Haptoglobin Type and the Development of Delayed Ischemic Neurological Deficits After Subarachnoid Hemorrhage

The Haptoglobin-Vasospasm Study Group**:**

Northern Neurosciences, Inc.: Mark Borsody, M.D., Ph.D.*; Tamara Heaton, M.S.; Chisa Yamada, M.D.

"La Sapienza" University of Rome: Federico Bilotta, M.D., Ph.D. (Department of Anesthesiology); Andrea Doronzio, M.D. (Department of Anesthesiology); Giovanni Rosa, M.D. (Department of Anesthesiology); Antonio Santoro, M.D. (Department of Neurosurgery)

Department of Pediatrics, Wayne State University, Detroit, Michigan: Dawn M. Bielawski, Ph.D.

Department of Neurology, Wayne State University / The Detroit Medical Center, Detroit, Michigan: Abraham Kuruvilla, M.D.

Department of Neurology, Northwestern Memorial Hospital, Chicago, Illinois: Allan Burke, M.D.

Rappaport Faculty of Medicine, Technion Institute of Technology, Haifa, Israel: Andrew Levy, M.D., Ph.D.; Rachel Miller-Lotan, Ph.D.

* author for correspondence and reprints: Northern Neurosciences, Inc.
1297 Hedgerow Dr.
Grayslake, IL 60030
mborsody@northern-neurosciences.com

Conflicts of Interest / Disclosures: The authors have no conflicts of interest or disclosures related to this study.

Acknowledgements: Support for this research was provided by grants from the American Heart Association and the Northwestern Memorial Hospital Women's Board, and by Northern Neurosciences, Inc. (www.northern-neurosciences.com), a not-for-profit organization dedicated to the advancement of neuroscience research.

Cover Title: Haptoglobin and DINDs

Figures: none

Tables: 5

Keywords: risk factors; subarachnoid hemorrhage; vasospasm; delayed ischemic neurological deficit

Word Count, Abstract: 246

Word Count, Text: 4473

Abstract

Object: Previously we have shown that angiographic vasospasm occurs at a higher rate in subarachnoid hemorrhage (SAH) patients who have haptoglobin containing the α2 subunit. Here we examine the relationship between haptoglobin type and the development of delayed ischemic neurological deficits (DINDs) after SAH. We hypothesized that SAH patients who have haptoglobin containing the α2 subunit are more likely to develop DINDs than are SAH patients who have haptoglobin α1-α1.

Methods: A total of 49 patients with Fisher grade 3 SAH were examined. DINDs was defined as a ≥ 2-point decrease in the modified Glasgow Coma Scale (mGCS) lasting at least 24 hours, or a ≥ 2-point increase in the NIH Stroke Scale (NIHSS) between measures on day 2 and day 14 / ICU discharge.

Results: None of the 8 patients who had haptoglobin α1-α1 developed DINDs by either mGCS or NIHSS criteria. In contrast, 8 of the 41 patients who had the haptoglobin α2 subunit developed DINDs by mGCS criteria (p=0.21 vs. haptoglobin α1- α1 group), whereas 10 of these patients developed DINDs by NIHSS criteria (p=0.13 vs. haptoglobin α1-α1 group).

Conclusions: Consistent with our previous observation that angiographic vasospasm is more common in SAH patients who have haptoglobin containing the α2 subunit, DINDs also appeared to be more common in SAH patients who have haptoglobin containing the α2 subunit. However, no statistical significance could be demonstrated, likely because of unexpectedly low rates of DINDs.

Introduction

In the days that follow aneurysmal subarachnoid hemorrhage (SAH), hemoglobin is released from decaying red blood cells that are trapped in the subarachnoid clot. It is this extracorpuscular 'free' hemoglobin that is thought to induce cerebral artery constriction ("angiographic vasospasm"). Angiographic vasospasm, in turn, may produce brain ischemia and additional neurological impairment generally termed "delayed ischemic neurological deficits" (DINDs).

The amount of blood in the subarachnoid space is a major risk factor for developing vasospasm after SAH [3,9,17,21], yet sufficient room exists for other factors to be influential therein. We hypothesized that the type of haptoglobin expressed by a SAH patient may also be a risk factor for developing vasospasm. Haptoglobin is an abundant serum protein whose chief actions are to bind, neutralize, and facilitate the elimination of extracorpuscular hemoglobin [1,10,14,25,28]. Because of its anti-hemoglobin activity and its presence in the subarachnoid space after SAH [18], haptoglobin likely counteracts extracorpuscular hemoglobin as the hemoglobin is released from the decaying red blood cells. Indeed, administration of haptoglobin into the subarachnoid space of SAH patients has been tested as a treatment for vasospasm in uncontrolled studies where it was claimed to have some therapeutic benefit [4,5,22].

Considering that a person can express only one of three types of haptoglobin (α1-α1, α1-α2, or α2-α2) according to the α subunit genes he inherits, we evaluated the possibility that haptoglobin type might affect the development of vasospasm after SAH. In a previous publication, we tested this association using measures of angiographic vasospasm (transcranial Doppler ultrasonography, cerebral angiography), and we found that SAH patients who have haptoglobin containing the α2 subunit had a four-fold greater likelihood of developing

angiographic vasospasm [8]. In this study, we examined the relationship between haptoglobin type and the development of DINDs.

Materials and Methods

Subjects: This study was a prospective, observational, multicenter study. Approval was obtained from the Institutional Review Boards of Northwestern University, Wayne State University (covering the Detroit Medical Center), and 'La Sapienza' University of Rome, where consecutive patients were enrolled from between 2005 - 2007. All patients enrolled in the study, or else their legal surrogates, provided informed consent. Patients were eligible for enrollment if they were greater than 18 years-of-age and if they had (i) a known date of onset of the SAH, (ii) aneurysmal rupture as the cause for the SAH, and (iii) SAH of Fisher grade 3 severity (i.e., greater than 1 mm-thick blood layers or clots [17]) as detected by CT scan within 48 hours of admission to the hospital. Extension of the hemorrhage into the brain parenchyma or ventricular system was acceptable as long as the other CT criteria were met. Patients were included in this study irrespective of sex or race. Patients with suspected non-aneurysmal SAH (e.g., perimesencephalic SAH), or patients who had diseases associated with abnormal haptoglobin expression or that can affect the likelihood of developing vasospasm (i.e., hemolytic conditions, liver disease or liver transplant, autoimmune diseases, leukemias, endometriosis), were planned exclusions from this study, however, no such exclusions occurred. The presence of one or more cerebral aneurysms was confirmed by angiography or CT angiography in all cases.

Enrollment in the study did not influence the patient's treatment course. Haptoglobin typing was performed after the completion of patient enrollment.

Measurements and Data Collection: This study used two measures to assess DINDs: a modified Glasgow Coma Scale (mGCS) without the verbal response component, and the NIH Stroke Scale (NIHSS). The mGCS was assessed every 8 hours by clinical intensivist fellows ('La Sapienza' University of Rome) or by the nursing staff of the neuro-ICU (Detroit Medical Center). The NIHSS score was assessed by clinical intensivist fellows and attending physicians ('La Sapienza' University of Rome) or by certified study nurses and stroke neurologists (Detroit Medical Center) on the 2nd day after SAH (a time at which vasospasm is highly unlikely [15]), and again 14 days after SAH or else at the time of discharge from the ICU.

For our study, DINDs was defined as a decrease in the mGCS of at least 2 points lasting at least 24 hours (i.e., over three consecutive 8 hour measures) or an increase in NIHSS score of at least 2 points between the day 2 measure and the day 14 post-SAH / ICU discharge measure. Point changes of this magnitude have been used previously in clinical trials of DINDs to reflect neurological worsening [33,34]. Before DINDs could be diagnosed a CT scan was required to rule-out recurrent SAH or hydrocephalus, and routine serum chemistries were required to rule-out electrolyte and glucose abnormalities. Confirmation of vasospasm by angiography or transcranial Doppler ultrasonography was recommended, but not required, as with previous clinical trials of DINDs [33,34]. Other laboratory and diagnostic testing to confirm DINDs and to rule-out other diagnoses were allowed at the discretion of the study sites according to local practice patterns.

Determination of Haptoglobin Type: Blood (1 mL) was drawn from the patients immediately upon their enrollment into the study. Following centrifugation, the plasma fraction was stored at -80°C until it was shipped on dry ice to the Technion Institute of Technology

(Haifa, Israel) for haptoglobin typing. Haptoglobin typing was performed without any knowledge of the patient's clinical course.

Haptoglobin typing was performed on 10 μL of plasma by means of polyacrylamide gel electrophoresis, as described elsewhere [11]. A signature banding pattern was obtained for each of the three possible haptoglobin types: haptoglobin α1-α1 (patients homozygous for the haptoglobin α1 allele), haptoglobin α2-α2 (patients homozygous for the haptoglobin α2 allele), or haptoglobin α1-α2 (heterozygous patients). Polyacrylamide gel electrophoresis has 100% concordance with the haptoglobin genotype as determined by polymerase chain reaction [6]. An unambiguous haptoglobin type was obtained in all samples.

Data Analysis: Between-group comparisons were made by Fisher's exact test. The incidences of DINDs according to mGCS and NIHSS definitions were evaluated separately and in additive combination. In all analyses, the haptoglobin α1-α1 group was compared against the combined group of patients who had haptoglobin α1–α2 or α2–α2; the decision to combine the haptoglobin α1–α2 or α2–α2 groups for analysis was prespecified and based on our observations in our previous study [8]. One-sided tests were performed given our *a priori* expectation of a lower incidence of DINDs in the haptoglobin α1-α1 group. A P value less than 0.05 was considered statistically significant.

The sample size for this study was predetermined. Based on the data from our previous study [8], we estimate a 75% difference in DINDs risk attributable to haptoglobin type. The assumptions underlying this prospective sample size estimation included: probability of DINDs in patients with haptoglobin α1-α1 = 0.1; probability of DINDs in patients with haptoglobin α1-α2 or α2-α2 = 0.4; $\alpha = 0.05$, power = 0.80. According to these parameters, a target of n = 50

enrolled SAH patients was set for this study.

Results

Of the 55 patients who were enrolled in the study, n=27 were treated at 'La Sapienza' University of Rome, n=26 were treated at the Detroit Medical Center, and n=2 were treated at Northwestern University. Six patients were removed after enrollment leaving 49 patients for the final analysis because insufficient data was available about their clinical course.

Our group of 49 patients included n=8 with haptoglobin α1-α1, n=25 with haptoglobin α1-α2, and n=16 with haptoglobin α2-α2. This asymmetric distribution of haptoglobin types is consistent with that of our previous study [8] and of the general populations of the regions in which our study enrolled patients [2,16,19].

The characteristics of the patients grouped according to haptoglobin type are noted in Table 1. Patients in the haptoglobin α1-α1 group appeared to be older than patients in the other groups by more than a decade. The groups were otherwise comparable. As with our previous study [8], there was a noticeable predominance of women (38 of 49 patients), which may reflect the higher rate of aneurysm rupture in women [26]. Generally, the groups were reasonably comparable with regard to race, premorbid medical conditions, or drug use. Apparent differences between groups in hypertension and smoking rates did not achieve statistical significance (Fisher's test = 0.44 and 0.42, respectively).

As shown in Table 2, the features of SAH were comparable between the groups. All patients were found to have at least one aneurysm and often multiple aneurysms were present in the same patient. More specifically, the occurrence of intraparenchymal or intraventricular extension of the subarachnoid clot and the number of aneurysms per patient identified by

angiography appeared to be similar across the three groups. Treatments employed in the management of the aneurysm and SAH, and the application of preventative therapies against vasospasm, were also evaluated (Table 3). A single patient in the haptoglobin α1-α1 group did not have any surgical procedure to control the site of hemorrhage, presumably because she was 85 years old and in excellent clinical condition after the SAH. Aside from this case, no obvious difference was observed between the groups in terms of any of the other common treatments employed for this sort of patient. Notably, the use of triple H therapy was similar across groups, and this apparently reflects the prophylactic use of this therapy at one of the study sites. As shown in Table 4, hospital complications were more common in patients who had haptoglobin α1-α2 or α2-α2 only to a small degree.

The development of DINDs appeared to correlate with haptoglobin type (Table 5). No SAH patient who had haptoglobin α1-α1 achieved study criteria for DINDs using either the mGCS or the NIHSS criteria. In contrast, 6 patients from the haptoglobin α1-α2 group and 2 patients from the haptoglobin α2-α2 group exhibited a decrease in mGCS $\geq$ 2 points lasting at least 24 hours. Similarly, 8 patients from the haptoglobin α1-α2 group and 2 patients from the haptoglobin α2-α2 group exhibited an increase in NIHSS scores $\geq$ 2 points between day 2 and day 14 / ICU discharges. However, comparison of the combined haptoglobin α1-α2 and α2-α2 groups against the haptoglobin α1-α1 group did not reveal a statistically significant difference in the incidence of DINDs using mGCS ($p=0.21$) or NIHSS ($p=0.13$) criteria, nor when considering the combination of the two criteria ($p=0.26$).

Table 5 also shows the disposition of the patients after their hospitalization for SAH. None of the SAH patients who had haptoglobin α1-α1 died, and none were sent to a nursing home or otherwise institutionalized. In comparison, four SAH patients who had haptoglobin α1-

α2 or α2-α2 died, and 8 were sent to nursing homes or were otherwise institutionalized.

Discussion

In all animals, haptoglobin is composed of two subunits - α and β - that form the four-subunit structure $(\alpha\beta)_2$. In man, the haptoglobin α gene locus is dimorphic with two alleles denoted "α1" and "α2" [19]. Because each person has an α subunit gene on each of two chromosomes that are both continuously transcribed to make protein subunits, three major types of haptoglobin are found in the general population: haptoglobin α1-α1, α1-α2, and α2-α2 (the β subunit is invariable and so is not written here). These three types of haptoglobin neutralize hemoglobin to different degrees. In general, haptoglobin containing the α2 subunit does not inhibit the biochemical actions of hemoglobin as well as does haptoglobin α1-α1 [10,14,24,25,28]. Furthermore, haptoglobin α2-α2 may worsen the local inflammatory response in the subarachnoid space after SAH, as is suggested by the observations that the complex of hemoglobin with haptoglobin α2-α2 is (i) more potent than haptoglobin α1-α1 at activating the monocyte / macrophage CD163 receptor [5] and (ii) less able to stimulate production of anti-inflammatory cytokines from cultured monocytes [29]. Finally, haptoglobin α1-α2 and α2-α2 likely have worse tissue permeability than haptoglobin α1-α1 because the α2 subunit promotes the aggregation of those types of haptoglobin into large polymers [11]. The tissue permeability of haptoglobin may be important in the context of vasospasm because extracorpuscular hemoglobin is known to penetrate deep into the walls of the cerebral arteries after experimental SAH [12]. Given these pronounced differences between the three haptoglobin types – all of which favor haptoglobin α1- α1 - we hypothesized that haptoglobin containing the α2 subunit would be

associated with higher rates of vasospasm than would haptoglobin α1-α1.

In a previous study [8], we tested that hypothesis using angiographic measures of vasospasm. Transcranial Doppler ultrasonography measures of blood flow velocity in 9 cerebral arteries were examined on a daily basis according to standard criteria for 'possible' vasospasm. In that study, 2 of 9 SAH patients (22%) with haptoglobin α1-α1 developed possible vasospasm, whereas 22 of 29 SAH patients (87%) with haptoglobin containing the α2 subunit developed possible vasospasm (Fisher's exact test, $p = 0.001$). This observation was confirmed with higher blood flow velocity thresholds that defined 'definite' vasospasm, and also with cerebral angiography measures of arterial constriction. Neurological condition was not assessed in that study as an outcome measure, yet the positive result we observed with angiographic vasospasm encouraged us to examine DINDs in a similar manner.

DINDs did not occur in a single case in the haptoglobin α1-α1 group, while it was detected in several patients who expressed haptoglobin α1-α2 or α2-α2. However, we were unable to demonstrate a statistically significant difference between groups with this data. This proved to be the case whether using the frequent, basic measures of neurologic function (i.e., the mGCS) or a more thorough, function-based measure collected before and after the vasospasm window (i.e., the NIHSS). The sample size of this study clearly limited the statistical comparison. Given the group sizes, the rate of DINDs in SAH patients with haptoglobin containing the α2 subunit (10 of 41 patients = 24%, when using NIHSS criteria) appeared to be simply too low to reveal a difference despite the complete absence of DINDs in the haptoglobin α1-α1 group.

Methodological issues could certainly have impacted the results of this study. Firstly, there are neither universally-accepted nor validated measurement tools to detect DINDs. To

define DINDs, we chose widely-used scales and set point-change thresholds that we believed would (i) define a minimal amount of neurological deterioration and (ii) be sufficiently sizable and durable to be reliably detected in the ICU setting. We chose to use the mGCS and NIHSS as our measures of DINDs because of their general currency in the clinical neurosciences. Both scales have been shown to detect DINDs due to cerebral artery vasospasm after SAH, with the NIHSS appearing to be superior for detecting early changes in clinical condition and subtle weakness, and the GCS providing a resilient clinical assessment in the presence of impaired consciousness [30]. Both scales have been previously adopted for the assessment of DINDs from other uses: the GCS, from the evaluation of head trauma patients, in whom it was used to monitor for changes in clinical condition [31], and which has been shown to be superior to traditional neurological condition scales (the Hunt and Hess Scale and the World Federation of Neurological Surgeons Scale) for outcome prediction in SAH patients [35]; and the NIHSS, a scale designed specifically for the closely-related condition of ischemic stroke, wherein it can also be used serially to monitor for changes in clinical condition [32]. Unfortunately, since there is no consensus as to how to define DINDs in the medical literature, we were forced to develop our own definitions for the purposes of this study.

In our study, the Glasgow Coma Scale was modified so as to remove the verbal response component, which has been shown to reduce the predictive value of the original scale in longitudinal assessments of hospitalized patients suffering from a variety of intracranial diseases including SAH [7]. The NIHSS is a standard and reproducible means of detecting the development of focal neurological injury after ischemic stroke, and it has been validated in this regard [23]; given that DINDs is a type of ischemic stroke, we believe the NIHSS has face validity in detecting DINDs. Another methodological concern with our study may be the

confounding of the measure of DINDs with other causes of neurological dysfunction, which may have evaded our attempts to exclude them with standard ancillary testing. According to study protocol, before DINDs could be diagnosed a CT scan was required to rule-out recurrent SAH or hydrocephalus, and routine serum chemistries were required to rule-out electrolyte and glucose abnormalities. These activities represent standard-of-care practices for SAH patients, nevertheless they cannot rule-out all other causes of neurological dysfunction and assure the accuracy of the diagnosis of DINDs.

Despite potential methodological limitations, and in spite of our previously published finding with angiographic measures of vasospasm, one must also recognize that DINDs is not invariably correlated with angiographic vasospasm [13,20,27]. Thus, the failure of the current study to show a statistically-significant association between DINDs and haptoglobin type given the latter's association with angiographic vasospasm may simply reflect the divergence between DINDs and angiographic vasospasm that is well-known in the SAH literature.

While our results may have suggested that haptoglobin containing the α2 subunit is associated with a higher rate of DINDs after SAH, due to the low rate of occurrence of DINDs as defined by our measurement tools, larger cohorts would need to be examined to adequately test the hypothesis. Along that line, we intend to examine this hypothesis with a larger sample size of patients, and in that endeavor we invite interested partners.

References

1. Allison AC, Blumberg BS, Rees AP. Haptoglobin types in British, Spanish Basque, and Nigerian African populations. **Nature 181**: 824-825, 1958.
2. Asleh R, Guetta J, Kalet-Litman S, Miller-Lotan R, Levy AP. Haptoglobin genotype- and diabetes-dependent differences in iron-mediated oxidative stress in vitro and in vivo. **Circ Res 96**: 435-441, 2005.
3. Asleh R, Marsh S, Shilkrut M, Binah O, Guetta J, Lejbkowicz F, et al. Genetically determined heterogeneity in hemoglobin scavenging and susceptibility to diabetic cardiovascular disease. **Circ Res 92**: 1193-1200, 2003.
4. Bell BA, Kendall BE, Symon L. Computed tomography in aneurysmal subarachnoid haemorrhage. **J Neurol Neurosurg Psychiatry 43**: 522-524, 1980.
5. Borsody M, Burke A, Coplin W, Miller-Lotan R, Levy A. Haptoglobin and the development of cerebral artery vasospasm after subarachnoid hemorrhage. **Neurology 66**: 634-40, 2006.
6. Delank HW. Clinical experience with polyacrylamide-electrophoretic analysis of cerebrospinal fluid proteins. **Klin Wochenschr 46**: 779-783, 1968.
7. Diringer MN, Edwards DF. Does modification of the Innsbruck and the Glasgow Coma Scales improve their ability to predict functional outcome? **Arch Neurology 54**: 606-11, 1997.
8. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. **Neurosurgery 6**: 1-9, 1980.
9. Geraud G, Tremoulet M, Guell A, Bes A. The prognostic value of noninvasive CBF measurement in subarachnoid hemorrhage. **Stroke 15**: 301-5, 1984.
10. Giblett ER. Haptoglobin Types in American Negroes. Nature 183: 192-193, 1959.

11. Guetta J, Strauss M, Levy NS, Fahoum L, Levy AP. Haptoglobin genotype modulates the balance of Th1/Th2 cytokines produced by macrophages exposed to free hemoglobin. **Atherosclerosis 191**: 48-53, 2007.

12. Gurusinghe NT, Richardson AE. The value of computerized tomography in aneurysmal subarachnoid hemorrhage. The concept of the CT score. **J Neurosurg 60**: 763-770, 1984.

13. Gutteridge JM. The antioxidant activity of haptoglobin towards haemoglobin- stimulated lipid peroxidation. **Biochim Biophys Acta 917**: 219-223, 1987.

14. Haurani FI, Meyer A. Iron and the reticuloendothelial system. **Adv Exp Med Biol 73**: 171-187, 1976.

15. Heros RC, Zervas NT, Varsos V. Cerebral vasospasm after subarachnoid hemorrhage: An update. **Ann Neurol 14**: 599-608, 1983.

16. Jue DM, Shim BS, Kang YS. Inhibition of prostaglandin synthase activity of sheep seminal vesicular gland by human serum haptoglobin. **Mol Cell Biochem 51**: 141-147, 1983.

17. Kistler JP, Crowell RM, Davis KR, Heros R, Ojemann RG, Zervas T, et al. The relation of cerebral vasospasm to the extent and location of subarachnoid blood visualized by CT scan: a prospective study. **Neurology 33**: 424-436, 1983.

18. Koch W, Latz W, Eichinger M, Roguin A, Levy AP, Schomig A, et al. Genotyping of the common haptoglobin Hp 1/2 polymorphism based on PCR. **Clin Chem 48**: 1377-1382, 2002.

19. Langlois MR, Delanghe JR. Biological and clinical significance of haptoglobin polymorphism in humans. **Clin Chem 42**: 1589-1600, 1996.

20. Laumer R, Steinmeier R, Gonner F, Vogtmann T, Priem R,Fahlbusch R. Cerebral hemodynamics in subarachnoid hemorrhage evaluated by transcranial Doppler sonography. Part 1. Reliability of flow velocities in clinical management. **Neurosurgery 33**: 1-8, 1993.

21. Liszczak TM, Varsos VG, Black PM, Kistler JP, Zervas NT. Cerebral arterial constriction after experimental subarachnoid hemorrhage is associated with blood components within the arterial wall. **J Neurosurg 58**: 18-26, 1983.

22. Meyer BC, Hemmen TM, Jackson CM, Lyden PD. Modified National Institutes of Health Stroke Scale for use in stroke clinical trials: prospective reliability and validity. **Stroke 33**: 1261-6, 2002.

23. Miyaoka M, Nonaka T, Watanabe H, Chigasaki H, Ishi S. Etiology and treatment of prolonged vasospasm --experimental and clinical studies. **Neurol Med Chir (Tokyo) 16**: 103-114, 1976.

24. Nonaka T, Watanabe S, Chigasaki H, Miyaoka M, Ishii S. Etiology and treatment of vasospasm following subarachnoid hemorrhage. **Neurol Med Chir (Tokyo) 19**: 53-60, 1979.

25. Ohmoto T. Current management of cerebral vasospasm. **No Shinkei Geka 6**: 229-234, 1978.

26. Okada Y, Nishida M, Yamane K, Hatayama T, Yamanaka C, Yoshida A. Comparison of transcranial Doppler investigation of aneurysmal vasospasm with digital subtraction angiographic and clinical findings. **Neurosurgery 45**: 443-9, 1999.

27. Rinkel GJ, Djibuti M, van Gijn J. Prevalence and risk of rupture of intracranial aneurysms: A systematic review. **Stroke 29**: 251-6, 1998.

28. Saeed SA, Mahmood F, Shah BH, Gilani AH. The inhibition of prostaglandin biosynthesis by human haptoglobin and its relationship with haemoglobin binding. **Biochem Soc Trans 25**: S618, 1997.

29. Wejman JC, Hovsepian D, Wall JS, Hainfeld JF, Greer J. Structure and assembly of haptoglobin polymers by electron microscopy. **J Mol Biol 174**: 343-368, 1984.

30. Doerksen K, Naimark BJ, Tate RB. Comparison of a standard neurological tool with a stroke scale for detecting symptomatic cerebral vasospasm. **J Neurosci Nursing 34**: 320-5, 2002.

31. Matis G, Birbilis T. The Glasgow Coma Scale - A brief review. Past, present, future. **Acta Neurol Belg 108**: 75-89, 2008.

32. Wityk RJ, Pessin MS, Kaplan RF, Caplan LR. Serial assessment of acute stroke using the NIH Stroke Scale. **Stroke 25**: 362-5, 1994.

33. Haley EC, Kassell NF, Torner JC et al. A randomized controlled trial of high-dose intravenous nicardipine in aneurysmal subarachnoid hemorrhage. **J Neurosurg 78**: 537-47, 1993.

34. Haley EC, Kassell NF, Apperson-Hansen C, Maile MH, Alves WM, et al. A randomized, double-blind, vehicle-controlled trial of tirilazad mesylate in patients with aneurysmal subarachnoid hemorrhage: A cooperative study in North America. **J Neurosurg 86**: 467-75, 1997.

35. Oshiro EM, Walter KA, Piantoadosi S, Witham TF, Tamargo RJ. A new subarachnoid hemorrhage grading system based on the Glasgow Coma Scale: A comparison with the Hunt and Hess and World Federation of Neurological Surgeons Scales in a clinical series. **Neurosurg 41**: 140-8, 1997.

Table 1: Characteristics of patients according to haptoglobin type.

	haptoglobin α1 – α1 (n=8)	*haptoglobin α1 – α2* (n=25)	*haptoglobin α2 – α2* (n=16)	*haptoglobin α1 – α2 or α2 – α2* (n=41)
age *(patients > 55 years-of-age)*	64.5 ± 4.0 yr (7)	53.7 ± 2.2 yr (11)	49.3 ± 2.9 yr (5)	52.0 ± 1.8 yr (16)
sex	7 women, 1 man	18 women, 7 men	13 women 3 men	31 women, 10 men
race				
white	6	16	12	28
black	2	8	2	10
other	0	1	2	3
premorbid medical conditions				
hypertension	1	10	2	12
ischemic stroke	2	1	0	1
hemorrhagic stroke	0	1	0	1
coronary artery disease	1	3	0	3
diabetes	0	2	0	2
dyslipidemia	0	3	2	5
arthritis	0	2	1	3
drug use ^				
smoking	2	13	5	18

alcohol	0	5	2	7
drugs-of-abuse	1 *	3 #	0	3#

^ reported or detected on urinalysis

* marijuana

n=1 marijuana, n=2 opioids

Table 2: SAH and aneurysm features according to haptoglobin type.

	haptoglobin α1 – α1 (n=8)	*haptoglobin α1 – α2* (n=25)	*haptoglobin α2 – α2* (n=16)	*haptoglobin α1 – α2 or α2 – α2* (n=41)
intraparenchymal or intraventricular hemorrhage	1	4	3	7
*# aneurysms per patient (range) **	1.3 ± 0.3 (1-3)	1.2 ± 0.1 (1-3)	1.2 ± 0.1 (1-3)	1.2 ± 0.1 (1-3)
aneurysm sites				
middle cerebral	6	8	4	12
anterior cerebral	2	5	3	8
anterior communicating	0	8	4	12
posterior communicating	0	4	2	6
*vertebrobasilar system ***	2	2	4	6
ophthalmic or terminal carotid	0	2	1	3

* includes ruptured and unruptured aneurysms

** includes cerebellar arteries

Table 3: Treatments administered according to haptoglobin type.

	haptoglobin α1 – α1 (n=8)	*haptoglobin α1 – α2* (n=25)	*haptoglobin α2 – α2* (n=16)	*haptoglobin α1 – α2 or α2 – α2* (n=41)
time to aneurysm surgery from diagnosis (days)	2 ± 1	5 ± 2	1 ± 1	4 ± 2
type of aneurysm surgery				
coiled	1	10	5	15
clipped	6	15	11	26
untreated	1	0	0	0
other treatments during hospitalization				
antiepileptics	3	17	8	25
calcium-channel blockers	6	18	12	30
triple-H therapy *	3	8	6	14
glucocorticoids	2	4	3	7
antibiotics	2	10	5	15
antihypertensives	4	14	7	21
anticoagulation	0	3	1	4
antiplatelets	0	4	2	6
statin agents	2	8	5	13

* triple-H therapy: hypertension, hypervolemia, hemodilution

Table 4: Hospital complications according to haptoglobin type.

	haptoglobin α1 – α1 (n=8)	*haptoglobin α1 – α2* (n=25)	*haptoglobin α2 – α2* (n=16)	*haptoglobin α1 – α2 or α2 – α2* (n=41)
infection				
peripheral	1	7	3	10
intracranial	0	2	3	5
deep venous thrombosis	0	1	1	2
hyponatremia	2	3	2	5
seizure	0	2	0	2
hydrocephalus	2	4	3	7
total number of complications (per patient average)	5 (0.6)	19 (0.8)	12 (0.8)	31 (0.8)

Table 5: Development of DINDs according to haptoglobin type.

	haptoglobin α1 – α1 (n=8)	*haptoglobin α1 – α2 (n=25)*	*haptoglobin α2 – α2 (n=16)*	*haptoglobin α1 – α2 or α2 – α2 (n=41)*
mGCS decrease ≥ 2 points for 24hr	0	6	2	8 (p=0.21 vs. *α1 – α1*)
NIHSS increase ≥ 2 points between day 2 and day 14 / ICU discharge	0	8	2	10 (p=0.13 vs. *α1 – α1*)
Both mGCS and NIHSS criteria achieved	0	6	1	7 (p=0.26 vs. *α1 – α1*)
Duration of hospitalization (days)	18 ± 4	20 ± 3	19 ± 2	19 ±2
Disposition from hospital				
dead	0	2	2	4
nursing home / institutionalized	0	6	2	8
home / rehabilitation	8	17	12	29

Patients with discordant mGCS and NIHSS results:

mGCS-positive, NIHSS-negative: n=1 from the haptoglobin α2 – α2 group

NIHSS-positive, mGCS-negative: n=2 from the haptoglobin α1 – α2 group; n=1 from the haptoglobin α2 – α2 group