



Diffusion-weighted magnetic resonance imaging for detection of postoperative intracranial pyogenic abscesses in neurosurgery

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Received: 27 September 2018 / Accepted: 8 March 2019 / Published online: 26 March 2019
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Abstract

Background Diffusion-weighted magnetic resonance imaging (MRI-DWI) is the modality of choice for detecting intracranial abscesses; however, it is unclear whether prior brain surgery has an influence on its diagnostic value. Thus, we assessed the robustness of MRI-DWI and determination of an ADC cutoff value for detecting intracranial abscesses in patients who underwent brain surgery.

Methods We retrospectively evaluated 19 patients prior to surgery for postoperative supratentorial parenchymal abscesses by means of MRI-DWI. Forty randomly selected patients with routine postoperative MRI-DWI were used for comparative analyses. Clinical and serum biomarkers (C-reactive protein, interleukin-6, white blood cell count) as well as from results of early postoperative imaging findings (computed tomography and/or MRI scan) were recorded. Additionally, ADC values, T1±gadolinium, and T2/fluid-attenuated inversion recovery sequences were investigated.

Results After initial surgery, early postoperative control imaging showed evidence of hemorrhage and/or hemostatic agents within the resection cavity in 10/19 patients of the abscess group and in 16/40 patients of the control group. No postoperative ischemia was detected. Neither hemostatic agents nor blood affected the mean ADC values in both the reference group (blood $2.96 \pm 0.22 \times 10^{-3} \text{ mm}^2/\text{s}$ vs. no blood $2.95 \pm 0.26 \times 10^{-3} \text{ mm}^2/\text{s}$, $p = 0.076$) and in the abscess group (blood $0.87 \pm 0.07 \times 10^{-3} \text{ mm}^2/\text{s}$ vs. no blood $0.76 \pm 0.06 \times 10^{-3} \text{ mm}^2/\text{s}$, $p = 0.128$). The mean ADC value within the resection cavity was significantly lower in the abscess group (1.5 T 0.88 ± 0.41 vs. $2.88 \pm 0.20 \times 10^{-3} \text{ mm}^2/\text{s}$, $p < .01$; 3.0 T 0.75 ± 0.24 vs. $3.02 \pm 0.26 \times 10^{-3} \text{ mm}^2/\text{s}$, $p < 0.01$). The optimal ADC cut-off for the differentiation of an abscess from normal postoperative findings was found at $1.87 \times 10^{-3} \text{ mm}^2/\text{s}$ (area-under-the-curve 1.0, sensitivity = 100%, specificity = 100%). Moreover, no differences between the abscess patients and the control group were seen with respect to the analyzed serum biomarkers.

Conclusion MRI-DWI provides a robust tool to discriminate postoperative abscess formation from normal postoperative changes.

Keywords Apparent diffusion coefficient · Diffusion-weighted MRI · Neurosurgery · Pyogenic intracranial abscess · Revision surgery · Serum biomarker

Christoph Schwartz, Markus Lenski, Robert Forbrig and Niklas Thon contributed equally to this work.

This article is part of the Topical Collection on *Infection*

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Abbreviations

ADC	Apparent diffusion coefficient
CT	Computed tomography
CRP	C-reactive protein
CSF	Cerebrospinal fluid
DWI	Diffusion-weighted imaging
IL-6	Interleukin-6
MRI	Magnetic resonance imaging
WBCC	White blood cell count

Introduction

Despite modern minimally invasive approaches, the use of perioperative antibiotics, and hygienic measures, postoperative infections remains a major concern in brain surgery and constitute a serious complication in neurosurgical patients [5, 6]. In brain tumor patients, postoperative infections cannot only influence the immediate outcome but can also delay adjuvant treatment with potential negative effects on the clinical course. Surgical site infections, abscess formation, and postoperative meningitis rates of 1.1 to 8.9% have been reported in unselected cohorts [1, 12, 17, 18, 30, 33]. Known risk factors are higher age, emergency and repeated operations, prolonged duration of surgery, use of steroids, and cerebrospinal fluid (CSF) leakage [1, 12, 17, 18, 30, 33].

In order to successfully treat these potentially life-threatening postoperative infections, early radiological identification is of utmost importance. Magnetic resonance imaging (MRI) is the imaging modality of choice for the detection of intracranial pyogenic abscesses, empyema, or ventriculitis [7, 23, 26]. Typical findings of abscess formations include ring enhancement with low T1-signal intensity of the central necrotic area, iso- to high on T2-weighted, frequently surrounded by extensive perifocal edema. Important additional information comes from diffusion-weighted imaging (DWI) [8, 10, 19, 21]. DWI is based on the effect dephasing of water molecules secondary to movement along a strong gradient during the measurement. Areas with restricted motion exhibit high signal intensity whereas free water such as the CSF becomes dark. The absolute value of diffusion restriction can be quantified by the apparent diffusion coefficient (ADC) and is independent of the MRI manufacturer as well as the applied field strength [3, 13, 25]. ADC values correlate to the viscosity of liquids and are typically low in abscesses and high in necrotic brain tumors [9, 28, 29]. It remains unclear, however, to what extent surgery-related alterations such as the use of hemostatic agents, postoperative blood in the resection cavity, and/or focal ischemia interfere with the diagnostic value and robustness of MRI-DWI for the detection of parenchymal infections.

The aim of this study was to address this specific question. We used the MRI measurements obtained from an unselected

group of patients after uneventful surgery, serving as a control and compared these with patients with postoperative bacterial infection. In addition, the impact of surgery-related alterations on the potential diagnostic value of MRI-DWI was assessed. Furthermore, we assessed the datasets for useful ADC cutoff values that would reliably differentiate between acute intracranial abscess formation and non-specific postoperative changes. In addition, these results were correlated to serologic indicators for acute inflammation.

Methods and materials

Abscess patients

By conducting a retrospective search (i.e., patients ≥ 18 years of age operated for brain tumors between 2009 and 2016) of our in-house database, we identified 19 eligible patients with proven brain abscess formation (eight males, median age 55.1 years), who also underwent in-house MRI-DWI scan prior to removal of the abscess. Indications for initial supratentorial surgery included tumors in 14 patients (eight meningiomas, two gliomas, two metastases, one hemangiopericytoma, and one fronto-basal carcinoma with brain infiltration), three intracerebral hematomas, one cavernoma, and one focal epilepsy. All patients received CT scans on the first day after surgery and an early postoperative MRI-DWI was additionally available for 7/19 patients. Initial wound healing was recorded as uneventful in all patients and no CSF leakage and/or extended cortisone treatment was documented. Further documented parameters included serum C-reactive protein (CRP), serum interleukin-6 (IL-6), white blood cell count (WBCC), and bacteriologic analysis.

The median time interval between the first operation and abscess formation was 59.0 days (range 17.0–417.0 days). The leading symptoms were secondary wound healing deficits in 13 (68.4%) patients (purulent discharge ($n=9$), swelling of the surgical site ($n=4$)), new neurological deficits in three (15.8%) patients (aphasia ($n=2$), hemiparesis ($n=1$)), and seizures and headaches, each affecting one (5.3%) patient. In another asymptomatic patient, the suspected infection was coincidentally detected by a routine follow-up MRI scan. Fever (> 38.5 °C) was seen in five (26.3%) patients; one (5.3%) patient presented with meningeal symptoms. No other infection of the lung, urinary tract, etc. was diagnosed. Except for two cases (15 and 20 days), surgical revision always was performed within 48 h after MRI-based diagnosis. Intraoperatively, a putrid infection of the resection cavity was confirmed in all cases. In 17 cases, the pathogenic agent was identified, with *Staphylococcus aureus* (five patients) and *Propionibacterium acnes* (four patients) being most common. Two patients were treated with antibiotics prior to surgery and no infective agent could be identified (Fig. 1; Table 1).

Fig. 1 Patient population. cCT, cranial computed tomography; DWI, diffusion-weighted imaging; pMRI, postoperative magnetic resonance imaging

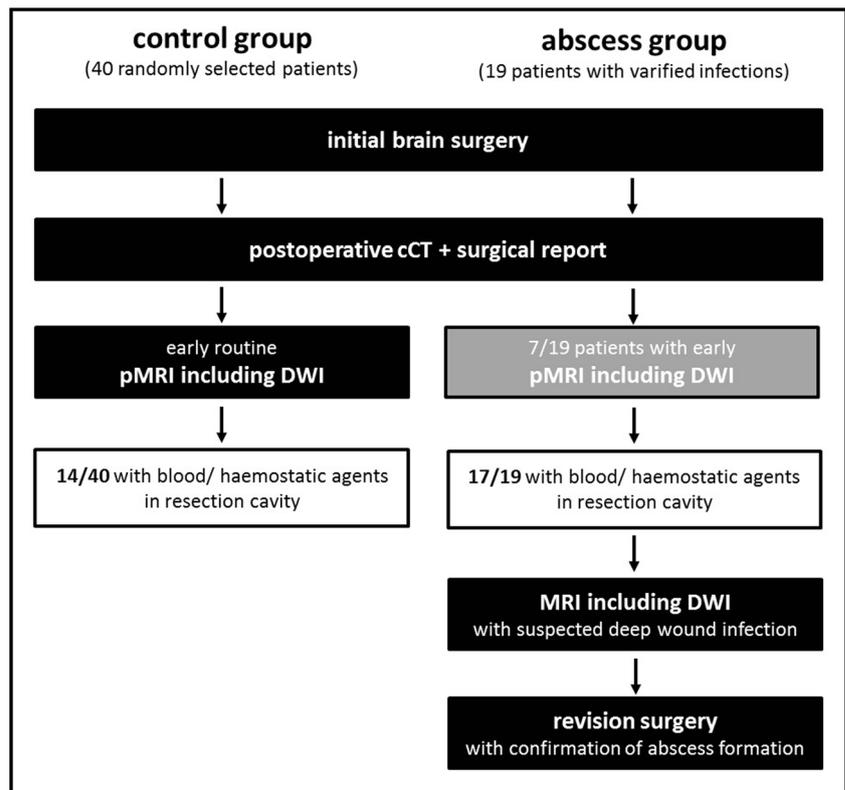


Table 1 MRI characteristics of the abscess patients

Pat. ID	Time span initial surgery and MRI (days)	Time span MRI and revision surgery (days)	MRI field strength (Tesla)	Mean ADC value ($\times 10^{-3}$ mm ² /s)	ADC standard deviation ($\times 10^{-3}$ mm ² /s)	Edema (0 = no edema/decrease, 1 = unchanged, 2 = increase)	Hemorrhage (0 = no blood, 1 = blood/hemorrhagic imbibition)
1	211	2	1.5 (Aera)	0.59	0.03	1	0
2	64	0	1.5 (Aera)	1.18	0.21	0	1
3	23	1	3.0 (GE Signa)	1.27	0.19	0	0
4	59	10	3.0 (GE Signa)	0.68	0.09	2	0
5	38	0	3.0 (GE Signa)	0.54	0.04	2	1
6	30	0	1.5 (Avanto)	0.96	0.10	0	1
7	17	20	3.0 (GE Signa)	0.72	0.18	0	1
8	71	1	1.5 (Symphony)	0.96	0.10	2	1
9	53	0	1.5 (Symphony)	1.16	0.15	2	1
10	42	1	3.0 (GE Signa)	0.67	0.08	1	0
11	31	0	1.5 (Symphony)	0.82	0.02	0	1
12	198	1	3.0 (GE Signa)	0.96	0.20	0	0
13	417	0	3.0 (GE Signa)	0.59	0.04	2	0
14	316	1	1.5 (Aera)	0.80	0.07	2	0
15	34	1	3.0 (GE Signa)	0.73	0.08	2	0
16	72	15	1.5 (Avanto)	0.71	0.10	2	1
17	38	0	1.5 (Symphony)	1.12	0.21	2	1
18	68	1	1.5 (Symphony)	0.58	0.17	2	1
19	111	1	1.5 (Aera)	0.59	0.02	2	0

Control group

The control group included 40 randomly selected patients (20 males, median age 49.5 years) with early MRI-DWI scans, who underwent uneventful supratentorial brain surgery. Twenty-nine patients had a glioma; eight had metastatic lesions, meningioma, hemangioblastoma, and cavernoma were resected in one patient each. Early postoperative CT scans and MRI-DWI were available for all controls. The median time span between surgery and postoperative MRI was 2.0 days (range 1.0–6.0 days) (Fig. 1; Table 1).

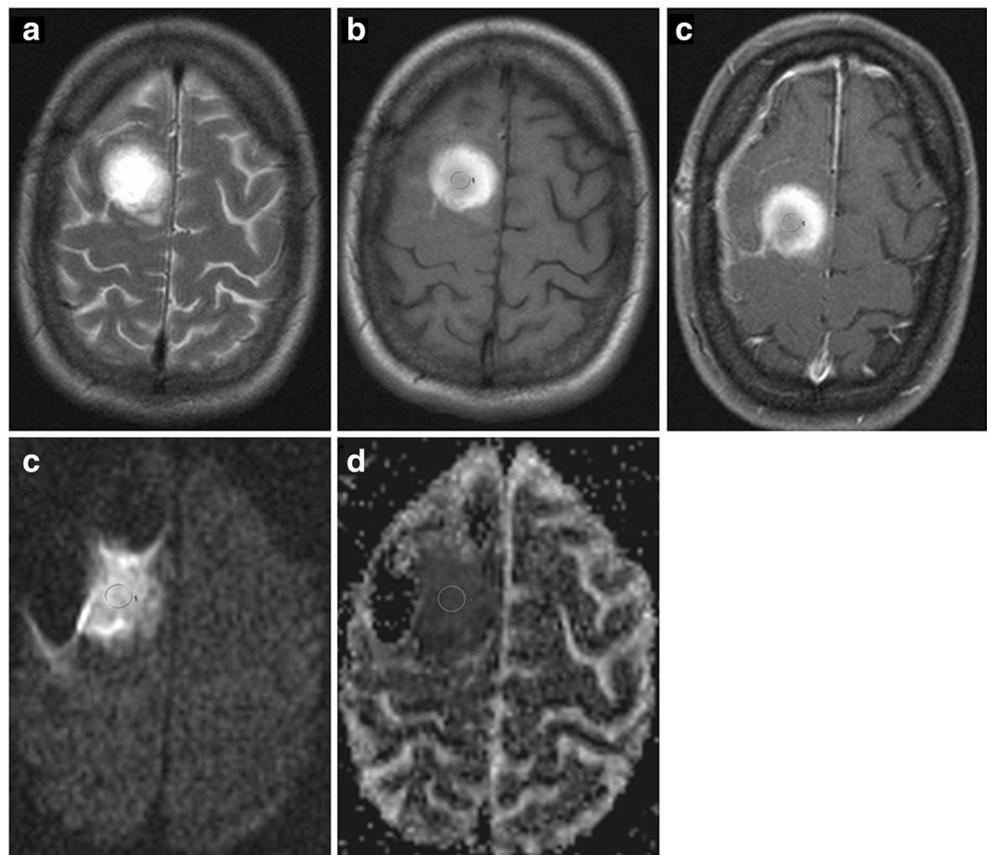
Computed tomography

According to in-house standards, a cranial computed tomography (CT) control the day after surgery was available for each patient. Two experienced neuroradiologists blinded to the actual clinical course of each patient independently performed retrospective evaluations of these CT scans. Blood degradation products within the resection cavity were judged visually and rated as either 0 = no blood or 1 = blood/hemorrhagic imbibition.

MRI and serum biomarker analysis

All MRI scans were retrospectively and independently evaluated by two neuroradiologists, who were both blinded to the patients' clinical course. The MRIs were obtained from three 1.5 T MRI scanners (Symphony, Avanto, and Aera; Siemens Healthcare, Erlangen, Germany) and one 3.0 T scanner (General Electric Medical Systems, Milwaukee, WI). The 8- and 20-channel head array coils were used. The protocol included non-contrast and contrast-enhanced (gadolinium) T1-weighted (T1w±ce), T2-weighted (T2w), fluid-attenuated inversion recovery (FLAIR) sequences, and DWI. The following DWI parameters were applied: DWI sequence type, spin-echo echo-planar imaging; repetition time ms/echo time ms, 5700/91; slice thickness, 5 mm with no gap; matrix, 130 × 130; and field of view, 23 cm². The ADC maps were generated from b0 and b1000 data. For calculation of the mean ADC value, a spherical/elliptical region of interest was placed within the resection cavity paying special attention to obvious b1000 signal hyperintensities. The ADC map was linked with the b1000 map by slice position; the region of interest was copied and transferred to the referring ADC map. Correct anatomic placement was verified linking the T2w with the b1000 images [31]. Attained ADC values were used for

Fig. 2 Suspected abscess after tumor resection. MRI shows no perifocal edema (**a** T2w), hyperintense blood degradation products (**b** T1w), and no contrast enhancement within the resection cavity (ROI in **b** and **c**) (**c** T1w+Gd). DWI reveals a homogenous bright signal (**d** b1000) with a hypointense corresponding ADC signal (**e**) indicating diffusion restriction



analyses; individual T1w±ce signals within the resection cavity were measured. Presence of perifocal edema was rated as 0 = no edema/decrease, 1 = unchanged, and 2 = increasing based on T2w/FLAIR images in comparison to the preoperative status (Figs. 2 and 3; Table 1).

Moreover, as per our clinical routine, we measured WBCC, CRP, and IL-6. These values measured on the day of the index MRI were used for the evaluation.

Statistical analysis

Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS, Inc., Chicago, USA) 17.0 and 23.0. Differences of mean DWI and ADC values for both groups (\pm blood/hemorrhagic imbibition in the resection cavity) were calculated using the Student's *t* test. Receiver operating characteristic (ROC) curves and area-under-the-curve (AUC) were used to determine the diagnostic potential of DWI/ADC and the serum markers WBCC, CRP, and IL-6. Outcome parameters were the determination of a cutoff value, the positive and likelihood ratios, sensitivities, and specificities. Significance levels were set at $p < 0.05$; maximized sensitivity and specificity were identified with Youden J statistics.

No study-specific investigations or surgeries were performed. Our local institutional review board approved this study.

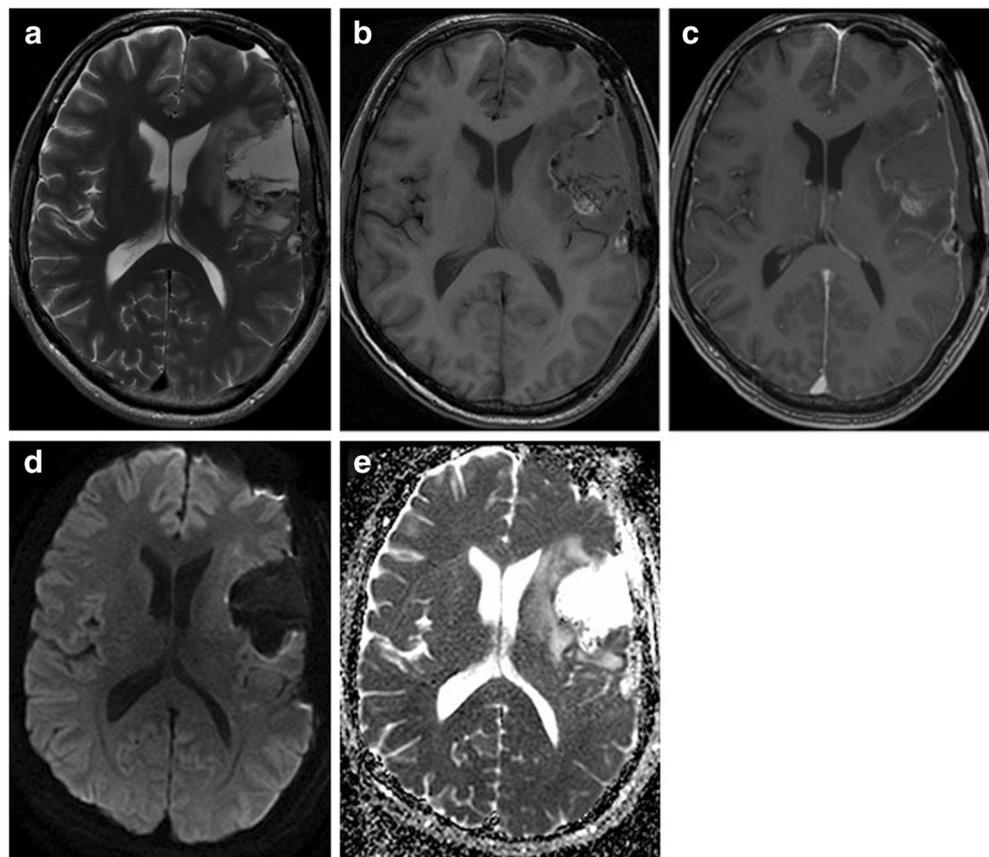
Results

MRI-DWI analysis

Abscess formation was associated with increasing perifocal edema in 11/19 patients; there was no significant accumulation of contrast media within the resection cavity in any of these patients. Furthermore, surgery-associated ischemia with relation to the resection cavity was not diagnosed in neither the abscess patients nor the control group. Postoperative imaging and surgical reports indicated blood/hemostatic agents in the resection cavity of 10/19 abscess patients and in 16/40 patients of the control group.

Mean ADC levels were significantly lower in patients with intracranial abscess formation compared to the aseptic controls ($0.82 \pm 0.23 \times 10^{-3} \text{ mm}^2/\text{s}$ vs. $2.95 \pm 0.24 \times 10^{-3} \text{ mm}^2/\text{s}$, $p < 0.01$). No significant correlation between the measured ADC values and the time intervals between the initial surgery and the abscess formation was found ($p = 0.233$) (Table 1).

Fig. 3 Unsuspectious postoperative control. Marked blood levels/hemorrhagic liquid within the resection cavity (**a** T2w hypointense; **b** native T1w intermediate-slightly hyperintense) and reactive contrast enhancement of the dura (**c**, T1w+Gd). The DWI (**d** b1000) and ADC-map (**e**) are without pathological signal alterations



The optimal ADC cutoff for the differentiation of an abscess from normal postoperative findings was found at $1.87 \times 10^{-3} \text{ mm}^2/\text{s}$ (area-under-the-curve 1.0, sensitivity = 100%; specificity = 100%) (Fig. 4). In case of the seven abscess patients, for whom an additional immediate postoperative MRI-DWI was available for further analyses, we also found the ADC values to be significantly lower in the MRI at the time of the abscess formation than in the immediate postoperative imaging. Moreover, the ADC values early after the initial surgery for these seven patients did not differ from the

respective values seen in the non-infectious controls ($2.95 \pm 0.24 \times 10^{-3} \text{ mm}^2/\text{s}$ vs. $2.77 \pm 0.20 \times 10^{-3} \text{ mm}^2/\text{s}$, *t* test, *p* = 0.11). The presence of blood/hemostatic agents in the resection cavity after first surgery did not affect mean ADC values, neither early after surgery (control group: blood/hemostatic material $2.96 \pm 0.22 \times 10^{-3} \text{ mm}^2/\text{s}$ vs. no blood/no hemostatic material $2.95 \pm 0.26 \times 10^{-3} \text{ mm}^2/\text{s}$, *t* test, *p* = 0.93) nor late at the time of abscess formation (abscess group: former blood/hemostatic material $0.87 \pm 0.66 \times 10^{-3} \text{ mm}^2/\text{s}$ vs. no former blood/hemostatic material $0.76 \pm 0.64 \times 10^{-3} \text{ mm}^2/\text{s}$, *t* test,

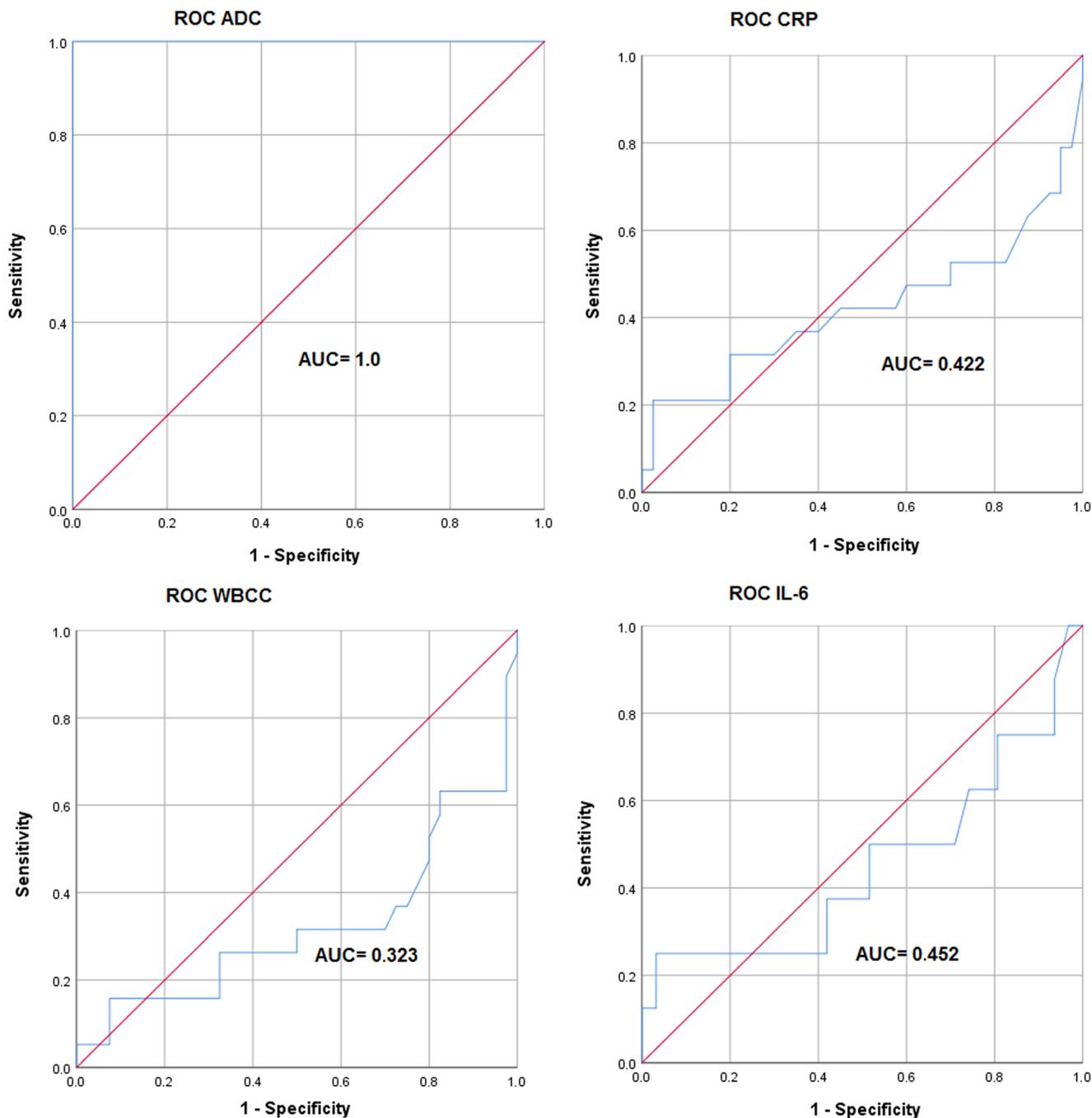


Fig. 4 Receiver operating characteristic (ROC) curves of ADC and inflammatory parameters for predicting postsurgical intracranial abscess formation

$p = 0.474$). No difference was seen between the respective values obtained from 1.5 to 3.0 T scanners (abscess group $0.88 \pm 0.41 \times 10^{-3} \text{ mm}^2/\text{s}$ vs. $0.75 \pm 0.24 \times 10^{-3} \text{ mm}^2/\text{s}$, t test, $p = 0.576$; control group $2.88 \pm 0.20 \times 10^{-3} \text{ mm}^2/\text{s}$ vs. $3.02 \pm 0.26 \times 10^{-3} \text{ mm}^2/\text{s}$, t test, $p = 0.382$) (Table 2).

Serum biomarkers

No difference between the abscess patients and the control group was seen with respect to WBCC ($16.4 \pm 33.6 \text{ G/L}$ vs. $11.3 \pm 3.6 \text{ G/L}$, $p = 0.34$), CRP ($4.9 \pm 6.9 \text{ mg/dL}$ vs. $3.8 \pm 3.8 \text{ mg/dL}$, $p = 0.41$), and IL-6 ($43.4 \pm 71.1 \text{ pg/ml}$ vs. $21.7 \pm 21.5 \text{ pg/ml}$, $p = 0.14$). The respective AUC values are summarized in Table 2.

Discussion

In the study at hand, we addressed the important issue of the diagnostic value and robustness of MRI-DWI for the detection of parenchymal infections in patients who underwent brain surgery. Our main findings were as follows: (1) MRI-DWI retains its robustness for detection of cerebral abscesses even in postoperative neurosurgical patients and (2) we were able to define an ADC cutoff value for differentiation between postoperative abscesses and normal postoperative changes/artifacts.

The overall low incidence of postoperative infections led us to opt for a retrospective design. Since indications for reoperation were made according to strict criteria and local infections were verified in each case, no false-positive MRI was seen in our series, which is in contrast to previous reports [11, 15, 16]. Patients, who had developed postoperative abscesses, underwent initial craniotomy for a wide range of indications including some which do not normally require early MRI follow-up examinations. Therefore, an early postoperative MRI was not available for all patients of the abscess cohort as an additional control for ADC measurements. In order to overcome this hindrance, we utilized a control group of random patients with early postoperative MRI after brain surgery. It is hereby important to point out that we recorded no statistically significant difference in ADC values on postoperative

MRI scans, obtained early after brain surgery, between the control group and the respective subpopulation of seven abscess patients for whom these data were available. In addition, the ADC values of these seven abscess patients were found to be significantly lower on MRI-DWI at the time of abscess formation than on their corresponding immediate postoperative MRI scans after the patients' initial neurosurgical procedures. Thus, this crucial aspect of our study design underscores that our taken approach, of utilizing and selecting a respective control group with routine postoperative MRI-DWI for conduction of our analyses, appears to be a feasible and reliable option. Furthermore, at the time of abscess formation, ADC values were significantly lower in the abscess group when compared to the controls. The respective measurements within the infected group were in line with those being found in case of spontaneous intraparenchymal abscesses [9, 27]. In accordance with the literature, ADC values were independent of the applied field strengths (1.5 vs. 3.0 T) [14–16]. An ADC cutoff value, highly indicative for abscesses, could be established at $1.87 \times 10^{-3} \text{ mm}^2/\text{s}$.

Only few previous studies have already addressed the usefulness of DWI for detecting intracranial infections in neurosurgical patients. It was often assumed that DWI may be non-specific in the postoperative setting and therefore insufficient for excluding postoperative intracranial abscesses [11, 16]. Unfortunately, no objective quantitative data and no ADC values were provided in these studies. One recent study provided quantitative data and found that ADC values were significantly higher (median ADC $1.34 \times 10^{-3} \text{ mm}^2/\text{s}$) in postoperative abscess formations compared with those in spontaneous abscesses (median ADC $0.68 \times 10^{-3} \text{ mm}^2/\text{s}$). Accordingly, a limited diagnostic value of DWI after brain surgery was assumed and influence of surgery-associated artifacts was suspected, although not systematically analyzed [22]. As stated above, in our study early postoperative, hemorrhagic imbibition and use of hemostatic agents did not alter the respective ADC values in either patient group. However, one important difference between our study and Lotan et al. study is that we did not include patients undergoing revision surgery for subdural/epidural infections, as results from ADC analysis may differ depending on the site of infection [11, 22]. Another study, which recently assessed MRI-DWI with regard

Table 2 Diagnostic potential of ADC and serum biomarkers for predicting intracranial abscess formation

	<i>N</i>	Abscess	Control	<i>p</i>	AUC	Cutoff	SE	SP	+LR	−LR
ADC ($\times 10^{-3} \text{ mm}^2/\text{s}$)	59	0.82 ± 0.23	2.95 ± 0.24	<0.01	1.0	1.87	100	100	∞	n.d.
WBCC (G/L)	59	16.4 ± 33.6	11.3 ± 3.6	0.34	0.323	17.4	11	93	2.1	0.9
CRP (mg/dL)	59	4.9 ± 6.9	3.8 ± 3.8	0.41	0.422	12.4	21	97	8.4	0.8
IL-6 (pg/ml)	39	43.4 ± 71.1	21.7 ± 21.5	0.14	0.452	12.7	50	48	1.0	1.0

N, number; *p*, *p* value; *AUC*, area-under-the-curve; *WBCC*, white blood cell count; *IL-6*, serum interleukin-6; *CRP*, serum C-reactive protein; *ADC*, apparent diffusion coefficient; *SE*, sensitivity; *SP*, specificity; +*LR*, positive likelihood ratio; −*LR*, negative likelihood ratio; *n.d.*, not defined

to its usefulness of detecting postoperative intracranial abscesses in neurosurgical patients, was published by Berndt et al. [4]. The study design utilized three subgroups consisting of patients with postoperative abscesses, spontaneous abscesses as well as controls to address this issue, and found the ADC values of postoperative abscesses to be significantly higher than that of spontaneous abscesses ($p < 0.001$), but still lower than in the control group ($p = 0.16$). The authors therefore concluded that MRI-DWI is only of limited value in evaluating postoperative brain infections and that MRI-DWI regains its validity at approximately 3 months after surgery. Thus, their findings are contrary to our results with regard to ADC values and our establishment of a cutoff value indicative of postoperative abscesses. The main reason for this might indeed be the difference in time intervals between the initial surgeries and abscess formations of their study population compared to ours (28 vs. 59 days). However, we found no significant correlation between the time intervals and ADC values in our patient cohort ($p = 0.233$).

From a clinical standpoint, patients with postoperative abscesses may become symptomatic with impaired wound healing, headache, signs of increased intracranial pressure, reduced physical resilience, and development of neurological deficits. The clinical signs and symptoms of our patients are in keeping with these usual findings. Laboratory tests including blood and CSF evaluation are only poorly investigated for predicting intracranial abscess formations [14, 24]. Approximately 60–90% of abscess patients have increased serum CRP levels, which may correlate with the severity of infection, but their specificity is low [24]. Only 30% of patients have a WBCC greater than 11 G/L [14]. Additionally, CSF is seldom helpful in establishing a differential diagnosis of brain abscess [20]. The role of serum IL-6 for predicting brain abscess has not been investigated previously. The low sensitivity and specificity of biomarkers might be explained by the assumed privileged shelter caused by the blood-brain barrier. In our study, CRP levels were not significantly altered in abscess patients when compared to early postoperative neurosurgical patients. Moreover, in our series, IL-6 levels in serum and WBCC were also not significantly increased in infectious patients when compared to early post-craniotomy patients.

Cranial neurosurgical interventions are associated with a postoperative inflammatory reaction, even in the case of a regular course of the surgery [2, 32]. At this point, the inflammatory parameters in the blood were similarly elevated when compared to patients with intracranial abscesses. Differentiation of the infectious pathology by means of biomarkers was therefore not possible in our patient population. After postoperative normalization of the inflammatory parameters, however, it can be assumed that a renewed increase of the inflammatory parameters (WBCC, CRP, IL-6) may be indicative of an infectious

disease, including intracranial infection and abscess formation.

Based on our above-reported findings, we do believe that MRI-DWI and ADC values are reliable diagnostic tools for detection of cerebral abscesses after brain surgery and probably superior to routine serum biomarkers. Nonetheless, all postoperative MRI-DWI and ADC data have to be interpreted with particular caution, and treatment decisions ought to be only made in the light of the patients' clinical status.

Conclusions

MRI-DWI represents a robust tool for identifying intracranial abscesses in patients after brain surgery. ADC values were significantly lower in the abscess than in the control group, were found to be independent of the applied field strength, and had a higher diagnostic potential than standard serum biomarkers. An ADC cutoff value, which was highly indicative for abscesses, could be established at $1.87 \times 10^{-3} \text{ mm}^2/\text{s}$. Postoperative changes did not impact ADC values. This study may help to reduce interobserver variability in DWI interpretation and may alleviate future treatment decisions in patients with suspected intracranial infections.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (ethics committee of the Ludwig-Maximilians-University Munich, Germany) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. For this type of study, formal consent is not required.

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