Quantification of tissue sodium concentration in the ischemic stroke: A comparison between external and internal references for $^{23}$Na MRI

Anne Adlung$^{a, *}$, Christian Licht$^a$, Simon Reichert$^a$, Safa Özdemir$^a$, Sherif A. Mohamed$^{b,c}$, Melina Samartzi$^d$, Marc Fatar$^d$, Achim Gass$^d$, Eva Neumaier Prost$^b$, Lothar R. Schad$^a$

$^a$Computer Assisted Clinical Medicine, Medical Faculty Mannheim, Heidelberg University, Germany
$^b$Department of Neuroradiology, Medical Faculty Mannheim, Heidelberg University, Germany
$^c$Clinic for Diagnostic and Interventional Radiology, University Hospital Heidelberg, Germany
$^d$Department of Neurology, Medical Faculty Mannheim and Mannheim Center of Translational Neurosciences (MCTN), Heidelberg University, Germany

ARTICLE INFO

Keywords:
$^{23}$Na MRI
Sodium MRI
Tissue sodium concentration
Stroke
Quantitative MRI

1. Introduction

Sodium ions are involved in many metabolic processes and they are especially important for signal transmission throughout the brain and body. The sodium-potassium pump (Na$^+$/K$^+$-ATPase) maintains a consistent gradient between intra- and extracellular concentration of both ions with the extracellular sodium concentration (ESC) being around a ten-fold higher compared to the intracellular concentration (Somjen, 2002). The tissue sodium concentration (TSC) is the volume-weighted average of intra- and extracellular sodium concentration (Madelin and Regatte, 2013; Burstein and Springer, 2019a; Boada et al., 1994; Thulborn et al., 1999). Therefore, it is sensitive to any changes in cell vitality and viability or any processes affecting the intracellular sodium concentration or the extra- to intracellular volume fraction. For example, an increase in TSC might indicate an increase in extracellular volume fraction, thus being an early indicator for cell apoptosis (Poku et al., 2021; Bottomley, 2007).

$^{23}$Na magnetic resonance imaging (MRI) non-invasively measures the signal of the $^{23}$Na ion in the body, and density-weighted $^{23}$Na MR sequences allow for in-vivo TSC quantification (Thulborn et al., 1999; Thulborn, 2018; Burstein and Springer, 2019b; Konstandin and Schad, 2014; Hu et al., 2020a). There have been numerous studies that investigated the sodium concentrations or $^{23}$Na relaxation times with $^{23}$Na MRI. Studies were performed in healthy brain tissue as well as within various pathologies in the human brain (Madelin et al., 2014; Gilles et al., 2017; Ridley et al., 2018; Ouwerkerk et al., 2003). In the healthy brain tissue (gray - and white matter), absolute TSC values were found to be between 20 and 60 mM in the white matter (WM) and between 30 and 70 mM in the gray matter (GM) (Madelin and Regatte, 2013; Gilles et al., 2017; Ridley et al., 2018; Ouwerkerk et al., 2003). Varying quantification techniques are susceptible to TSC quantification biases, yielding the urgent need for a robust and established gold standard.

Currently, it is common to perform TSC quantification by using reference vials (phantoms) with a known sodium and agarose concentration (Madelin and Regatte, 2013; Hu et al., 2020b; Paschke et al., 2018). The signal amplitude of each voxel is linearly interpolated from the signal of multiple reference phantoms. This method is intrinsically prone to numerous different (human) errors, such as misplacement of...
the vials and potential movements of the patient during the measurement. Further potential risks include inaccuracies and uncertainties of the sodium and agar concentration within the reference vials (which depend on the precise fabrication) and potential partial volume effects (PVE). The stability of the concentration over time within the reference vials remains uncertain, yielding the need for regular renewal of the phantoms. Furthermore, \(^{23}\)Na phased array coils sometimes do not even allow for external quantification vials due to restricted space, which have already warranted internal references previously (Gerhalter et al., 2021; Wilferth, et al., 2022). Therefore, it is necessary to investigate alternative quantification techniques that use internal reference regions within the body - in proximity to the investigated tissue. Previously, the cerebrospinal fluid (CSF) and the vitreous humor (VH) have been suggested and used as quantification references (Gerhalter et al., 2021; Gao et al., 2017). Pre-depositions of, e.g., ischemic stroke (Lee and Packer, 1986; Wan et al., 2010) or various pathologies, and alterations have already been linked to the assumption of ESC and TSC within CSF being equal (Olsen and Rudolph, 1955; Harrington et al., 2010). The blood serum sodium concentration (SSC) is an indicator of the amount of sodium relative to the amount of water within the extracellular fluid (DiBartola, 2011), suggesting the SSC corresponds to the ESC. SSC might be influenced by different quantification methods is still missing.

1.1. Cerebrospinal fluid

The CSF surrounds brain tissue and the spinal cord. It fills the ventricles in the brain, which expand with age, consequently leading to an increased volume of CSF in older individuals (Matsumae et al., 1996; Zhang et al., 2005). The fluid does not contain any vital cells, justifying the assumption of ESC and TSC within CSF being equal (Olsen and Rudolph, 1955; Harrington et al., 2010). The blood serum sodium concentration (SSC) is an indicator of the amount of sodium relative to the amount of water within the extracellular fluid (DiBartola, 2011), suggesting the SSC corresponds to the ESC. SSC might be influenced by various pathologies, and alterations have already been linked to pre-depositions of, e.g., ischemic stroke (Lee and Packer, 1986; Wannameethee et al., 1994; Gao et al., 2017).

Results from previous \(^{23}\)Na MRI studies support this assumption as TSC within CSF was found to be around 140–145 mM (Madelin and Regatte, 2013; Ouwerverkerk et al., 2003; Niesporek et al., 2015; Inglese et al., 2010; Wilferth et al., 2022).

Relaxation times and sodium concentration within CSF differ from those of brain tissue, which is why it is well distinguishable on most MR images. Because of that, it was previously suggested as a reference for TSC quantification (Haneder et al., 2013). This manuscript focuses on further exploring that quantification method.

1.2. Vitreous humor

The vitreous humor (VH) is a gel-like material in the vitreous chamber. It is located in the space between the lens and retina. Its diameter is approximately 16 mm, yielding a volume of around 4 ml (Ruby et al., 2006; Siggers and Ether, 2012; Modarreszadeh and Abouali, 2014).

The VH - analog to the CSF - is assumed to be exclusively extracellular space (Smith et al., 2020), and is therefore referred to as trans-cellular fluid in the eyeball (Sampaolesi et al., 2014; Maeda et al., 2016). The VH not containing any vital cells justifies the assumption of its sodium concentration being equivalent to the ESC. This is supported by findings of 145–148 mM \(^{23}\)Na in the VH (with a mean of 146.7 ± 3.3 mM) by Kokavec et al. (2016) and Pigaiani et al. (2020). The natural regularization of electrolytes in the human body maintains a constant sodium concentration in the extracellular space (Somjen, 2002; Woll, 2013), which is thus also assumed for the VH. Wenz et al. explained that because of the VH’s gel-like consistency, similar \(^{23}\)Na relaxation times compared to CSF can be assumed with T1 and T2 ranging between 50 and 55 ms (Madelin and Regatte, 2013; Wenz et al., 2018).

1.3. Stroke

An ischemic stroke is defined by a restricted blood supply in areas of the brain. This results in an insufficient supply of oxygen and glucose, which may cause brain cell apoptosis. Diagnosis of the ischemic stroke is commonly performed with CT or \(^1\)H MRI. Studies exploring additional \(^{23}\)Na MRI have suggested improved penumbra (perfused brain tissue with the capacity to recover) detection. Some publications have even suggested correlations between TSC and the stroke onset, which would be of high interest for the treatment decisions for patients with a wake-up stroke (Thulborn et al., 2005; Hussain et al., 2009; Shimizu et al., 1993; Lin et al., 2001; Kim et al., 2014; Neumaier-Probst et al., 2015; Wetterling et al., 2015). Thus, accurate TSC quantification is of high importance to further explore \(^{23}\)Na MRI for its eventual establishment in the clinic.

In the here presented study, \(^{23}\)Na MRI datasets from a study investigating patients with ischemic stroke were used to evaluate in-depth the feasibility of using internal references for accurate, absolute TSC quantification. Quantification was performed based on two different internal reference regions: the cerebrospinal fluid and the vitreous humor. The results of both methods were compared to the currently most common TSC quantification method; the usage of external reference vials.

2. Methods

The presented study analyzed data that was acquired for a prospective in-vivo study, investigating the TSC within patients with an ischemic stroke. The study was approved by the local ethical review board and it was funded by Dietmar-Hopp Stiftung. Written informed consent was obtained from all patients or their next of kin. The prospective study included 62 patients out of which the data from 50 patients was included in the here presented study. Twelve patients were excluded for data evaluation of which four patients had aborted the measurement and the data of eight patients did not allow for proper TSC quantification as there were problems with the data acquisition, such as missing or misplaced reference phantoms. The exams were performed within the first 72 h after the onset of symptoms of the ischemic stroke.

2.1. Data acquisition and post-processing

All MRI data acquisition was performed at 3 T with the Magnetom Trio (Siemens Healthineers, Erlangen, Germany) with a dual-tuned \(^1\)H/\(^{23}\)Na birdcage coil (Rapid Biomedical, Rimpau, Germany). The study protocol was described previously (Neumaier-Probst et al., 2015; Adlung et al., 2021a, 2021b).

The MRI examination included a fluid-attenuated inversion-recovery (FLAIR) and a diffusion-weighted \(^1\)H MRI sequence, based on which the absolute diffusion coefficient (ADC)-map was calculated. Additionally, the protocol consisted of a 3D radial density-adapted \(^{23}\)Na MRI sequence (Nagel et al., 2009). The sodium sequence used TR/TE = 100/0.2 ms and 6000 spokes with 384 samples each. The pulse duration was set to 320 µs, the gradient amplitude was 4.6 mT/m with a bandwidth of 50 Hz/Px and the flip angle was set to 90. This resulted in a measurement time of 10 min per 3D \(^{23}\)Na MRI dataset and the image had a nominal resolution of 4 × 4 × 4 mm\(^3\). The reconstruction was performed offline in MATLAB 2018a (MathWorks, Natick, MA, USA). The reconstruction algorithm used a Kaiser Bessel window with a width of 4 and a Hanning filter in k-space. Regridding was applied before the inverse discrete Fourier transform. Furthermore, zero-filling with a factor of 2 was introduced to enhance the apparent resolution to 2 × 2 × 2 mm\(^3\). The reconstruction considered a field of view of 241 × 241 × 241 mm\(^3\). During the measurements, two reference vials were attached to the patients’ heads to enable absolute TSC quantification according to the current state of the art. The vials contained 50 and 100 mM NaCl respectively and 2% agarose to imitate brain sodium concentrations and relaxation times (Pascke et al., 2018; Neumann et al., 2017a). They had a volume of 14 ml and a diameter of 15 mm. The \(^{23}\)Na MR image and the ADC-map were co-registered to the patient’s FLAIR using SPM12 (The Wellcome Centre for Human
Neuromaging, UCL, London, UK). Image segmentation into WM, GM, and CSF was performed based on the FLAIR, also using SPM12. Manual segmentation of the patient’s stroke region was performed by a neuroradiologist with 3 years of experience based on the co-registered ADC-map. The stroke region was defined by lowered values on the ADC-map in correlation with high-intensity regions on the FLAIR.

2.2. TSC quantification

Absolute TSC quantification based on SI within the reference vials (TSC\textsubscript{Vials}) was used as a baseline (ground truth) to further compare it with the absolute TSC quantification based on other (internal) references.

Two additional reference regions were defined as three-dimensional ROIs, which were segmented within the patient’s left vitreous humor (VH) and within the lateral ventricle (CSF) as depicted in Fig. 1. Thus, a total of three TSC-maps per patient were calculated based on SI within those reference regions:

I. TSC\textsubscript{Vials}: TSC quantification based on SI within manually segmented regions within the reference vials
II. TSC\textsubscript{CSF}: TSC quantification based on SI within the manually segmented region within the cerebrospinal fluid
III. TSC\textsubscript{VH}: TSC quantification based on SI within the manually segmented region within the vitreous humor

Within the clinical routine, the SSC was determined for most patients and TSC within VH and CSF was assumed to be equivalent to the patients’ SSC as both regions are entirely extracellular and as SSC was described to indicate the amount of sodium relative to the amount of water within the extracellular fluid (DiBartola, 2011). For the remaining patients, where no information about SSC was given, a mean SSC of 145 mM was assumed, corresponding to the extracellular sodium concentration according to literature values (Madelin and Regatte, 2013; Somjen, 2004; Petracca et al., 2016). Sodium levels in the CSF were also found by Wilferth et al. through laboratory analyses (Wilferth et al., 2022).

CSF has longer relaxation times compared to vital brain tissue (T1 (CSF) is around 50 ms) (Madelin and Regatte, 2013) so that T1(CSF) exceeded 5 TR substantially, indicating the necessity of a T1 bias correction. The T1 relaxation of VH was assumed to be equivalent to T1 of CSF (thus, T1(VH) = 50 ms was estimated) as both are purely extracellular fluids. The assumption was previously suggested by Wenz et al. (2018).

Therefore, SI within CSF and VH was corrected for T1-weighting with

\[
SI_{T1\text{corr}} = SI \frac{1}{1 - \exp(-TR/T1)}
\]

The same correction factor was, thus, used for both regions. T1 relaxation time corrections were exclusively performed for VH and CSF but not for WM, GM or the reference vials. This was because T1 relaxation times within WM and GM were reported to be lower. Literature suggests values between 15 and 35 ms (Madelin and Regatte, 2013). With the applied TR, T1 bias is expected to be between 1% and 6% within WM and GM. Reference vials were composed to mimic the relaxation times of brain tissue, which was approached by the addition of 2% agarose to the NaCl solution (Paschke et al., 2018; Neumann et al., 2017a; Mitchell et al., 1986). Correction for T2\textsuperscript{*} -weighting was not performed as a very short TE = 0.2 ms was used and mono-exponential T2\textsuperscript{*} (CSF) > 55 ms. Thus, the impact of T2\textsuperscript{*} -weighting was below 1%.

Mean TSC in the whole head (the mask was determined via thresholding and thus included brain matter and CSF, as well as the skull) was evaluated for all three TSC-maps. Differences between the TSC\textsubscript{Vials}-maps and the other two TSC-maps were evaluated by calculating the absolute TSC differences voxel-wise. Mean TSC values within the manually segmented internal reference regions were evaluated based on the TSC\textsubscript{Vials}-map. Furthermore, the standard deviation (SD) within the VH, the CSF and within one of the reference vials (100 mM) was calculated within their respective TSC-maps to evaluate and compare the stability of the quantification method.

Mean absolute TSC within the masks of WM, and GM, and within the mask of the stroke region were evaluated on all three TSC-maps. The masks included a 4 mm (WM and GM) or a 2 mm (stroke) cut-off of the outer border to reduce PVE. The segmented masks and the evaluated masks including the cut-off are depicted in Fig. 2.

2.3. Evaluation of quantification stability

To evaluate the stability of quantification based on VH or CSF, three healthy control (HC) (two female, 25 and 53 years old, one male, 29 years old) underwent three 23Na MRI measurements each. 23Na MR images and an additional T2w \textsuperscript{1}H MR image were acquired of each HC. All three 23Na MR images were co-registered to the respective \textsuperscript{1}H MR image. Absolute TSC quantification was performed within all 23Na MR images based on SI within ROIs in the CSF and the VH. As no blood sample was available from the HCs, ESC was assumed to be 145 mM. Due to the image co-registration, the same ROIs in CSF and VH were usable within all three 23Na MR images of each HC. Image co-registration and automatic image segmentation into WM, GM, and CSF were performed with SPM12. Absolute TSC was calculated within WM and GM on the resulting TSC-maps from all three images of each HC. It was compared voxel-wise within both tissues between the measurements.

2.4. Statistical analysis

The paired student t-test was performed to evaluate whether TSC differences between the three absolute TSC quantification methods were statistically significant. Significance was considered for \( p < 0.05 \). The t-test was applicable as the Kolmogorov-Smirnov test showed normal distribution. The correlation was tested between SSC and mean absolute TSC within VH and CSF on the TSC\textsubscript{Vials}-map using the Pearson correlation test.

3. Results

The patients in the here presented study had a mean age of 73 ± 13 years. Out of the 50 patients that were included, 24 were women and 26 were men. Using the quantification method based on the SI within the

![Fig. 1. One representative transverse slice of the quantified 23Na MR image of one patient with the segmentation of the different reference regions, which were used for the quantification methods. The reference region within both reference phantoms are encircled in black, the CSF reference region is encircled in magenta, and the VH reference region is encircled in red.](image-url)
reference vials (TSC\textsubscript{Vials}), for all included 50 patients, the absolute TSC quantification showed a mean of 42 ± 6 mM in the whole head, averaged for all patients.

### 3.1. TSC quantification differences

Mean absolute TSC in the whole head averaged over all evaluated patients was 38 ± 3 mM with the quantification based on SI within the CSF region (TSC\textsubscript{CSF}), and it was 35 ± 4 mM with the quantification based on SI within the VH region (TSC\textsubscript{VH}). Mean TSC\textsubscript{CSF} and mean TSC\textsubscript{VH} in the whole head of all evaluated patients were both significantly lower compared to TSC\textsubscript{Vials} (both \( p < 0.0001 \)) and mean TSC\textsubscript{VH} was significantly lower than mean TSC\textsubscript{CSF} (\( p < 0.0001 \)).

Mean absolute TSC values in the whole head of all evaluated patients are depicted as boxplots of all three TSC-maps in Fig. 3.

Mean absolute TSC differences within the whole head, averaged over all evaluated patients (\( \Delta \text{TSC} \)) between TSC\textsubscript{CSF} and TSC\textsubscript{Vials} was 6 ± 6 mM, which was significantly lower than the mean \( \Delta \text{TSC} \) between TSC\textsubscript{VH} and TSC\textsubscript{Vials}, which was 8 ± 5 mM (\( p = 0.0019 \)). \( \Delta \text{TSC} \) of TSC\textsubscript{CSF} and of TSC\textsubscript{VH} within the whole head of all evaluated patients are depicted as boxplots in Fig. 4.

The SSC was available for 44 out of 50 patients and it was at 139 ± 2 mM. No information about SSC was available for the remaining six patients. Considering those six patients, \( \Delta \text{TSC} \) was 10 ± 9 mM within the TSC\textsubscript{CSF}-map and 9 ± 6 mM within the TSC\textsubscript{VH}-map. \( \Delta \text{TSC} \) of that subgroup was not significantly different compared to \( \Delta \text{TSC} \) of all patients within the respective TSC-map (\( p = 0.14 \) for TSC\textsubscript{CSF} and \( p = 0.40 \) for TSC\textsubscript{VH}).

On the TSC\textsubscript{Vials}-map, after the introduction of the correction factor,
3.2. TSC quantification within tissues

Mean absolute TSC was evaluated within the segmented masks - after subtraction of their outer border. Mean TSC values, together with the SD and the coefficient of variation in WM, GM, and stroke region on all three TSC-maps are listed in Table 1.

Averaged over all patients, mean absolute TSC within GM was significantly higher compared to mean absolute TSC in WM on all three TSC-maps (all p < 0.0001) and mean absolute TSC within the stroke region was significantly higher compared to mean absolute TSC in WM and GM (all p < 0.0001). The TSC maps of the three different methods for one stroke patient are shown in Fig. 5. The stroke region is well distinguishable on all three maps. Fig. 6 depicts the boxplots of TSC in WM, GM and the stroke region based on all three quantification references. The figure visualizes how differentiation of TSC within the different tissues is possible on all three TSC-maps.

Fig. 7 depicts the Bland-Altman plot and correlation plots for both quantification methods with internal references compared to the quantification with external reference vials. The figure illustrates how quantification based on the VH exhibits a smaller deviation than quantification based on CSF when compared to the quantification with external references. In addition, TSC\textsubscript{VH} consistently shows a lower sodium concentration compared to TSC\textsubscript{Vials}, whereas TSC\textsubscript{CSF} does not exhibit a constant offset but instead alternates.

3.3. Evaluation of quantification stability

TSC quantification based on SI within reference regions in the CSF and within the VH was performed on three MRI scans of three HC. All TSC differences between scans (within both tissue types and on both TSC-maps) were lower than the SD of the respective tissue within all HC. Again, the TSC\textsubscript{Vials}-maps showed lower TSC levels than the TSC\textsubscript{CSF}-map (Table 2). Fig. 8 shows two transverse slices of one \textsuperscript{23}Na MR image from one HC (female, 53 years old). The respective reference regions within CSF and VH are encircled in magenta (CSF) and red (VH).

4. Discussion

In this study, different absolute TSC quantification methods were performed with datasets from a study investigating the sodium concentration within the ischemic stroke of patients within the first 72 h after the onset of symptoms. The absolute TSC quantification was performed based on the average SI within the reference vials (TSC\textsubscript{Vials}), as it is currently considered state-of-the-art (Madelin and Regatte, 2013; Hu et al., 2020a; Ouwerkerk et al., 2003). This quantification method is prone to various technical and human errors and positioning external reference vials is not always feasible, e.g., when using phased array coils due to space restrictions. Thus, two other, less established, absolute quantification methods were performed additionally. Instead of using external phantoms, TSC quantification was performed based on internal quantification references: the cerebrospinal fluid (CSF) and the vitreous humor (VH).

Previously, it had been suggested to evaluate the sodium concentration relative to other organs or tissues (Haneder et al., 2013; Marli et al., 2006). CSF and VH both do not contain vital cells and are therefore assumed to have a stable sodium concentration, equivalent to the ESC (Olsen and Rudolph, 1955; Harrington et al., 2010; Smith et al., 2020; Pigiani et al., 2020). Furthermore, previous laboratory analyses by Wilferth et al. have supported this assumption as they have found stable sodium concentration around 147 mM in the CSF (Wilferth et al., 2022). This motivated the consideration of either region as a potential reference for the absolute TSC quantification. In the presented study, TSC in VH and in CSF were assumed to be equivalent to SSC if the value was available. If SSC was not available, it was assumed to be 145 mM, corresponding to previously reported ESC (Somjen, 2002; Skou and Esmann, 1992).

Overall, the results of all three quantification methods were within the range of previously reported values (Madelin and Regatte, 2013). However, they were at the upper bound of literature values, which could have derived from white matter lesions as previously reported by our group (Adlung et al., 2021b). Image segmentation allowed for TSC evaluation within WM, GM and as a stroke region separately. On all three TSC-maps, the stroke regions presented significantly higher TSC values than WM and GM. Furthermore, TSC within WM was lower than TSC within GM. Both tendencies were present with all three quantification methods and they correspond to previously reported findings (Hussain et al., 2009; Ridley et al., 2018; Lin et al., 2001; Neumaier-Probst et al., 2015; Madai et al., 2012; Maarouf et al., 2017; Liao et al., 2019).

However, the TSC\textsubscript{CSF}-map and TSC\textsubscript{VH}-map showed lower values in the whole head and within every tissue than the TSC\textsubscript{Vials}-map. The manually segmented regions within CSF and VH showed generally high mean absolute TSC values in CSF and VH on the TSC\textsubscript{Vials}-map. There were n = 4 outliers who even presented values above 200 mM, which is far above previously reported or physiologically reasonable values (Somjen, 2002, 2004; Skou and Esmann, 1992; Falcioni et al., 1994). The size of the used reference vials was rather small, with a volume of 14 ml and a diameter of 15 mm, making them sensitive to PVE. PVE - especially from the air surrounding the phantoms - can decrease the SI within the phantoms and consequently increase TSC values within the brain on the TSC\textsubscript{Vials}-map offering a possible explanation for the physiologically unreasonably high TSC values. CV within all evaluated regions was higher compared to previously published values by Wilferth et al. (2022) or Riemer et al. (2019). However, those studies evaluated brain matter of healthy volunteers Riemer et al. (2019) or the healthy-appearing brain matter of patients Wilferth et al. (2022), whereas the entire gray and white matter was included for the evaluation in this study. Thus, pre-conditions in the patients’ brain might explain the higher CV values compared to previous studies.

T1 relaxation time correction was exclusively performed within the internal reference regions but not within the WM or GM, resulting in an error of up to 6% within those regions. This could be further corrected if more precise information about the relaxation times within those regions was available. However, quantification of the T1 relaxation times would require further data acquisition and thus longer measurement times. Furthermore, the added gaseous within the reference vials was supposed to achieve relaxation times within the reference phantoms which are similar compared to the relaxation times within the brain matter (Paschke et al., 2018; Neumann et al., 2017b).

Misplacement of the vials, e.g. not parallel to the image plane, could potentially increase the PVE’s impact, which might explain the outliers. In this study, particularly larger in diameter, could have helped to reduce inaccuracies in TSC quantification deriving from PVE in the reference phantoms. However, this might also increase discomfort for the patient, as the narrow coil already does not provide a lot of space around the head. There have been suggestions to reduce PVE with algorithms that are based on the geometric transfer matrix approach (Niesporek et al., 2015). Adding such an algorithm to the image post-processing could decrease the PVE arising from the point-spread function in the reconstruction algorithm, which would

| Tissue | TSC\textsubscript{Vials} | TSC\textsubscript{CSF} | TSC\textsubscript{VH} |
|--------|-----------------|-----------------|------------------|
|        | Mean ± SD | CV   | Mean ± SD | CV   | Mean ± SD | CV   |
| WM     | 53 ± 9    | 0.17 | 48 ± 7    | 0.15 | 44 ± 7    | 0.16 |
| GM     | 58 ± 8    | 0.14 | 53 ± 6    | 0.12 | 48 ± 6    | 0.13 |
| Stroke | 73 ± 16   | 0.21 | 66 ± 15   | 0.23 | 60 ± 12   | 0.21 |
prospectively further improve the quantification accuracy. Further investigations would be necessary to re-evaluate whether quantification, particularly based on ROIs in the CSF, might improve even further when applying such algorithm.

Furthermore, TSC within VH was higher than TSC within CSF despite literature describing both to be strongly associated with ESC (Olsen and Rudolph, 1955; Harrington et al., 2010; Smith et al., 2020; Pigaiani et al., 2020). The VH is a spherical body with a diameter of approx. 16 mm, which remains relatively stable in adulthood (Ruby et al., 2006) whereas CSF expands with age, and ventricles are significantly larger in older patients (Matsumae et al., 1996; Zhang et al., 2005). Smaller ventricles make the manual definition of a CSF region more challenging. Thus, CSF in younger patients is more prone to the introduction of PVE compared to regions within the VH where segmentation is easier because of its stable size. The impact of PVE was further explored by considering the SD of TSC within the segmented regions of one of the reference phantoms, the CSF, and the VH. SD was significantly lower within VH than within the two other regions, which emphasizes the homogeneity of the TSC in VH. This finding allows the conclusion that TSC quantification based on the SI within the VH is more stable and robust.

SSC (and therefore ESC) might vary between different patients and a significant positive correlation was found between SSC and TSC in VH (on the TSCVH map) whereas the correlation between SSC and TSC in

![Fig. 5.](image1) One representative transverse slice of the quantified $^{23}$Na MR image of one patient with an ischemic stroke. The figure shows the quantified image slice with quantification being based on external reference phantom (TSCVials, current state of the art), based on a manually segmented region within the cerebrospinal fluid (TSCCSF) and based on a manually segmented region within the vitreous humor (TSCVH).

![Fig. 6.](image2) Boxplots of the mean absolute tissue sodium concentration (TSC) in white matter (WM, blue), gray matter (GM, red), and in the stroke region (Stroke, yellow) on the three TSC-maps: TSCVials, TSCCSF, and TSCVH. The middle line in the box depicts the median value and the blue box’ top and bottom edges represent the 25th and 75th percentiles of the data, respectively. The whiskers extend to the most extreme data points, not considering outliers, which are depicted as red +. Statistically significant differences are indicated with a *.

![Fig. 7.](image3) Top: Bland-Altman comparing the absolute TSC in the stroke region on the TSCCSF and the TSCVials-map (left) and on the TSCVH and the TSCVials-map (right). Bottom: Correlation plot of the absolute TSC within the stroke region on the TSCVials and the TSCCSF-map (left) and on the TSCVials and the TSCVH-map (right).

CSF was not significant. However, for TSCVH and for TSCCSF, TSC differences to TSCVials were not significantly different between patients where SSC was available and those where an ESC was estimated to be 145 mM. Apparently, even with no SSC available, the TSC quantification accuracy did not seem to suffer substantially.

Those results justify a TSC quantification based on internal references even if no blood sample of the patient is available, which further improves patient comfort significantly.

4.1. Evaluation of quantification stability

To evaluate the absolute TSC quantification stability, three $^{23}$Na MR images each were acquired of three HC. TSC quantification was performed based on SI within ROIs in the CSF and in the VH. Again, TSCVH showed lower values than TSCCSF. Results within WM and GM on all TSC-maps show values, which were within the range of literate values (Madelin and Regatte, 2013). However, for all TSCCSF, they are at the upper limit, whereas the results within the TSCVH-maps were more
Table 2
Mean absolute tissue sodium concentration (TSC) within white matter (WM) and gray matter (GM) in three healthy control (HC), and averaged over all three HC at three measurements, including the mean voxel-wise differences (MeanΔ) between all three measurements within both tissue types. TSC values are given for quantification based on SI within cerebrospinal fluid (TSCCSF) and vitreous humor (TSCVH).

| Scan | HCl | TSCCSF | TSCVH | HC2 | TSCCSF | TSCVH | TSC [mM] | HCl | TSCCSF | TSCVH | AI | TSCCSF | TSCVH |
|------|-----|--------|-------|-----|--------|-------|---------|-----|--------|-------|----|--------|-------|
| WM   |     |        |       |     |        |       |         |     |        |       |    |        |       |
| I    | 58.7 ± 8.7 | 46.0 ± 6.9 | 56.3 ± 8.6 | 47.4 ± 7.3 | 48.7 ± 5.9 | 48.4 ± 5.9 | 54.5 ± 7.8 | 47.3 ± 6.7 |       |       |       |    |        |       |
| II   | 56.7 ± 8.6 | 47.7 ± 7.2 | 55.2 ± 8.4 | 48.1 ± 7.4 | 48.1 ± 6.0 | 48.1 ± 6.0 | 53.3 ± 7.7 | 48.0 ± 6.8 |       |       |       |    |        |       |
| III  | 57.7 ± 8.5 | 47.7 ± 7.1 | 57.1 ± 8.8 | 47.2 ± 7.2 | 47.7 ± 5.9 | 49.1 ± 6.1 | 54.2 ± 7.8 | 48.0 ± 6.8 |       |       |       |    |        |       |
| Mean | 57.7 ± 8.6 | 47.1 ± 7.0 | 56.2 ± 8.6 | 47.5 ± 7.3 | 48.2 ± 5.9 | 48.5 ± 6.0 | 54.0 ± 7.7 | 47.7 ± 6.8 |       |       |       |    |        |       |
| MeanΔ| 3.3 ± 2.5  | 2.8 ± 2.1  | 3.3 ± 2.5  | 2.7 ± 2.0  | 2.7 ± 2.1  | 2.8 ± 2.1  | 3.1 ± 2.3  | 2.7 ± 2.1  |       |       |       |    |        |       |
| GM   |     |        |       |     |        |       |         |     |        |       |    |        |       |
| I    | 70.8 ± 9.4 | 55.5 ± 7.4 | 68.0 ± 9.5 | 57.2 ± 8.0 | 56.2 ± 6.9 | 55.8 ± 6.9 | 65.0 ± 8.6 | 56.2 ± 7.4 |       |       |       |    |        |       |
| II   | 68.5 ± 9.3 | 57.5 ± 7.8 | 66.8 ± 9.6 | 58.2 ± 8.3 | 55.6 ± 6.9 | 55.7 ± 6.9 | 63.6 ± 8.6 | 57.1 ± 7.7 |       |       |       |    |        |       |
| III  | 69.5 ± 9.3 | 57.4 ± 7.7 | 69.0 ± 9.6 | 57.0 ± 7.9 | 55.4 ± 6.7 | 56.9 ± 6.9 | 64.8 ± 8.5 | 57.1 ± 7.5 |       |       |       |    |        |       |
| Mean | 69.6 ± 9.3 | 56.8 ± 7.6 | 67.9 ± 9.6 | 57.5 ± 8.1 | 55.7 ± 6.9 | 56.1 ± 6.9 | 64.4 ± 8.6 | 56.8 ± 7.5 |       |       |       |    |        |       |
| MeanΔ| 3.4 ± 2.6  | 2.9 ± 2.2  | 3.3 ± 2.5  | 2.7 ± 2.1  | 2.8 ± 2.1  | 2.8 ± 2.1  | 3.2 ± 2.4  | 2.8 ± 2.1  |       |       |       |    |        |       |

Fig. 8. 23Na MR image of one healthy control (female, 53 years old) from one scan with two transverse slices. The reference regions (for the quantification of the tissue sodium concentration) within cerebrospinal fluid (magenta) and vitreous humor (red) are encircled.

similar to the globally reported values (Niesporek et al., 2015; Liao et al., 2019; Zaaraoui et al., 2012; Eisele et al., 2019; Weber et al., 2021). TSC differences between the three measurements were below the SD within the respective tissues, on all TSC-maps of all three HC. Thus, the results indicate high quantification stability. The TSC differences were lower on the TSCVH-maps than on the TSCCSF-maps for two out of three HC, and overall.

Overall, the VH appeared as a reliable reference for the TSC quantification. Quantification based on SI within VH seemed to be more robust and reproducible than quantification based on SI within CSF, which is despite the fact that the TSCVH-map of the patients with an ischemic stroke presented higher TSC differences to the TSCCSF-map compared to the TSCVH-map. Additionally, the great variations in ventricle size and shape must be considered a confounder when considering reference regions within the CSF. Considering the VH as a reliable quantification reference is justified because of the low differences between the TSCVH-maps of scans at different time points.

Furthermore, previously it was shown that, especially around Larmor frequency, mobility of the ionic salts dominates conductivity calculations (Foster and Schepps, 1981). Based on that knowledge, several other publications claimed there is a positive linear correlation between conductivity and tissue sodium concentration (Liao et al., 2019; van Lier et al., 2013) with conductivity imaging being another emerging technique for the diagnosis of various diseases, especially different cancer types (Hancu et al., 2015; Shin et al., 2015). The obtained tissue sodium concentration could be considered together with conductivity and might improve diagnosis and treatment decisions, prospectively. As a future direction, the relationship between tissue sodium concentration and conductivity could also be further explored, particularly with the here presented possibility of a simplified measurement setup using the VH as quantification reference.

5. Conclusion

This study compared TSC quantification in the ischemic stroke based on two different internal references to the currently most common approach, which is TSC quantification based on external reference vials. The stable sodium concentration within the CSF and the VH were used as references. Quantification based on external reference vials has led to questionable TSC values in the CSF and VH for some individuals. This might be because it may suffer from human errors in the fabrication process and placement of the vials. Also, it is prone to PVE, which influences the quantification substantially.

Internal references prove to be a stable alternative. Different pathologies and the age of the patient potentially influence the quantification based on TSC in CSF as its size varies with age. The VH, however, provides a stable and reliable reference point that is not reported to change significantly. Therefore, using the VH as internal reference for TSC quantification seems to provide a reliable alternative that could overcome some of the problems, which exist with the currently most commonly performed method.

Declarations of interest

None.

Data availability

The data that has been used is confidential.

Acknowledgements

The study was funded by Dietmar-Hopp Stiftung.

References

Adlung, A., et al., 2021a. 23Na MRI in ischemic stroke: acquisition time reduction using postprocessing with convolutional neural networks. NMR Biomed. 34 (4), e4474. Adlung, A., et al., 2021b. Tissue sodium concentration within white matter correlates with the extent of small vessel disease. Cerebrovasc. Dis. 50 (3), 347–355. Boada, F.E., et al., 2012. Sodium MRI and the assessment of irreversible tissue damage during hyper-acute stroke. Transl. Stroke Res. 3 (2), 219–223. Bottomley, P.A., 2007. Sodium MRI in Man: Technique and Findings. eMagRes. Burstein, D., Springer Jr, C.S., 2019a. Sodium MRI revisited. Magn. Reson. Med. 82 (2), 521-524. Burstein, D., Springer Jr, C.S., 2019b. Sodium MRI revisited. Magn. Reson. Med. 82 (2), 521-524. Dilbaro, S.P., 2011. Fluid, Electrolyte, and Acid-base Disorders in Small Animal Practice. Elsevier Health Sciences.
Eisele, P., et al., 2019. Temporal evolution of acute multiple sclerosis lesions on serial sodium (23Na) MRI. Multi. Scler. Relat. Disord. 29, 48-54.
Falcion, J., et al., 1994. Role of cell membrane Na, K-ATPase for survival of human lymphocytes in vitro. Biosci. Rep. 14 (4), 189-204.
Foster, K., Schepp, J., 1981. Dielectric properties of tumor and normal tissues at radio through microwave frequencies. J. Microsc. Power 16 (2), 107-119.
Gao, S., et al., 2017. Cross-sectional positive association of serum lipids and blood pressure with serum sodium within the normal reference range of 135-145 mmol/L. Arterioscler. Thromb. Vasc. Biol. 37 (3), 598-606.
Gerhalter, T., et al., 2021. Global decrease in brain sodium concentration after mild traumatic brain injury. Brain Commun. 3 (2), e00511.
Gilles, A., Nagel, A.M., Madelin, G., 2017. Multipulse sodium magnetic resonance imaging for multicompartment quantification: Proof-of-concept. Sci. Rep. 7 (1), 17435.
Hancu, I., et al., 2015. On conductivity, permittivity, apparent diffusion coefficient, and their usefulness as cancer markers at MRI frequencies. Magn. Reson. Med. 73 (5), 2025-2029.
Haneder, S., et al., 2011. Quantitative and qualitative 23Na MRI imaging of the human kidneys at 3 T: before and after a water load. Radiology 260 (3), 857-865.
Haneder, S., et al., 2013. Assessment of the renal corticomedullary 23Na gradient using isotropic data sets. Acad. Radiol. 20 (4), 407-413.
Harrington, M.G., et al., 2016. Cerebrospinal fluid sodium rhythms. Cereb. Fluid Res. 7 (1), 1-9.
Hillal, S.K., et al., 1985. In vivo NMR imaging of sodium-23 in the human head. J. Comput. Assist. Tomogr. 9 (1), 1-7.
Hu, R., et al., 2020a. X-nuclei imaging: current state, technical challenges, and future directions. J. Magn. Reson Imaging 51 (2), 355-376.
Hu, R., et al., 2020b. X-nuclei imaging: current state, technical challenges, and future directions. J. Magn. Reson. Imaging 51 (2), 355-376.
Hussain, M.S., et al., 2009. Sodium imaging intensity increases with time after human ischemic stroke. Ann. Neurol. Off. J. Am. Neurol. Assoc. Child Neurol. Soc. 66 (1), 55-62.
Inglese, M., et al., 2010. Brain tissue sodium concentration in multiple sclerosis: a sodium imaging study at 3 tesla. Brain 133 (Pt. 3), 847-857.
Insko, E.K., Clayton, D.B., Elliott, M.A., 2002. In vivo sodium MR imaging of the intervertebral disk at 4 T. Acad. Radiol. 9 (7), 800-804.
Kim, B.J., et al., 2014. Magnetic resonance imaging in acute ischemic stroke treatment. Magn. Reson. Imaging 32 (4), 925-932.
Kokavec, J., et al., 2016. Biochemical analysis of the living human vitreous. Clin. Exp. Ophthalmol. 44 (4), 295-303.
Kokavec, J., et al., 2018. Dielectric properties of tumor and normal tissues at radio through microwave frequencies. J. Microsc. Power 16 (2), 107-119.
Kokavec, J., et al., 2019. Quantitative and qualitative 23Na MRI imaging of the human kidneys at 3 T: before and after a water load. Radiology 260 (3), 857-865.
Kim, B.J., et al., 2014. Magnetic resonance imaging in acute ischemic stroke treatment. Magn. Reson. Imaging 32 (4), 925-932.
Kokavec, J., et al., 2016. Biochemical analysis of the living human vitreous. Clin. Exp. Ophthalmol. 44 (4), 295-303.
Kokavec, J., et al., 2018. Dielectric properties of tumor and normal tissues at radio through microwave frequencies. J. Microsc. Power 16 (2), 107-119.