per se widely precludes inclusion of truly healthy persons. Thus, it has to be emphasised that our groups 0 and 1, used as controls, rather represent the lowest grade of cSVD detectable, which is a common limitation found in trials investigating cSVD. Nonetheless, direct comparisons of our results with those from other studies based on pure visual scoring should be done very carefully.

Furthermore, our study lacks an age-matched group of younger patients with severe WMH-manifestations, which is widely owed to the fact that hereditary vasopathies were excluded. Nevertheless, in a sonographic study, the hemodynamic impairment in younger patients suffering a hereditary vasopathy was comparable to group 3 in our sample.\(^{27,28}\) Thus, we do not expect a significant effect on our results.

Finally, gender is known to show an effect on cerebral perfusion.\(^34\) While groups 0 and 1 contained equal numbers of male and female patients, in group 2 predominantly male patients were represented. Multiple comparisons between male and female
subgroups within and between groups including cross-over testing (i.e. male vs. female) did not reveal any effect from gender differences in our sample. This could result from the fact that TDC evaluates the global cerebral bolus transit, which might turn out as more robust to this confounding effect than the voxel-wise evaluations usually performed. It is important to note that age, also yielding a strong effect on both transit time and nWMH-volume, was comparable between groups 1 and 2. This additionally underlines a strong effect from presumably microvascular alterations on the measured differences of cerebral hemodynamics between these groups.

In conclusion, our findings support the hypothesis of a direct relationship between the extent of normalised WMH-volume, attributable to underlying cSVD, and its presumably direct influence on the arterio-venous transit time measured by TDC derived from DSC-MRI. This is of great interest since, on the one hand, TDC could be used to support further adjustment of the DSC-MRI derived perfusion time parameters, which depend on the arterio-venous transit time. On the other hand, TDC could provide a novel approach to robust assessment of early cSVD. As a future prospect, our approach could provide a more accurate analysis of early stages of cSVD that might help to improve therapeutic treatment.

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Authors’ contributions
NC contributed to preparation of the paper, MR measurements and data evaluation; BRN and KK contributed to preparation of the paper and data evaluation; and ME contributed to preparation of the paper and final approval of the manuscript.

Statement on underlying research materials
All underlying research materials related to our paper can be accessed anonymously upon request. Otherwise, all relevant data are included in the manuscript.

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