Vascular Endothelial Growth Factor Concentration in Chronic Subdural Hematoma Fluid Is Related to Computed Tomography Appearance and Exudation Rate

Ralf Weigel,^{1,2} Axel Hohenstein,^{1,3} and Lothar Schilling¹

Abstract

Chronic subdural hematoma (CSH) is characterized by a net increase of volume over time. Major underlying mechanisms appear to be hemorrhagic episodes and a continuous exudation, which may be studied using labeled proteins to yield an exudation rate in a given patient. We tested the hypothesis that the concentration of vascular endothelial growth factor (VEGF) in hematoma fluid correlates with the rate of exudation. Concentration of VEGF was determined in 51 consecutive patients with CSH by the sandwich immune enzyme-linked immunosorbent assay technique. Mean values were correlated with exudation rates taken from the literature according to the appearance of CSH on computed tomography (CT) images. The CT appearance of each CSH was classified as hypodense, isodense, hyperdense, or mixed density. Mean VEGF concentration was highest in mixed-density hematomas ($22,403 \pm 4173$ pg/mL; mean $\pm$ standard error of the mean; $n=27$), followed by isodense (9715 ± 1287 pg/mL; $n=9$) and hypodense (5955 ± 610 pg/mL; $n=18$) hematomas. Only 1 patient with hyperdense hematoma fulfilled the inclusion criteria, and the concentration of VEGF found in this patient was 24,200 pg/mL. There was a statistically significant correlation between VEGF concentrations and exudation rates in the four classes of CT appearance ($r=0.98$). The current report is the first to suggest a pathophysiological link between the VEGF concentration and the exudation rate underlying the steady increase of hematoma volume and CT appearance. With this finding, the current report adds another piece of evidence in favor of the pathophysiological role of VEGF in the development of CSH, including mechanisms contributing to hematoma growth and CT appearance.

Key words: chronic subdural hematoma; CT classification; exudation; VEGF

Introduction

CHRONIC SUBDURAL HEMATOMA (CSH) is considered a rather benign disease of the elderly, although mortality rates up to 13% are reported in the contemporary literature.¹ Minor head trauma preceding development of CSH is documented in two thirds of patients; however, a CSH may also occur without such an obvious insult.² Clinically, patients present with neurological impairment of varying intensity, which is classified using the scoring system suggested by Markwalder some 30 years ago.³ Diagnosis is usually corroborated by computed tomography (CT) scanning showing different levels of density within the hematoma,² commonly based on the classification suggested by Nomura and co-workers.⁴ According to this classification, CSH images may appear as hypodense, isodense, hyperdense, or display mixed density.

The pathophysiology of CSH is not yet clear. Minor head trauma may lead to shear stress on the loose junction between the inner and outer layer of the dura and may initiate a cascade of bleeding,

rebleeding, and exudation, which finally results in the formation of an increasing cavity within the dura mater. In this sequence of pathophysiological events, the parietal membrane adjacent to the subdural space appears to play an important role. Morphologically, the microvasculature of the parietal layer is grossly altered with an increase of vessel density, capillary diameter, and the occurrence of large intercellular gaps between the endothelial cells (ECs).^{5,6} These alterations have been related to increased local inflammatory activity,⁷ intermittent episodes of (re)bleeding,⁸ and exudation of proteins and plasma water.⁹ Recently, our group has reported extremely high concentrations of vascular endothelial growth factor (VEGF) in hematoma fluid.^{10,11} In addition, we found an inverse angiopoietin-1/angiopoietin-2 (Ang-1/Ang-2) messenger RNA ratio in tissue samples obtained from the parietal membrane.¹¹ Based on these data, we suggested CSH to be an angiogenic disease.¹¹

Previously, Tokmak and coworkers⁹ have related CT scan characteristics of CSH to rate of exudation into the hematoma cavity.

¹Division of Neurosurgical Research, Medical Faculty Mannheim, Ruprecht-Karls-University Heidelberg, Heidelberg, Germany.

²Current address: Department of Neurosurgery, St. Katharinen Hospital, Frankfurt/Main, Germany.

³Current address: Bioassay, Biological Analytics GmbH, Heidelberg, Germany.

These measurements were based on the detection of technetium-99m-labeled human serum albumin in hematoma fluid after intravenous injection. Exudation rate was found to be highest in CSH with hyperdense appearance, whereas it was lowest when the hematoma was hypodense. Given the well-established permeability-promoting effect of VEGF, we have reevaluated our patient database and related the concentrations of VEGF in hematoma fluid to the CT characteristics and the related exudation rates from the literature.

Methods

We have previously initiated a prospective study according to the principles of the local ethics committee (Faculty of Clinical Medicine Mannheim Ruprecht-Karls-University Heidelberg, Heidelberg, Germany) to measure concentrations of different growth factors in the hematoma fluid of patients suffering from CSH.^{10–12} Each patient had a careful clinical and neurosurgical assessment on admission, using a standard protocol. The protocol also included patient data, history of trauma, actual medication, and the presence of hematoma fluid in CT images. For the current study, 51 patients equivalent to 55 cases of hematoma (in patients with bilateral hematomas [$n=4$] each side was counted as one case) were included. The study was approved by the medical ethics committee II of the Medical Faculty Mannheim (approval no. 2012-292N-MA). Exclusion criteria included any neurological impairment not related to CSH, hereditary coagulation disorders (e.g., hemophilia), hepatic diseases, a known malignancy, and any anticoagulation treatment, most notably, warfarin and acetylsalicylic acid. These criteria were defined to exclude any systemic coagulation impairment as a potential cause of bleeding into the hematoma cavity. Treatment with an angiotensin-converting enzyme (ACE) inhibitor was noted, but not considered an exclusion criterion. The appearance of CSH on CT was assessed before surgery (by R.W.), according to the classification proposed by Nomura and colleagues⁴ and modified by Tokmak and coworkers,⁹ using the terms hypodense, isodense, hyperdense, and mixed density.

Neurosurgical procedure

All patients underwent surgical evacuation of hematoma fluid under general anesthesia, using a standard burr hole procedure, as described previously.¹¹ After excision of the dura and opening of the outer membrane, hematoma fluid was aspirated into ethylenediaminetetraacetic acid-containing vials (Monovette; Sarstedt, Nümbrecht, Germany). The vials were quickly transferred to the laboratory, divided for further investigations, and centrifuged (10 min, 1700g, 4°C). Supernatants were removed and stored at -80°C until assayed.

Measurement of vascular endothelial growth factor in hematoma fluid and plasma

Measurement of VEGF concentration was performed using a sandwich enzyme immunoassay (R&D Systems, Wiesbaden,

Germany). Briefly, hematoma fluid was diluted up to 1:100 with assay diluent reagent. The test results in generation of a yellow reaction product, and light absorption was measured using a microplate reader (Multiskan Plus MKII; Titertek Instruments, Huntsville, AL) equipped with a 450-nm filter. A standard curve was generated and used to determine the actual VEGF concentration in individual samples with the background intensity subtracted. All measurements were performed in duplicates. The person to do the VEGF analysis (A.H.) was not aware of the CT classification.

Statistical analysis

Concentration of VEGF in hematoma fluid was related to the CT classes of appearance and to the mean exudation rates available. Differences of mean concentrations between groups were evaluated using one-way analysis of variance. Statistical analysis was performed using SigmaStat 2000 (Systat Software, Inc., San Jose, CA) and Microsoft Excel statistical software (Mac Version 2008; Microsoft Corporation, Redmond, WA).

Results

Epidemiological and clinical data of the patients included are presented in Table 1. In the majority of cases, a CSH with mixed-density appearance was found ($n=27$), followed by hypodense CSH ($n=18$) and isodense CSH ($n=9$). A homogeneously hyperdense hematoma was noted in only 1 patient who fulfilled the inclusion criteria.

Average concentration of VEGF was highest in mixed-density hematomas ($22,403 \pm 4173$ pg/mL; mean $\pm$ standard error of the mean [SEM]). The patient presenting with hyperdense hematoma had a comparably high level of VEGF in hematoma fluid ($24,200$ pg/mL). The concentration of VEGF was markedly lower in isodense hematomas (9715 ± 1287 pg/mL; $p < 0.05$ vs. mixed-density hematomas) and was lowest in hypodense hematomas (5955 ± 610 pg/mL; $p < 0.05$ vs. mixed-density hematomas).

Subgroup analysis showed that VEGF concentration was generally lower in patients with ACE inhibitor treatment for arterial hypertension. In the mixed-density hematoma group, VEGF concentrations were $10,735 \pm 2525$ pg/mL in patients with ACE treatment ($n=5$), as opposed to $25,055 \pm 4937$ pg/mL without ACE treatment ($n=22$; $p < 0.05$ vs. ACE-treated patients). Similar differences, although not reaching statistical significance, were observed in the isodense hematoma group (8186 ± 814 pg/mL with ACE treatment [$n=3$] and $10,479 \pm 1868$ pg/mL without ACE treatment; $n=6$) and for hypodense hematomas (5747 ± 541 pg/mL with ACE treatment [$n=5$] and 6035 ± 831 pg/mL without ACE treatment; $n=13$).

We then related VEGF concentrations in hematoma fluid of the different CT classes to the relative exudation rates published by Tokmak and coworkers.⁹ Respective data for all patients are shown

TABLE 1. DEMOGRAPHIC DATA OF 55 CASES OF CHRONIC SUBDURAL HEMATOMA INCLUDED IN THE PRESENT STUDY

Demographic data	Hypodense	Isodense	Hyperdense	Mixed density	Significance
<i>n</i>	18	9	1	27	
Age (years)	73 ± 3	71 ± 3	75	72 ± 3	n.s.
Gender	15 m, 3 f	6 m, 3 f	1 m	23 m, 4 f	n.s.
Pre-OP score	1.6 ± 0.2	1.7 ± 0.2	1	1.7 ± 0.1	n.s.
Post-OP score	0.4 ± 0.1	0.7 ± 0.3	2	0.7 ± 0.1	n.s.
Recurrence	1	2	0	4	n.s.

Pre- and post-OP score refers to the Markwalder scoring, which was noted before surgery and at the day of discharge. Mean $\pm$ standard error of the mean are given.

Pre-OP, preoperative; post-OP, postoperative; m, male; f, female; n.s., not significant.

FIG. 1. Mean concentrations $\pm$ standard error of the mean (SEM) of vascular endothelial growth factor (VEGF) in hematoma fluid and mean exudation rates $\pm$ SEM in classes of patients suffering from chronic subdural hematoma. Patients were classified according to the computed tomography (CT) appearance of the hematoma. The appropriate exudation rates were obtained from data published by Tokmak and coworkers.⁹ The correlation coefficient for mean VEGF concentrations and mean exudation rates is highly significant.

in Figure 1. Exudation rates and VEGF concentrations proved to be clearly related, yielding a highly significant correlation ($r=0.987$; $p=0.01$). This significance persisted even in the subgroup analysis with respect to ACE treatment (results not shown).

Finally, we also looked at the recurrence rate necessitating reoperation in the different patient groups. The overall low number of recurrences allows only descriptive analysis. A total of 7 patients had a recurrent hematoma. The sequence of CT classes was hypodense hematomas (1 case of recurrence) < isodense hematoma (2 cases of recurrence) < mixed density (4 cases of recurrence). This sequence is in agreement with the increase of average VEGF concentrations in hematoma fluid.

Discussion

The results of the present study demonstrate a clear relationship between VEGF concentration in hematoma fluid and appearance of CSH in CT scans. Further, concentrations of VEGF also showed a statistically significant correlation with rates of plasma exudation into hematoma fluid reported on previously.⁹ Significance of correlation was independent of ACE inhibitor treatment for arterial hypertension. Thus, the data lend further support to the assumption of the concentration of VEGF in hematoma fluid being a highly important mechanistic factor in the pathophysiology of CSH and its steady enlargement.

In the present study, we divided CSH patients according to the widely accepted classification of CT appearance (for a short description, see below), as suggested by Nomura and coworkers.^{4,9} Most CT images from our CSH patients showed hematomas with mixed density, followed by hypo- and isodense hematomas. This distribution of CSH types is largely comparable with previously published reports.^{4,9,13} However, there was only 1 patient in the current study with a homogeneously hyperdense hematoma who met the inclusion criteria. Obviously, most patients presenting with hyperdense hematomas in our clinic received anticoagulation treatment with warfarin or similar compounds. Yet, anticoagulation therapy was an exclusion criterion in the current study because we

aimed at characterizing local, instead of systemic, mechanisms potentially involved in the pathophysiology of CSH enlargement.

It is generally accepted that CT appearance is related to the exudation and occurrence of bleeding episodes. Thus, fresh blood results in a hyperdense appearance while a long period without hemorrhage would allow breakdown and clearance of blood products, resulting in a subsequent iso- or hypodense appearance. Repeated bleeding events alternating with periods of exudation lead to a sedimentation-like layering and, consequently, a mixed-density CT appearance. In accord, we obtained a great variability of VEGF concentrations in mixed-density hematomas, which might well depend on sampling issues resulting from sedimentation and pseudochambering of hematomas.

A steady increase of hematoma volume is a hallmark of CSH. In the first empirical description based on microscopic studies, Virchow¹⁴ suggested an inflammatory pathogenesis. Subsequently, Gardner¹⁵ postulated an osmotic gradient between the subdural and intravascular space as the driving force for the steady growth of a CSH. Since the 1970s, a local disproportion of coagulation and fibrinolysis was assumed.¹⁶ During the last few years, a growing body of evidence has been accumulated, pointing to an involvement of angiogenic mechanisms in the propagation of CSH.¹⁷ This assumption is based on several major results, including 1) a specific pattern of growth factor distribution within the hematoma with an extremely high concentration of VEGF, but a lower than plasma level of platelet-derived growth factor (PDGF),¹⁰ 2) a relative overexpression of Ang-2 over Ang-1 in tissue samples taken from the parietal membrane during surgery,¹¹ and 3) a lower recurrence rate in hypertensive patients receiving ACE inhibitor treatment, which has antiangiogenic side effects.¹² The major effects of Ang-2 include a destabilization of the wall of mature blood vessels and, in combination with VEGF, an induction of new vessel sprouting. Together with the low concentration of PDGF, which is known to play a major role in maturation of newly formed blood vessels, this angiogenic activity results in the formation of immature macrocapillaries displaying a pathological wall structure with fenestrations and large gaps between their ECs. These ultrastructural features, resulting in a leaky vessel wall, have already been described in the late 1970s by Friede and Schachenmayr.⁵

Secretion of VEGF from local sources, including cells within hematoma fluid and the parietal membrane,¹¹ may well be considered an important pathophysiological factor resulting from the proinflammatory environment in the CSH cavity. A high secretion rate and accumulation of VEGF in hematoma fluid will lead to continuous formation of immature, fragile blood vessels, which are prone to hemorrhagic events. In addition, high concentration levels of VEGF will also result in an increased transfer of plasma components, including proteins into the hematoma cavity (i.e., a high exudation rate), because enhancement of vascular permeability is a major feature of VEGF. In the absence of recent bleeding episodes, concentration of VEGF as well as other proteins, such as albumin, in hematoma fluid is regulated by the balance of secretion and/or extravasation, on the one hand, and breakdown and clearance mechanisms, on the other hand, leading to a "moderately" increased level (i.e., approximately 50-fold over plasma) of VEGF. Further, breakdown of erythrocytes and elimination of hemoglobin will result in hypodense CT appearance. Any bleeding episode may increase inflammatory activity in the hematoma cavity and stimulate VEGF release, leading to a massive concentration rise. Thus, it is conceivable that the highest VEGF levels are found in hematomas of hyperdense appearance. Similarly, high levels may also be expected in mixed-density hematomas if a sample is taken from a

layer with recent bleeding. In contrast, lower levels will be found in layers in which exudation prevailed. This assumption is supported by the conspicuously high variation of VEGF values measured in patients presenting with mixed-density CT appearance. The results of the current study lend further support to the hypothesis that CSH might well be considered a member of the so-called angiogenic diseases, as suggested previously.^{10,11,18} In this class of diseases, the relation between pathological angiogenesis and rebleeding or exudation is widely accepted.¹⁹ In diabetic retinopathy, antiangiogenic treatment is, meanwhile, a routine part of successful disease management.²⁰ Unfortunately, to date, there is no valuable animal model to test the relationship between angiogenesis and enlargement of CSH. Therefore, clinical data are of elementary importance. In this respect, treatment of patients with drugs carrying antiangiogenic side effects might become a worthwhile approach. In a recent analysis of 320 patients, we have provided evidence in favor of ACE inhibitors used for treatment of arterial hypertension to lower the risk of recurrence in CSH. This decrease was related to a lower concentration of VEGF in hematoma fluid and therefore may be ascribed to antiangiogenic properties of ACE inhibitors.¹² Moreover, the group of CSH patients treated with ACE inhibitors appeared to represent a negative selection, because the number of patients with ACE inhibition was significantly lower in the CSH group, compared to a representative age-matched control group.

In the present study, we describe a significant correlation between concentrations of VEGF in the different classes of CT appearance and the respective exudation rates previously reported by Tokmak and coworkers.⁹ This is another piece of evidence suggesting that the high VEGF concentration in the hematoma fluid is of major pathophysiological importance in the generation and steady increase of the hematoma volume and in determining the CT appearance. The pathomechanisms involved are repeated bleeding episodes due to ongoing pathological angiogenesis and exudation of plasma components resulting from high vessel permeability. Although this does not exclude some contribution of other factors, the use of compounds to interfere with the action of VEGF, such as receptor blockers or inhibitors of the second messenger pathways, may well be considered a worthwhile approach for clinical testing in CSH, which, despite being a benign disease, is nevertheless accompanied by a high rate of mortality in the predominantly affected group of old patients.²¹

Acknowledgment

This study was supported by a grant from the Forschungsfonds of the Faculty of Clinical Medicine Mannheim, University Heidelberg (FF 932031.1).

Author Disclosure Statement

No competing financial interests exist.

References

1. Weigel, R., Schmiedek, P., and Krauss, J.K. (2003). Outcome of contemporary surgery for chronic subdural haematoma: evidence based review. *J. Neurol. Neurosurg. Psychiatry* 74, 937–943.
2. Krauss, J.K., Marshall, L.F., and Weigel, R. (2011). Medical and surgical management of subdural hematomas. in: *Youmans Neurological Surgery*. H.R. Winn (ed). Elsevier Saunders: Philadelphia, pps. 535–543.
3. Markwalder, T.M. (1981). Chronic subdural hematomas: a review. *J. Neurosurg.* 54, 637–645.
4. Nomura, S., Kashiwagi, S., Fujisawa, H., Ito, H., and Nakamura, K. (1994). Characterization of local hyperfibrinolysis in chronic subdural hematomas by SDS-PAGE and immunoblot. *J. Neurosurg.* 81, 910–913.
5. Friede, R.L., and Schachenmayr, W. (1978). The origin of subdural neomembranes. II. Fine structural of neomembranes. *Am. J. Pathol.* 92, 69–84.
6. Yamashima, T., Yamamoto, S., and Friede, R.L. (1983). The role of endothelial gap junctions in the enlargement of chronic subdural hematomas. *J. Neurosurg.* 59, 298–303.
7. Frati, A., Salvati, M., Mainiero, F., Ippoliti, F., Rocchi, G., Raco, A., Caroli, E., Cantore, G., and Delfini, R. (2004). Inflammation markers and risk factors for recurrence in 35 patients with a posttraumatic chronic subdural hematoma: a prospective study. *J. Neurosurg.* 100, 24–32.
8. Ito, H., Yamamoto, S., Saito, K., Ikeda, K., and Hisada, K. (1987). Quantitative estimation of hemorrhage in chronic subdural hematoma using the ⁵¹Cr erythrocyte labeling method. *J. Neurosurg.* 66, 862–864.
9. Tokmak, M., Iplikcioglu, A.C., Bek, S., Gokduman, C.A., and Erdal, M. (2007). The role of exudation in chronic subdural hematomas. *J. Neurosurg.* 107, 290–295.
10. Weigel, R., Schilling, L., and Schmiedek, P. (2001). Specific pattern of growth factor distribution in chronic subdural hematoma (CSH): evidence for an angiogenic disease. *Acta Neurochir.* 143, 811–818.
11. Hohenstein, A., Erber, R., Schilling, L., and Weigel, R. (2005). Increased mRNA expression of VEGF within the hematoma and imbalance of angiopoietin-1 and-2 mRNA within the neomembranes of chronic subdural hematoma. *J. Neurotrauma* 22, 518–528.
12. Weigel, R., Hohenstein, A., Schlickum, L., Weiss, C., and Schilling, L. (2007). Angiotensin converting enzyme inhibition for arterial hypertension reduces the risk of recurrence in patients with chronic subdural hematoma possibly by an antiangiogenic mechanism. *Neurosurgery* 61, 788–792.
13. Kostasian, V., Choi, J.C., Liker, M.A., Go, J.L., and Zee, C.S. (2000). Computed tomographic characteristics of chronic subdural hematomas. *Neurosurg. Clin. N. Am.* 11, 479–489.
14. Virchow, R. (1857). Das hämatom der dura mater (Hematoma of the dura matter). *Verh. Phys. Med. Ges. Würzburg* 7, 134–142.
15. Gardner, W.J. (1932). Traumatic subdural hematoma with particular reference to the latent interval. *Arch. Neurol. Psychiatry* 27, 847–858.
16. Ito, H., Yamamoto, S., Komai, T., and Mizukoshi, H. (1976). Role of local hyperfibrinolysis in the etiology of chronic subdural hematoma. *J. Neurosurg.* 45, 26–31.
17. Weigel, R., and Krauss, J.K. (2004). Chronic subdural hematoma in the elderly. in: *Advances in Clinical Neurosciences*. K.K. Sinha, and P. Chandra (eds). East Zone Neuro DME: Ranchi, India, pps. 231–252.
18. Leibovitz, A., Baumohl, Y., Segal, R., and Habot, B. (2001). Age-associated neovasculopathy with recurrent bleeding. *Med. Hypotheses* 57, 616–618.
19. Folkman, J. (1995). Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat. Med.* 1, 27–31.
20. Hogeboom van Buggenum, I.M., Polak, B.C., Reichert-Thoen, J.W., de Vries-Knoppert, W.A., van Hinsbergh, V.W., and Tangelde, G.J. (2002). Angiotensin converting enzyme inhibiting therapy is associated with lower vitreous vascular endothelial growth factor concentrations in patients with proliferative diabetic retinopathy. *Diabetologia* 45, 203–209.
21. Miranda, L.B., Braxton, E., Hobbs, J., and Quigley, M.R. (2011). Chronic subdural hematoma in the elderly: not a benign disease. *J. Neurosurg.* 114, 72–76.

Address correspondence to:

Lothar Schilling, MD, PhD

Division of Neurosurgical Research

Medical Faculty Mannheim

Ruprecht-Karls-University Heidelberg

Theodor Kutzer-Ufer 1–3

68165 Mannheim, Germany

E-mail: lothar.schilling@medma.uni-heidelberg.de