Stroke

BRIEF REPORT

Acute Microbleeds and Microinfarcts Within the Perihematomal Area After Intracerebral Hemorrhage

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BACKGROUND: To further our understanding of the pathophysiology of spontaneous intracerebral hemorrhage (ICH) and related injury, we provided a postmortem neuropathological examination of acute microvascular lesions (microbleeds and microinfarcts) within the perihematomal area.

METHODS: We included all consecutive cases (2005–2019) from the Lille University Hospital brain bank of ICH patients who died within the first month. Paraffin-embedded tissue sections from the perihematomal area were processed for several stainings and immunolabelings to investigate the presence of acute microbleeds and microinfarcts in the perihematomal area and to characterize surrounding neuronal and systemic inflammatory reaction (macrophages and neutrophils).

RESULTS: We included 14 ICH cases (median age, 78 years; 10 females). Acute microbleeds were observed in the perihematomal area in 12/14 patients (86%, ranging from 1 through >10) and microinfarcts in 5/14 (36%, ranging from 1 through 4). Microbleeds were observed whatever the delay from ICH onset to death was, while most cases with acute microinfarcts were observed between day 3 and day 7 (n=3/5). Both lesions were characterized by an abundant accumulation of systemic inflammatory cells and necrotic areas.

CONCLUSIONS: Acute microbleeds and microinfarcts might contribute to the propagation of secondary brain tissue damages after ICH. Our examinations also question the potential role of massive systemic inflammatory cells recruitment in the genesis of these microvascular injuries.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: edema ■ inflammation ■ intracerebral hemorrhage ■ microbleeds ■ microinfarcts

In spontaneous intracerebral hemorrhage (ICH), evidence of acute microvascular changes in the vicinity of bleeding site are scarce and has been mainly studied through the Fisher's avalanche theory.[1] In this theory, the mass effect of the initial hematoma may tear surrounding arterioles by shearing, leading to secondary bleeding. Beyond hematoma expansion, it is unclear whether microvascular injury may also contribute to secondary brain tissue damages within the perihematomal area (PHA). Here, we investigate the neuropathological correlates of both acute microbleeds (aMBs) and acute microinfarcts (aMIs) within the PHA in a postmortem study of ICH cases. We hypothesized that microvascular injury is frequently observed in the PHA and might contribute to its development through the extension of inflammatory response and secondary brain tissue damages.

METHODS

This study adheres to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) cross-sectional

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This manuscript was sent to Claire L. Gibson, Guest Editor, for review by expert referees, editorial decision, and final disposition.
Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/STROKEAHA.123.042098.
For Sources of Funding and Disclosures, see page e62.

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study guidelines. All data relevant to the study are included in the article or uploaded as Supplemental Material. Other data are available upon reasonable request.

Human Brain Sampling

Human brains were obtained from the Lille Neurobank (CRB/CIC1403 Biobank, BB-0033-00030, agreement DC-2008-642), which fulfills the criteria of the local laws and regulations on biological resources with donor consent, data protection, and ethical committee review. We included all consecutive cases (2005–2019) of ICH patients who died within the first month after stroke onset. Details of autopsy protocol are provided elsewhere.[2]

Histology and Microvascular Lesions Definition

We investigated in each case paraffin-embedded tissue blocks from the PHA (2–4 blocks per case). Sections of formalin-fixed and paraffin-embedded tissue were processed for hematoxylin-eosin, Martius scarlet blue, and Perl's staining of 5-µm thick adjacent slices.[2] We defined aMBs by the presence of microvessel disruption accompanied by fresh red blood cells extravasation.[3] We used Perl's staining to date the occurrence of MBs. In case of similar iron deposition between ICH core and aMBs (eg, negative or positive), aMBs formation was considered concurrent to ICH onset. We defined aMIs by the presence of a circumscribed region characterized by a myelin loss (tissue pallor), vacuolization, and reduction of neuron density in the vicinity of a small vessel (<200 µm).[3] Those regions were notably distinct from the large necrotic areas at the immediate border of the hematoma. Myeloperoxydase, hemoglobin scavenger receptor CD163 (monocyte-macrophage scavenger receptor), and NeuN (neuronal marker) were immunolabeled to identify neutrophils, activated macrophages, and neurons, respectively, on 5-µm thick serial sections. Details of automated imunolabelings and slices examination are reported elsewhere.[2,4] Intravascular investigation of the presence of neutrophils and monocytes was assessed by light microscopy with oil immersion at 63- and 100-fold magnification.

Statistical Analysis

Continuous and categorical variables were expressed as median (interquartile range) or number (percentage), respectively.

RESULTS

Study Population

We included 14 ICH cases (median age, 78 [76–85] years; 10 females). Median delay between ICH onset and death was 8 days (5–15). Median sICH volume was 90 (61–112) cm^3. Thirteen ICH were associated with underlying cerebral amyloid angiopathy (confirmed by an experienced neuropathologist, V.D.) and 1 case with deep perforating vasculopathy. Two patients had a diagnosis of probable cerebral amyloid angiopathy before the index ICH.

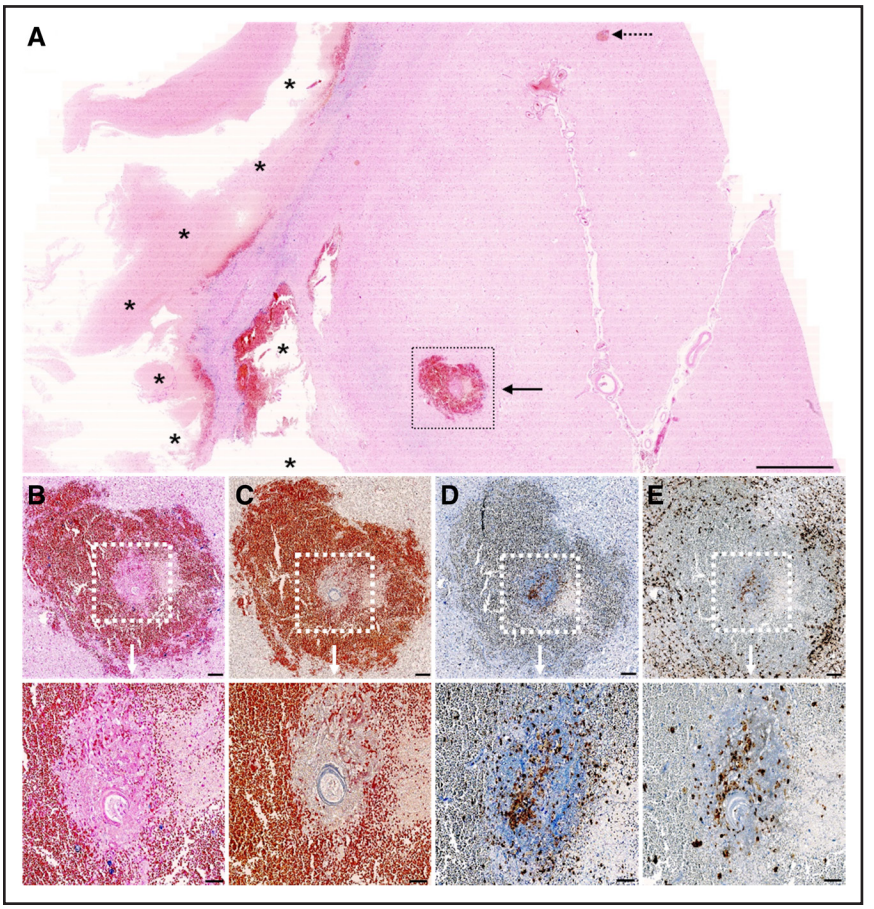

Figure 1. Acute microbleeds within the perihematomal area.
Perihematomal area of a patient who died 9 d after intracerebral hemorrhage onset. The asterisks indicate the hematoma site. **A**, This corresponds to an overview of the observed area after Perl's staining. Arrow with solid line indicates the acute microbleed (aMB) of interest. Another smaller aMB can be seen on the top right of the section (arrow with dotted line). **B**, The aMB shows first iron deposits. **C**, Martius Scarlet Blue staining. Injured small vessel (the blue ring corresponds to the vessel wall collagen) surrounded by fresh erythrocytes (orange). **D**, Myeloperoxydase immunolabeling. Abundant presence of neutrophils in the lumen of the disrupted vessel, perivascular space, and adjacent bleeding area. **E**, CD163 (monocyte-macrophage scavenger receptor) immunolabeling. Activated macrophages specifically localized at the perivascular space. Scale bars=2 cm; 100 µm; 50 µm.

Stroke. 2023;54:e58–e62. DOI: 10.1161/STROKEAHA.122.040908

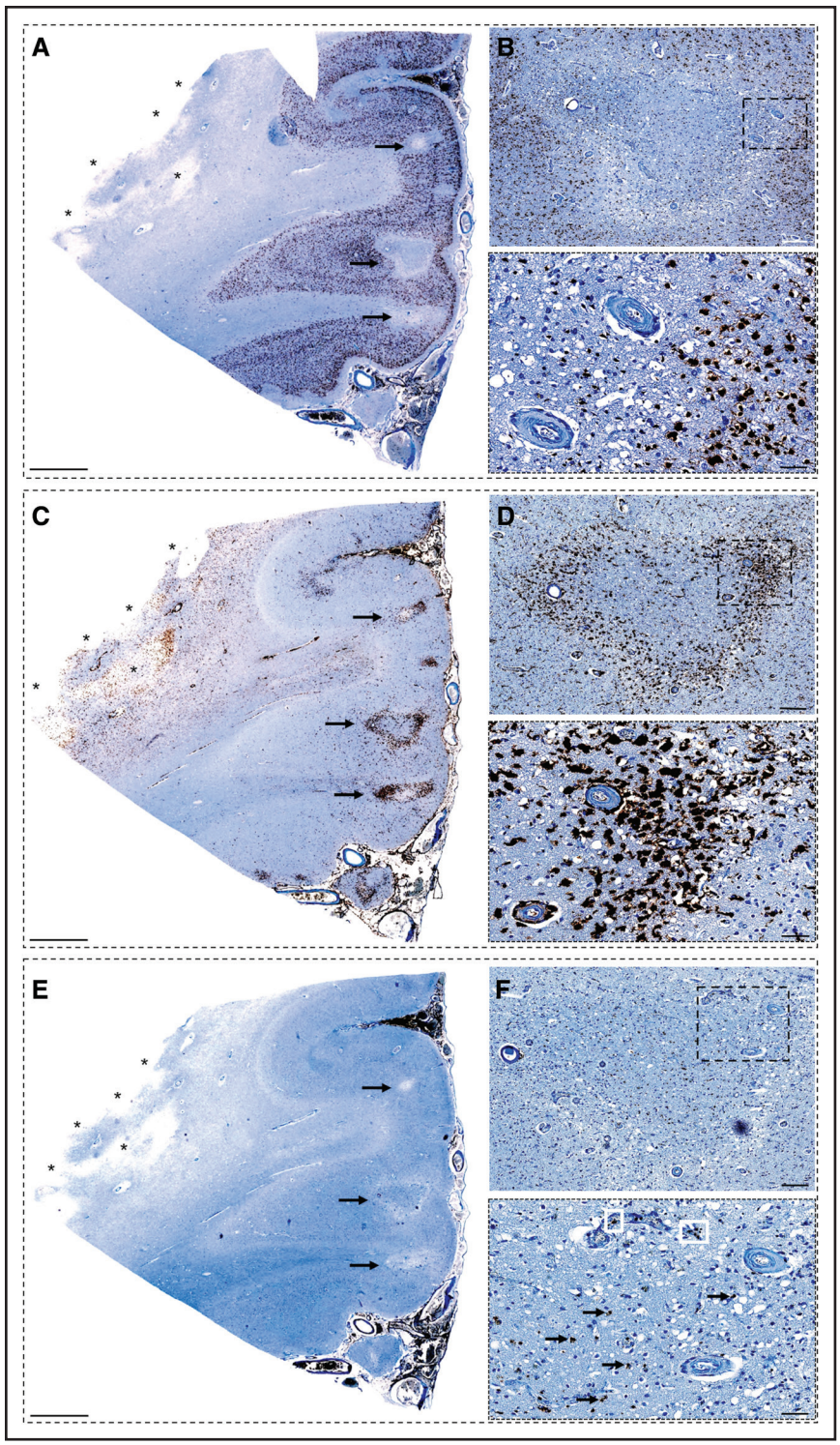

Figure 2. Acute microinfarcts within the perihematomal area.
Perihematomal area of a patient who died 5 d after intracerebral hemorrhage onset. The asterisks indicate the hematoma site. Three remote acute microinfarcts (aMIs) are in the gray matter (black arrows). **A** and **B**, NeuN (neuronal marker) immunolabeling. Circumscribed aMIs are characterized by a tissue pallor with tissue vacuolization and neuronal loss. **C** and **D**, CD163 (monocyte-macrophage scavenger receptor) immunolabelings. Abundant presence of recruited macrophages were found in the vicinity of small vessels within aMI. **E** and **F**, Myeloperoxydase immunolabeling. Some neutrophils were observed within the lumen of microvessel (white squares) and within the necrotic area (black arrows). Scale bars=2 cm; 100 µm; 50 µm.

Assessment of Microvascular Lesions

aMBs were observed in 86% of the cases (n=12/14). The number of aMBs ranged from 1 through >10. aMBs were observed in the immediate adjacent border of the hematoma but also within the PHA (Figure 1). aMBs were observed whatever the time from ICH onset to death was, including the first 24 hours. All aMBs (except 2) showed similar Perl's status than ICH core. aMIs were observed in 36% of our study population (n=5/14; Figure 2), ranging from 1 to 4. At the immediate ICH border, aMIs could not be distinguished from necrosis and edema. Therefore, aMIs were mostly observed in the PHA, remotely from the ICH core. We did not observe any aMI in cases who died within the first 48 hours after ICH onset. Most of the aMIs positive cases were observed between day 3 and day 7 (n=3/5). The estimated size of lesions ranged from 200 µm through 1.8 mm.

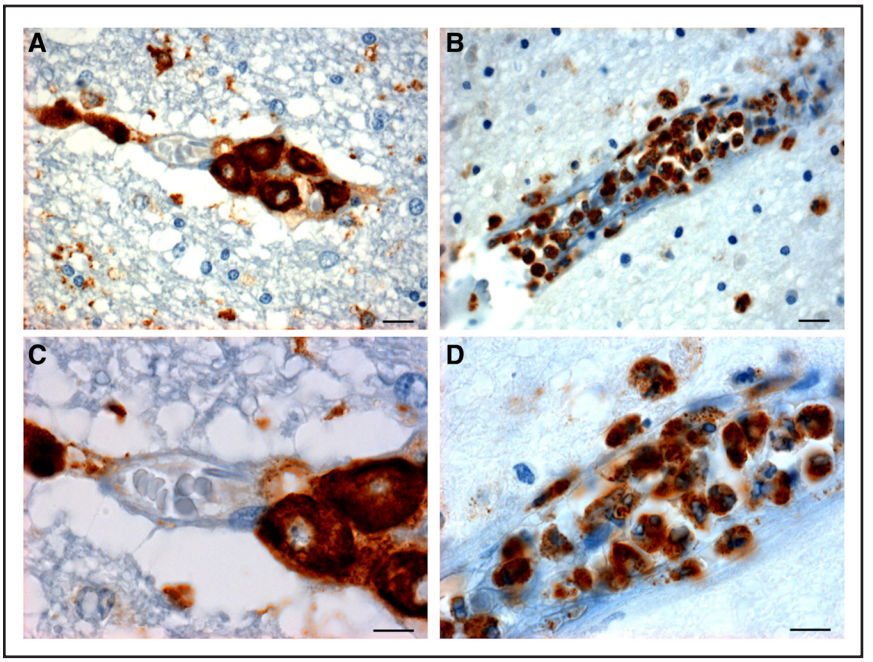

Figure 3. Intramicrovascular accumulation of systemic inflammatory cells.
A and **C**, Examination of a microvessel within the perihematomal area of a patient who died 14 d after intracerebral hemorrhage onset. **A**, Bulky accumulation of CD163 (monocyte-macrophage scavenger receptor)-positive macrophages (brown) in the lumen of a microvessel. At higher magnification (**C**), sequestered red blood cells, and dilated shape of the microvessel can be observed. The vacuolization of the adjacent tissue indicates an ischemic process. **B** and **D**, Examination of a microvessel within the perihematomal area of a patient who died 8 d after intracerebral hemorrhage onset. **B**, Accumulation of myeloperoxydase positive neutrophils in the lumen and the wall of a microvessel close to an acute microbleed (aMB). Slightly dilated vessel at higher magnification (**D**). Captures obtained in light microscopy with oil immersion. Scale bars: **A** and **B**=50 µm; **C** and **D**=10 µm.

Intravascular and Parenchymal Inflammatory Cells Associated With aMBs and aMIs

Both aMBs and aMIs were characterized by the presence of systemic inflammatory cells. We observed intra and perivascular CD163 positive cells that corresponds to activated macrophages and neutrophils were readily observed within the lumen of small vessels, in the perivascular spaces, or scattered in adjacent parenchyma surrounding the disrupted or occluded microvessels of interest (Figures 1 through 3). In addition, aMIs were visually characterized by a neuronal loss (Figure 2). Of note, intravascular examination showed accumulation of macrophages and neutrophils associated with microvascular remodeled shape and occlusion (Figure 3).

DISCUSSION

Both aMBS and aMIs are commonly observed in the PHA and might contribute to secondary brain tissue damage following ICH. Nearly 90% of our cases exhibited 1 to >10 aMBs within the PHA, suggesting a vulnerability of nearby arterioles to mechanical tear, therefore prone to rupture. Two cases displayed aMBs without iron deposition adjacent to a Perl's positive ICH, suggestive of a secondary microbleeding. These findings support the Fisher's avalanche theory[1] and this latter might also apply to aMIs. More than one third of the cases exhibited at least 1 aMI, indicative of a drop in the microvascular patency in the PHA. This proportion is consistent with a recent pooled meta-analysis of >1700 ICH patients,[5] in which 31.3% of cases displayed punctuate ischemic lesions in the PHA and beyond. While we were unable to perform an exhaustive examination of the entire sICH area, our assessment of microvascular lesions on 2 to 4 sections within the PHA may have led to an underestimation of the number of lesions. In addition, we cannot rule out the effect of the nature of the underlying vasculopathy. Whether the presence of these microvascular injuries had contributed to the poor prognosis of our patients remains unknown. We found a distinct temporal distribution between aMBs (observed whatever the delay from ICH onset to death was) and aMIs (mostly observed between day 3 and day 7) that should be carefully interpreted given the cross-sectional nature of postmortem investigations.

ICH triggers a systemic response leading to the massive recruitment of inflammatory cells. The intraluminal and extravasated neutrophils and macrophages observed in areas of neuronal loss, which were found around microvascular lesions, suggest that neuroinflammatory damage can concentrate around microvessels where the tissue was not necessarily infarcted before ICH onset. Such a localized feature of neuroinflammation, observed both in hemorrhagic and occlusive microlesions, provides novel insight into the mechanisms involved in the development of PHA. When too numerous, macrophages and neutrophils are known to generate free oxygen radicals and various proteolytic enzymes that can precipitate vessel wall disruption and then aMBs.[6] In addition, thrombo-inflammation is an emerging concept linking dysregulated inflammatory response to micro-occlusive processes.[7,8] Inflammation stimulates thrombosis, which in turn, promotes inflammation. This indicates a negative spiral of local inflammation establishing the contribution of microvascular insults to secondary brain tissue damage after ICH.

Stroke. 2023;54:e58–e62. DOI: 10.1161/STROKEAHA.122.040908

CONCLUSIONS

Both acute aMBs and aMIs are frequently observed in the PHA. By expanding inflammatory process and necrotic areas, these microvascular injuries might contribute to the spread of secondary brain tissue damages following ICH. Our findings also question the potential role of systemic inflammation in their genesis.

ARTICLE INFORMATION

Received August 10, 2022; final revision received November 18, 2022; accepted December 21, 2022.

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Acknowledgments

Drs Puy, Bérézowski, Deramecourt, Rauch, and Cordonnier contributed to the acquisition, analysis of the data, and drafted the manuscript.

Sources of Fundings

This work was supported by Fondation Recherche sur les Accidents Vasculaires Cérébraux (project FRAVC180713012), Fondation pour la Recherche Médicale (FDM201806006375).

Disclosures

Dr Cordonnier declares the following type of interests: consultant for Bristol-Myers Squibb; data and safety monitoring for the University of Glasgow; Professor of Neurology for the University of Lille. Dr Cordonnier reports grant funding from the French ministry of health (Programme Hospitalier de Recherche Clinique, A3ICH and TICH-3 Fr studies). Dr Cordonnier received speaker fees from BMS and Pfizer. The other authors report no conflicts.

Supplemental Material

CARE checklist

REFERENCES

1. Fisher CM. Pathological observations in hypertensive cerebral hemorrhage. *J Neuropathol Exp Neurol*. 1971;30:536–550. doi: 10.1097/00005072-197107000-00015
2. Puy L, Corseaux D, Perbet R, Deramecourt V, Cordonnier C, Bérézowski V. Neutrophil extracellular traps (NETs) infiltrate haematoma and surrounding brain tissue after intracerebral haemorrhage: a post-mortem study. *Neuropathol Appl Neurobiol*. 2021;47:867–877. doi: 10.1111/nan.12733
3. van Veluw SJ, Scherlek AA, Freeze WM, Ter Telgte A, van der Kouwe AJ, Bacskai BJ, Frosch MP, Greenberg SM. Different microvascular alterations underlie microbleeds and microinfarcts. *Ann Neurol*. 2019;86:279–292. doi: 10.1002/ana.25512
4. Puy L, Perbet R, Figeac M, Duchêne B, Deramecourt V, Cordonnier C, Bérézowski V. Brain peri-hematomal area, a strategic interface for blood clearance: a human neuropathological and transcriptomic study. *Stroke*. 2022;53:2026–2035. doi: 10.1161/strokeaha.121.037751
5. Murthy SB, Cho S-M, Gupta A, Shoamanesh A, Navi BB, Avadhani R, Gruber J, Li Y, Greige T, Lioutas VA, et al. A pooled analysis of diffusion-weighted imaging lesions in patients with acute intracerebral hemorrhage. *JAMA Neurol*. 2020;77:1390–1397. doi: 10.1001/jamaneurol.2020.2349
6. Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A. Neutrophil function: from mechanisms to disease. *Annu Rev Immunol*. 2012;30:459–489. doi: 10.1146/annurev-immunol-020711-074942
7. Bray MA, Sartain SE, Gollamudi J, Rumbaut RE. Microvascular thrombosis: experimental and clinical implications. *Transl. Res*. 2020;225:105–130. doi: 10.1016/j.trsl.2020.05.006
8. Jackson SP, Darbousset R, Schoenwaelder SM. Thromboinflammation: challenges of therapeutically targeting coagulation and other host defense mechanisms. *Blood*. 2019;133:906–918. doi: 10.1182/blood-2018-11-882993