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Bacterial Brain Abscesses Expand Despite Effective Antibiotic Treatment: A Process Powered by Osmosis Due to Neutrophil Cell Death

Daniel Dahlberg, MD, PhD[*], Sverre Holm, MSc, PhD[‡], Ellen Margaret Lund Sagen, BSc[‡§], Annika Elisabet Michelsen, MSc, PhD[‡§], Maria Stensland, MSc, PhD[‖], Gustavo Antonio de Souza, MSc, PhD[‖¶], Ebba Gløersen Müller, MD, PhD[§#], James Patrick Connelly, MD, PhD[#], Mona-Elisabeth Revheim, MD, PhD[§#], Bente Halvorsen, MSc, PhD[‡§], Bjørnar Hassel, MD, PhD[§††‡‡]**

*Department of Neurosurgery, Oslo University Hospital, Oslo, Norway; [‡]Research Institute of Internal Medicine, Oslo University Hospital, Oslo, Norway; [§]Institute of Clinical Medicine, University of Oslo, Oslo, Norway; [‖]Institute of Immunology and Centre for Immune Regulation, Oslo University Hospital, Oslo, Norway; [¶]Department of Biochemistry, Universidade Federal Do Rio Grande Do Norte, Natal, Brazil; [#]Division of Radiology and Nuclear Medicine, Department of Nuclear Medicine, Oslo University Hospital, Oslo, Norway; **The Intervention Centre, Oslo University Hospital, Oslo, Norway; [††]Department of Neurohabilitation, Oslo University Hospital, Oslo, Norway; [‡‡]Norwegian Defence Research Establishment (FFI), Kjeller, Norway

Correspondence: Bjørnar Hassel, MD, PhD, Department of Neurohabilitation, Oslo University Hospital, Ullevål, Kirkeveien 166, 0450 Oslo, Norway.
Email: bjornar.hassel@medisin.uio.no

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BACKGROUND AND OBJECTIVES: A bacterial brain abscess is an emergency and should be drained of pus within 24 hours of diagnosis, as recently recommended. In this cross-sectional study, we investigated whether delaying pus drainage entails brain abscess expansion and what the underlying mechanism might be.

METHODS: Repeated brain MRI of 47 patients who did not undergo immediate pus drainage, pus osmolarity measurements, immunocytochemistry, proteomics, and ^{18}F-fluorodeoxyglucose positron emission tomography.

RESULTS: Time from first to last MRI before neurosurgery was 1 to 14 days. Abscesses expanded in all but 2 patients: The median average increase was 23% per day (range 0%-176%). Abscesses expanded during antibiotic therapy and even if the pus did not contain viable bacteria. In a separate patient cohort, we found that brain abscess pus tended to be hyperosmolar (median value 360 mOsm; range 266-497; n = 14; normal cerebrospinal fluid osmolarity is ~290 mOsm). Hyperosmolarity would draw water into the abscess cavity, causing abscess expansion in a ballooning manner through increased pressure in the abscess cavity. A mechanism likely underlying pus hyperosmolarity was the recruitment of neutrophils to the abscess cavity with ensuing neutrophil cell death and decomposition of neutrophil proteins and other macromolecules to osmolytes: Pus analysis showed the presence of neutrophil proteins (protein-arginine deiminases, citrullinated histone, myeloperoxidase, elastase, cathelicidin). Previous studies have shown very high levels of osmolytes (ammonia, amino acids) in brain abscess pus. ^{18}F-fluorodeoxyglucose positron emission tomography showed focal neocortical hypometabolism 1 to 8 years after brain abscess, indicating long-lasting damage to brain tissue.

CONCLUSION: Brain abscesses expand despite effective antibiotic treatment. Furthermore, brain abscesses cause lasting damage to surrounding brain tissue. These findings support drainage of brain abscesses within 24 hours of diagnosis.

KEY WORDS: Brain abscess, Neutrophil extracellular traps, Citrullination, Osmolarity, Suppuration, Pus, Abscess expansion

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ABBREVIATIONS: FDG-PET, ^{18}F-fluorodeoxyglucose positron emission tomography; **HMG,** high mobility group; **MMP,** matrix metalloproteinase; **MNDA,** myeloid cell nuclear differentiation antigen; **NET,** neutrophil extracellular traps; **NGAL,** neutrophil gelatinase-associated lipocalin; **PAD,** protein-arginine deiminase.

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A bacterial brain abscess is a collection of pus within the brain parenchyma.[1] The pus is encased in a fibrous capsule that limits the spread of pus into the surrounding brain tissue (Figure 1). A brain abscess may cause serious sequelae, such as epilepsy, cognitive disability, and fatigue.[2-5] Recent treatment recommendations include neurosurgical pus drainage within 24 hours of diagnosis.[6] One study found that delaying neurosurgery was associated with greater postoperative disability.[7] The reason for this effect of delaying abscess evacuation has never been addressed with respect to the underlying mechanism(s).

We hypothesized that, with time, a brain abscess would increase in size because the pus would be hyperosmolar, drawing water into the abscess cavity. A previous study of abscesses in the head and neck found pus to be highly hyperosmolar.[8] Similarly, previous studies have shown that brain abscess pus has high concentrations of ammonia, amino acids, and potassium together with physiological concentrations of sodium,[9-13] suggesting that brain abscess pus, too, is hyperosmolar. We further hypothesized that pus hyperosmolarity is caused by the continuous recruitment of neutrophils to the abscess cavity followed by neutrophil cell death and release of intracellular osmolytes. Research during the past 2 decades has highlighted a role for neutrophil cell death in the formation of an extracellular meshwork of deconvoluted DNA, histones, and other neutrophil proteins, termed neutrophil extracellular traps (NETs). NETs limit the spread of bacteria and facilitate the bactericidal activity of neutrophils.[14] NET formation could also contribute to the viscosity of pus. NET formation may occur during neutrophil cell death, which has been termed NETosis.[15]

To see whether brain abscesses expand with time, we examined repeated MRI of 47 patients who did not undergo pus evacuation immediately after the establishment of the brain abscess diagnosis. Of the 47 patients, 14 underwent ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) 1 to 8 years after brain abscess treatment to see whether the abscesses had caused tissue damage evident as neocortical hypometabolism. FDG-PET is well suited to identify focal brain damage evident as neocortical hypometabolism in conditions such as traumatic brain injury, stroke, and epilepsy.[16-18] We measured osmolarity in 14 brain abscess pus samples and, assuming that pus hyperosmolarity was caused by neutrophil cell death, looked for NETs and NET-related proteins in 20 brain abscess pus samples by immunocytochemistry and proteomics.

PATIENTS AND METHODS

Patients

This cross-sectional study was approved by The Regional Committee for Medical Research in South East Norway (Concessions #19305 and #371760); it was conducted in accordance with the World Medical Association's Declaration of Helsinki.[19] Patients were identified retrospectively (2000-2022) from the records at the Department of Neurosurgery, The National Hospital, Oslo, Norway. Patients who underwent MRI on 2 occasions, spaced at least 1 day apart, before neurosurgery were invited to participate. All patients experienced clinical deterioration between the 2 MRIs. All patients gave written informed consent. No patients withdrew from this study. Consent was waived for deceased patients.

MRI

All patients underwent preoperative MRI, including T1-weighted imaging before and after intravenous infusion of a gadolinium-based contrast agent (Clariscan, GE Healthcare), T2-weighted imaging, diffusion-weighted imaging, and apparent diffusion coefficient mapping, as described.[20] We calculated abscess volumes semiautomatically from postcontrast T1-weighted images with the Smartbrush program (Brainlab, Feldkirchen, Germany).

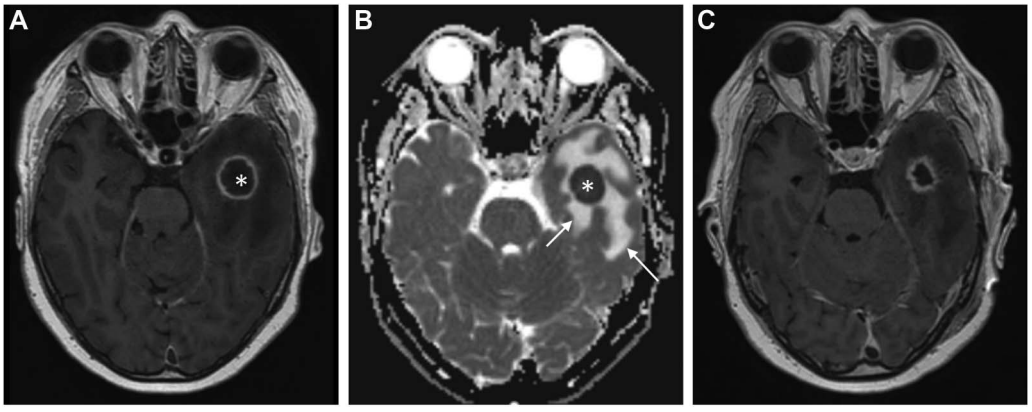

FIGURE 1. *Brain abscess in left temporal lobe.* **A**, *T1-weighted MRI before surgery, showing a brain abscess in the left temporal lobe (asterisk) as well as leakage of gadolinium contrast medium into the brain abscess capsule, which therefore has a bright appearance.* **B**, *Apparent diffusion coefficient map before surgery showing low diffusivity of the (dark) pus (asterisk) and the surrounding (light) edema (arrows).* **C**, *T1-weighted MRI 1 day after surgery, showing collapse of the abscess cavity.*

^{18}F-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography

For FDG-PET/computed tomography, patients fasted for at least 6 hours before the investigation. The median serum glucose value was 5.5 mmol/L (range 4.5-11.2) before intravenous injection of FDG (median value 154 MBq; range 99-261). After 69.5 minutes (median value; range 40-102), an 8-minute (median value; range 8-10) PET acquisition was performed together with a low-dose computed tomography scan.

In the acute stage of their brain abscess disease, these patients underwent pus evacuation through a burr hole less than 1 cm in diameter in the cranium with a steel cannula (diameter 2 mm).

Pus Examination

Microbial identification was achieved by polymerase chain reaction technique or bacterial culture, as per hospital routine.[11,20] Bacteria that grew on culture of pus samples were termed "viable," while bacteria that could not be cultured were termed "nonviable."

For osmolarity measurements, pus was centrifuged at 3000 *g* for 10 minutes at 4°C, and supernatants were analyzed with a K-7400S Knauer osmometer (Knauer, Berlin, Germany).

Freshly obtained pus was smeared onto microscope slides and air-dried before fixation in acetone. Some smears were stained with May-Grünewald-Giemsa stain, which verified the presence of neutrophils. Other smears were used for immunocytochemistry: After blocking (1% bovine serum albumin in Tris-buffered NaCl, 0.15 mol/L, pH 7.4, with 0.5% Tween 20) for 1 hour, the following antibodies were used for immunofluorescent staining: antineutrophil elastase (Clone NP57; mouse monoclonal, 1:100, MO752, DAKO) and antihistone H3 (citrulline R2 + R8 + R17; rabbit polyclonal, 1:100, NB100-57135, Novus-Biologicals). Appropriate Alexa-conjugated secondary antibodies were used (Alexa-488 goat anti-rabbit, 1:200, A11008, Alexa-568 donkey anti-mouse, 1:200, A10037). Samples were mounted in Slow Fade Gold antifade reagent (Invitrogen) containing diamidino-2-phenylindole (Thermo Fisher Scientific Inc) for staining of DNA. Images were taken with a Nikon Eclipse E400 microscope using the NIS-Elements BR Software.

For proteomics analysis, some of the pus sample was frozen without prior centrifugation (referred to as "cell-rich pus"), while some was centrifuged at 3000 *g* for 10 minutes at 4°C, and the supernatant was harvested. The pus samples (cell-rich and supernatants) were stored at −80°C until proteomics analysis, which was performed, as described earlier.[21] The proteomics data on pus supernatants have been published previously and are reproduced with permission from the publisher, The Journal of Neurosurgery Publishing Group.[21]

Pus formed clots after being drained from the abscess cavity. For clot dissolution, pus samples that had been stored at −80°C were weighed, thawed, and mixed with water 1:1. Vortexing (2500 rpm for 1 minute), trituration with a Pasteur glass pipette, and incubation with plasmin,[22] which targets fibrin, did not cause clot dissolution. We then incubated clotted pus (12-370 mg) with bovine DNase (Quantabio, Beverly, MA): 30 U/mL, final volume 1 mL, at 37°C for 30 minutes.

Outcome Measures, Data Presentation, and Statistics

Outcome measures were defined as changes in brain abscess volume presurgery, signs of focal neocortical hypometabolism postsurgery, pus osmolarity, and signs of NETosis. Abscess expansion is given as percent increase between first and last brain imaging or as average percent increase per day between the first and last brain imaging. Group comparisons were done with the Mann-Whitney *U* test, Fisher exact test, or Student *t*-test, paired or unpaired, as appropriate as according to Kolmogorov-Smirnoff normality testing. Correlations were determined with the Spearman test. A P value <.05 was considered statistically significant.

RESULTS

Patients, Microbes, and Antibiotics

Forty-seven patients, 17 women (8-80 years) and 30 men (6-86 years), had a bacterial abscess that primarily affected a frontal lobe (n = 15 patients), a temporal lobe (n = 9), a parietal lobe (n = 9), an occipital lobe (n = 6), cerebellum (n = 2), or the basal ganglia (n = 2). Four patients had more than 1 abscess. The most common microbial agent(s) identified were *Streptococcus intermedius* (27 patients), *Fusobacterium nucleatum* (4 patients), and *Aggregatibacter aphrophilus* (3 patients).

Of the 47 patients, 38 received antibiotic treatment before neurosurgery. Twenty of these 38 patients were subsequently drained of pus that did not contain viable bacteria, meaning that no bacteria grew in culture, aerobic or anaerobic. These patients had received antibiotics for a median of 11 days (Table 1). Twenty-seven patients had pus that contained viable bacteria. Of these, 18 patients had received antibiotic treatment, but only for a median of 3 days. All 9 patients who did not receive antibiotic treatment had viable bacteria in their brain abscess pus. The antibiotics that were most frequently used are presented in Table 1.

Brain Abscess Expansion and Effect of Antibiotic Treatment

The abscesses were initially of similar size in the groups of patients with and without viable bacteria in their pus; median values were 6.7 and 6.8 cm^3, respectively (Table 2). Abscesses increased significantly in size in both groups of patients (Table 2; Figures 2A and 2B); however, the increase was a near-significantly lower in the group with nonviable bacteria. The percent increase correlated with the number of days between the first and second brain imaging (r = 0.46; P = .012, for all 47 patients); this tendency was strongest among patients with pus containing viable bacteria (Table 2). Abscesses expanded irrespective of their volume on the first brain imaging. However, percent abscess expansion correlated inversely with abscess volume on first brain imaging (r = −0.56; P = .0004, for all 47 patients), meaning that, percentwise, smaller abscesses increased more than larger ones. This tendency was significant only for patients with pus containing viable bacteria (Table 2). In each patient group, there was 1 patient whose abscess did not expand.

The average increase in abscess size per day was 23% (median value; range 0-176) in the whole group of 47 patients. In patients with viable bacteria in their pus, the increase was 28% per day; in patients without viable bacteria, it was 13% per day (Table 2).

TABLE 1. Presurgery Treatment of Patients With Viable or Nonviable Bacteria in Brain Abscess Pus

	Viable bacteria in pus samples?	
Parameter	**Yes**	**No**
No. of patients (female/male)	27 (8/19)	20 (9/11)
Age of patients (y); median value (range)	58 (8-81)	52 (6-86)
Days of treatment of patients who received antibiotics before neurosurgery; median value (range)	3 (2-9)	11[a](2-18)
Ratio of patients on antibiotic treatment before neurosurgery	18/27	20/20
Ratio of patients on cefotaxime + metronidazole (range of days of treatment)	7/18 (1-9)	7/20 (2-17)
Ratio of patients on ceftriaxone + metronidazole (range of days of treatment)	4/18 (1-8)	4/20 (4-13)
Ratio of patients on penicillin + chloramphenicol + metronidazole (range of days of treatment)	2/18 (1-3)	5/20 (5-15)
Ratio of patients on corticosteroid treatment before neurosurgery	14/27	9/20
Days of corticosteroid treatment before neurosurgery; median value (range)	4.5 (1-90)	10 (1-15)[b]

Forty-seven patients underwent neurosurgical brain abscess pus drainage. Pus samples underwent culturing to see if the pus contained viable bacteria. Data are number of women and men in the 2 groups, patient age, and number of patients who received antibiotic treatment before neurosurgery. The 3 most common combinations of antibiotics are given together with the range of treatment duration (days). The 2 lower rows give the number of patients that received corticosteroid treatment before neurosurgical pus evacuation and corticosteroid treatment duration.

[a]P = .012 (Student unpaired t-test).

[b]Different from patients whose pus samples contained viable bacteria, P = .078 (Mann-Whitney U test).

Thus, antibiotic treatment did not prevent brain abscess expansion. The number of days between the first and last brain imaging differed between patients with and without viable bacteria in their pus; the median values were 3 and 4.5 days, respectively.

Some patients in each group received corticosteroid treatment before neurosurgery on the assumption that the abscess was a malignant tumor with a large peritumoral edema (Table 1). Duration of corticosteroid treatment did not correlate with percent abscess expansion per day (r = 0.06; P = .68).

There was no correlation between patient age on the one hand and abscess volume on first brain imaging (r = 0.06; P = .7), number of days between first and last brain imaging (r = 0.06; P = .7), or percent increase in abscess volume between first and last brain imaging (r = −0.10; P = .50) on the other. Nor was there a difference between women and men with respect to abscess volume on first brain imaging (P = .89), number of days between first and last brain imaging (P = .65), or percent increase in abscess volume between first and last brain imaging (P = 1.0).

TABLE 2. Brain Abscess Expansion in Patients With Viable or Nonviable Bacteria in Brain Abscess Pus at the Time of Neurosurgery

	Viable bacteria in pus samples?	
Abscess expansion with time	**Yes (n = 27)**	**No (n = 20)**
Abscess volume (cm^3) on first brain imaging; median value (range)	6.7 (1.0-40)	6.8 (1.2-36)
Abscess volume (cm^3) on last brain imaging; median value (range)	12.3 (3.5-48)[a]	14.1 (1.7-71)[a]
No. of days between first and last presurgery brain imaging; median value (range)	3 (1-8)	4.5 (1-14)[b]
Correlation of brain abscess expansion (%) with no. of days between first and last presurgery brain imaging	r = 0.53; P = .0050	r = 0.46; P = .042
Correlation of brain abscess expansion (%) with abscess volume on first brain imaging	r = −0.76; P = .00001	r = −0.26; P = .27
Percent brain abscess expansion per day; median average value (range)	28 (0-176)	13 (0-150)[c]

Forty-seven patients underwent neurosurgical brain abscess pus drainage. Pus samples underwent culturing to evaluate whether the pus contained viable bacteria or not. Data are abscess volume (cm^3) on first and second (last) brain imaging, number of days between the first and last brain imaging, and the percent brain abscess expansion per day between the first and last brain imaging investigation.

[a]Difference from abscess volume on first brain imaging, P < .01 (Student paired t-test).

[b]Difference from corresponding value for patients with nonviable bacteria in their brain abscess pus, P = .012 (Student unpaired t-test).

[c]Difference from patients whose pus samples contained viable bacteria, P = .056 (Mann-Whitney U test). Correlations were calculated with the Spearman test.

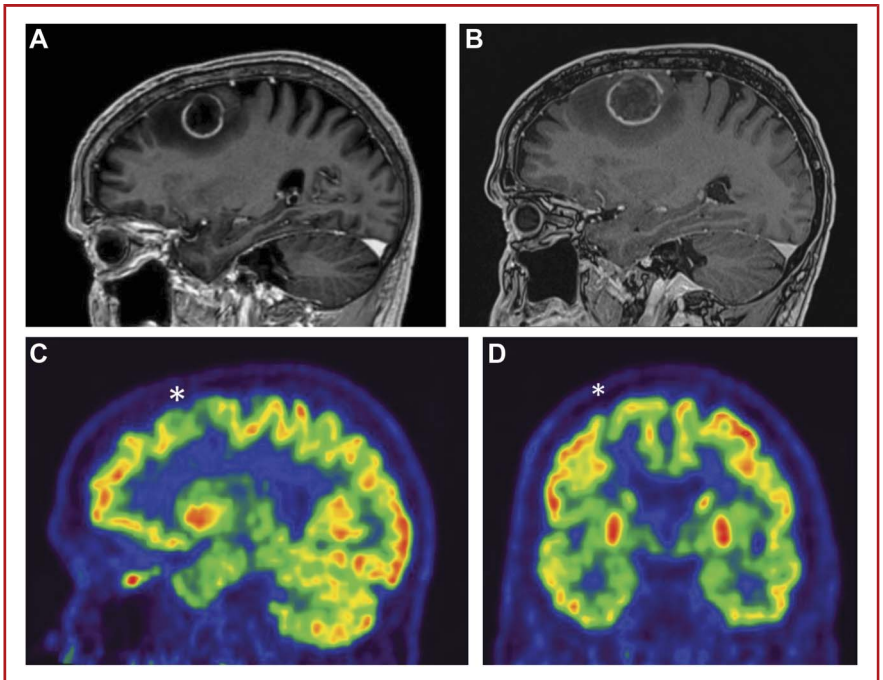

FIGURE 2. *Brain abscess expansion and neocortical hypometabolism.* **A**, *Sagittal T1-weighted MRI before surgery, showing gadolinium contrast enhancement of the brain abscess capsule.* **B**, *T1-weighted MRI 2 days after the first MRI, showing abscess expansion.* **C**, *and* **D**, *^{18}F-fluorodeoxyglucose positron emission tomography (sagittal and coronal, respectively) of the patient in* **A** *and* **B** *4 years and 7 months after brain abscess treatment, showing neocortical hypometabolism in the area where the abscess was located (asterisks).*

Pus Osmolarity

To see whether pus hyperosmolarity could be a cause of brain abscess expansion, we measured the osmolarity of brain abscess pus in a separate cohort of 14 patients. Pus osmolarity was 360 mOsm (median value; range 266-497). For comparison, a recent study found average osmolarity in cerebrospinal fluid at approximately 290 mOsm.[23]

We were able to investigate pus osmolarity in 3 patients whose brain abscess expanded 12.8%, 8.5%, and 10.4% per day over the course of 5, 4, and 1 days, respectively. Pus osmolarity at surgery was increased at 309, 315, and 389 mOsm, respectively. These patients were part of the 14 patients whose pus was examined for osmolarity, and they were part of the cohort of 47 patients who underwent repeated MRIs. All patients had received antibiotic treatment before neurosurgery, and in 2 of the patients, pus did not contain viable bacteria; in the third, *S. intermedius* grew sparingly on anaerobic culture.

Proteomics Analysis of NETosis-Related Proteins in Brain Abscess Pus

To see whether NETosis could be a mechanism underlying pus osmolarity, we performed a proteomics analysis. In 20 cell-rich brain abscess pus samples from a separate patient cohort, we found several proteins associated with NETs and NETosis (Table 3). The enzyme protein-arginine deiminase 4 (PAD4), which catalyzes citrullination of histone arginine residues in neutrophils, leading to decondensation of chromatin before extrusion,[36] was detected in most samples. Similarly, PAD2, which catalyzes citrullination of histones in macrophages in the formation of macrophage extracellular traps,[25] was detected in all 20 samples.

We compared proteomics results in cell-rich pus to those in supernatants obtained after sample centrifugation to see whether some NETosis-related proteins would be more strongly associated with the extracellular phase of pus (Table 3). Complement C5, complement factor H, thrombin, collagen α-1(I), and collagen α-3(VI) chains were detected in significantly more supernatants in line with these proteins being extracellular as well as being associated with extracellular traps.[30-33,35] Conversely, PAD4 and PAD2 were more strongly associated with cell-rich pus in agreement with their intracellular roles.[24,25,36,37]

Immunocytochemical Analysis of NETosis-Related Proteins in Brain Abscess Pus. Effect of DNase on Pus Clots

Immunocytochemical investigation of pus smears showed NETs in the form of extracellular decondensed DNA as well as citrullinated histone H3 and extracellular neutrophil elastase (Figure 3A). The density of these pus components varied from sample to sample.

TABLE 3. NET-Related and NETosis-Related Proteins and Some Interacting Complement and Coagulation Factors in Brain Abscess Pus

	NETosis-related proteins in cell-rich pus		NETosis-related proteins in pus supernatants	
Proteins	**n**	**Log-10 values**	**n**	**Log-10 values**
Histone 1.4[14]	19	8.3 ± 0.8	20	8.2 ± 0.5
Histone H2A[14]	16	8.2 ± 0.3	20	9.6 ± 0.5
Histone H2B[14]	20	9.7 ± 0.5	20	10.1 ± 0.6
Histone 3[14]	20	8.8 ± 0.4	20	9.5 ± 0.4
Histone H4[14]	20	9.5 ± 0.4	20	9.9 ± 0.9
PAD4[24]	18	7.3 ± 0.4	8[a]	7.2 ± 0.7
PAD2[25]	20	7.6 ± 0.5	15[b]	7.3 ± 0.8
HMG B1[26,27]	16	7.7 ± 0.6	17	7.6 ± 0.6
HMG B2[26,27]	20	8.1 ± 0.4	19	8.2 ± 0.5
MNDA[28]	18	8.1 ± 0.3	19	7.6 ± 0.8
α-Enolase[26]	20	9.1 ± 0.4	20	9.5 ± 0.6
Myeloperoxidase[14]	20	10.1 ± 0.3	20	10.8 ± 0.3
Cathepsin G[14]	20	9.3 ± 0.5	20	9.0 ± 0.6
Neutrophil elastase[14]	18	7.9 ± 0.4	20	8.7 ± 0.6
Lactotransferrin[14]	20	9.7 ± 0.4	20	10.5 ± 0.3
BPI[14]	20	8.5 ± 0.5	20	9.0 ± 0.7
Lipocalin2/NGAL[14]	20	8.7 ± 0.3	20	9.9 ± 0.3
S100-A9 } Calprotectin[28]	20	11.0 ± 0.1	20	11.5 ± 0.3
S100-A8 } Calprotectin[28]	20	10.2 ± 0.2	20	10.8 ± 0.2
Azurocidin[28]	17	8.9 ± 0.4	20	9.5 ± 0.4
Cathelicidin LL37[26,27]	20	8.0 ± 0.7	20	8.4 ± 0.8
MMP9[26,29]	20	8.7 ± 0.5	20	9.3 ± 0.6
Complement C3[30,31]	20	9.0 ± 0.5	20	10.3 ± 0.4
Complement C5[30,31]	3	7.9 ± 0.6	19[c]	8.1 ± 0.6
Complement factor B[30,31]	15	7.9 ± 0.6	20	8.8 ± 0.5
Complement factor H[30,31]	3	8.3 ± 0.3	18[c]	7.5 ± 0.9
von Willebrand factor[30,31]	1	8.6	10[a]	7.3 ± 0.7
Thrombin[32,33]	4	8.1 ± 0.3	17[c]	7.4 ± 0.5
Fibrinogen α chain[32,33]	18	8.1 ± 0.9	20	9.0 ± 0.6
Fibrinogen β chain[32,33]	20	8.6 ± 0.7	20	9.5 ± 0.7
Fibrinogen γ chain[32,33]	18	8.4 ± 0.8	20	9.1 ± 0.7
Fibronectin[32,34]	18	8.0 ± 0.8	20	9.1 ± 0.5
Collagen α-1(I) chain[35]	3	6.8 ± 0.3	12[a]	7.3 ± 0.6

TABLE 3. Continued.

Proteins	NETosis-related proteins in cell-rich pus		NETosis-related proteins in pus supernatants	
	n	Log-10 values	n	Log-10 values
Collagen α-2(IV) chain[35]	4	7.3 ± 0.3	6	6.9 ± 0.4
Collagen α-3(VI) chain[35]	1	6.5	18[c]	7.4 ± 0.5

BPI, bactericidal permeability-increasing protein; HMG, high mobility group; MMP, matrix metalloproteinase; MNDA, myeloid cell nuclear differentiation antigen; NET, neutrophil extracellular traps; NGAL, neutrophil gelatinase-associated lipocalin; PAD, protein-arginine deiminase.

[a]$P < .01$.

[b]$P = .047$.

[c]$P < .001$

Pus from 20 brain abscess patients was analyzed by mass spectrometry-based proteomics analysis, which identified several proteins that are associated with NET formation and NETosis. The samples were cell-rich, ie, not centrifuged, or centrifuged to obtain a supernatant. The columns show the number of positive samples (n) of the 20 samples and mass spectrometry raw data that were log-10 transformed; data are mean ± SD values for the positive samples. All proteins may originate from several cellular sources,[21,31] but neutrophils dominate pus and will be the major source, possibly with the exception of PAD2, which is highly expressed in macrophages and involved in formation of macrophage extracellular traps.[25] NET formation interacts with the complement and coagulation systems[30,31] and causes collagen formation by fibroblasts.[33,35] Protein S100-A9 and A8 make up calprotectin, which is a constituent of NETs. Note that some NET-related proteins are more highly represented in the cell-rich pus, whereas others are more highly represented in the extracellular pus fluid. Fifteen of the 20 patients received antibiotics before pus drainage, 5 did not. There was no difference in pus protein levels between these 2 groups ($P = .55$). Data on proteins in pus supernatants are from ref. 21, reproduced with permission from The Journal of Neurosurgery Publishing Group. Asterisks: difference from number of positive values in cell-rich pus. Fisher exact test. Superscript numbers refer to the reference list.

Cell-rich pus (23 samples) formed a clot that was highly viscous and resistant to mechanical dissolution. Incubation with bovine DNase for 30 minutes at 37°C led to complete dissolution of these clots (**Supplemental Digital Content 1**, **Supplemental Figure 1**, http://links.lww.com/NEU/E43), whereas attempts to achieve this with plasmin[22] were unsuccessful (see Methods). This observation illustrated a role for DNA in pus viscosity and clot formation.

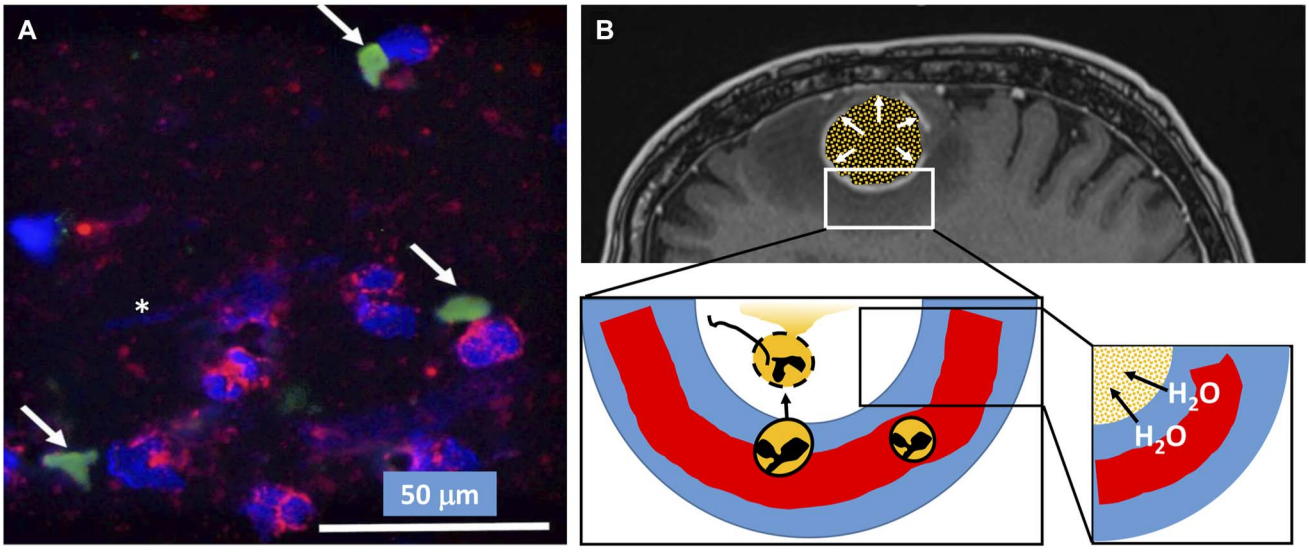

FIGURE 3. *NET formation in brain abscess pus and a model of brain abscess expansion caused by NETosis.* **A**, *Fresh brain abscess pus was smeared onto glass slides, air-dried and fixed in acetone before incubation with antibodies against citrullinated histone H3 (green; arrows) and neutrophil elastase (pink). DNA is stained with diamidino-2-phenylindole (blue). Note the presence of extracellular, deconvoluted DNA (asterisk) and extracellular elastase (small pink dots).* **B**, *A brain abscess in a frontal lobe with hyperosmolar pus (yellow) exerting pressure (arrows) on the abscess capsule. Left close-up: Neutrophils entering the abscess cavity from a capillary (red) in the abscess capsule (blue). Once inside the abscess, the neutrophils undergo NETosis, extruding DNA (black threads) and intracellular proteins (yellow), causing the formation of NETs. Decomposition of neutrophil macromolecules, eg to ammonia and amino acids, creates a hyperosmolar environment. Right close-up: Water is drawn from capillaries in the abscess capsule into the hyperosmolar pus, creating the pressure depicted by arrows in* **B**. *NET, neutrophil extracellular traps.*

FDG-PET Investigations

FDG-PET results were available for 14 of the 47 patients in whom brain abscesses expanded. These patients, who all had harbored abscesses in the forebrain, underwent FDG-PET 1 to 8 years after brain abscess surgery. In all the patients, hypometabolism was evident in the neocortex overlying the previous abscess, indicating permanent hypoactivity of the affected brain tissue (Figures 2C and 2D; **Supplemental Digital Content 2**, **Supplemental Figure 2**, http://links.lww.com/NEU/E44).

DISCUSSION

Brain Abscesses Increase in Size and Cause Tissue Damage if They Are Not Drained Neurosurgically

We show here that brain abscesses increase in size day by day. This expansion occurs in most patients even if they receive antibiotic treatment. We also show that brain abscesses cause lasting hypoactivity of the overlying neocortex, probably reflecting tissue damage. Whether the abscess expansion causes increasing damage to the surrounding brain tissue has not been conclusively shown in this study, but our findings support recent recommendations of rapid pus evacuation in brain abscess patients.[6]

NETosis Causes Hyperosmolarity of Brain Abscess Pus, Powering the Ballooning of Brain Abscesses

One likely mechanism underlying brain abscess expansion was pus hyperosmolarity, which would draw water into the abscess cavity; the water would probably come from capillaries in the highly vascularized brain abscess capsule (for model, see Figure 3B).[38,39] Pus hyperosmolarity and its attraction of water implies that high pressures are generated inside the abscess cavity. In abscesses in the head and neck area, Wiese measured pressures around 50 mm Hg,[8] which, if similar pressures occur in brain abscesses, may be compared with a mean intracranial pressure in the supine position of 11 mm Hg and to negative intracranial pressures in the upright position.[40,41] The pressure difference across the abscess capsule would be the force behind osmolarity-driven brain abscess expansion. However, for such pressures to occur in the abscess, the capsule must to some extent resist the physical pressure; otherwise, the abscess would rupture into the brain parenchyma. Indeed, brain abscess capsules contain a reticulin-collagen meshwork that would provide some degree of tensile strength.[38,42]

The mechanism behind the high osmolarity of pus probably relies on 2 factors. First, the continuous recruitment of neutrophils to the abscess cavity and their death by NETosis would supply the pus with macromolecules (protein, nucleic acids) that on their degradation would become potent osmolytes, such as amino acids and ammonia (Figure 3). Previous studies on brain abscess pus found high levels of amino acids (4-109 mmol/L), ammonia (1.7-69 mmol/L), and lactate (3-26 mmol/L) on a background of physiological concentrations of sodium.[11-13] Hyperosmolarity was not seen in all the examined pus samples; however, this observation, which may explain why abscess expansion varied between patients, echoes previous findings that the pus concentration of osmolytes varies among brain abscess patients.[11-13]

The second factor behind the high osmolarity of brain abscess pus has to be some form of restriction on the diffusion of small molecules out of the abscess cavity for the osmotic gradient to remain high enough to attract water. We currently do not know the mechanisms behind this restriction of diffusion, but the fact that the pus itself is not vascularized probably restricts osmolyte washout. The low diffusivity of the pus, which could be a result of NET formation,[14] probably also reduces washout of pus osmolytes by inhibiting the movement of small molecules.

NETosis may be triggered by gram-positive and gram-negative bacteria and interleukin 8 (IL-8).[14,24,43] In this study, we identified gram-positive (*S. intermedius*) and gram-negative (*F. nucleatum*, *A. aphrophilus*) bacteria that could induce NET formation. With respect to IL-8, we have recently reported high concentrations of this cytokine in brain abscess pus.[20] *In* vitro, Yang et al induced NET formation in human neutrophils with IL-8 at 10 000 pg/mL,[44] which is well within the concentration range found in brain abscess pus.[20]

CONCLUSION

We conclude that brain abscesses expand with time, even during effective antibiotic treatment. These findings support drainage of brain abscesses within 24 hours of diagnosis.

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Supplemental Digital Content 1. Supplemental Figure 1. Dissolution of pus clots by DNase treatment. A, Pus clots were resistant to mechanical dissolution and treatment with plasmin (which attacks fibrin). Please note that the pus clots remain undissolved at the bottom of the Eppendorf tubes. B, Treatment of the same samples in a DNase for 30 minutes at 37°C led to complete dissolution of the pus clots.

Supplemental Digital Content 2. Supplemental Figure 2. 14 Figures. Neocortical hypometabolism years after treatment for brain abscess. Fourteen brain abscesses patients underwent neurosurgical pus drainage followed by FDG-PET 1-8 years after neurosurgery to evaluate neocortical metabolism after brain abscess. The figures are MRIs and FDG-PET images. The images are presented in inverse chronological order, those with the shortest interval between presurgery MRI and postsurgery FDG-PET appearing first. Please note that black and blue colors indicate no or low uptake of ^{18}F-deoxyglucose and, consequently, no or low PET signal, whereas green-yellow, red, and white indicate progressively higher uptake of ^{18}F-deoxyglucose and PET signal.