“Self-Fulfilling Prophecy” or Recognition Requires a Concept of Perception

To the Editor:

The NINDS rt-PA Stroke Trial did not prospectively assess quantity and quality of ischemic brain parenchyma as detected by baseline CT.1 In this trial, no CT reading panel graded CT scans on the basis of predefined definitions. Now the trialists hypothesized that there would be disagreement among them about the presence of early CT changes.2 They randomly selected 70 baseline scans from the trial, and 16 of them reread the scans in a 1-day session. Among the investigators was 1 neuroradiologist who served as the “gold standard.” He was able to predict the lesion location at 24 hours in 96% (95% CI 92% to 100%) of the scans based on the information provided by the baseline CT. In comparison, the 16 raters of the NINDS group were much less sensitive (78%) and specific (57%) in detecting any early CT change or in identifying changes involving >33% of the MCA territory. With this poor performance, it is not at all surprising that the agreement beyond chance among these raters was somewhat low and heterogeneous. Moreover, the authors did not design and power their study to detect an effect of the method of film viewing, the effect of image quality, or an effect of the raters’ different experiences and training on agreement. To our surprise, however, they conclude from their data that all these factors do not affect the raters’ agreement.

Can we generalize this experience? Correct interpretation of CT is a problem not only for the experienced stroke specialists of the NINDS rt-PA Stroke Trial Group. Emergency physicians in another study had an error rate in stroke detection by CT twice that of neurologists and radiologists, and only 17% of emergency physicians and 40% of neurologists achieved a 100% sensitivity for identification of intracranial hemorrhage.3 Missing the ischemic edema in its early stage on CT could result from technical limitations or the lack of a perceptual concept.4 Moreover, we do not agree with Dr Grotta’s assertion that the technique is the main problem. At least the group’s neuroradiologist was able to predict ischemic tissue damage from the baseline CT with high specificity, confirming the experience of others.5–7 The problem is the feature of findings in acute stroke and a lack of training to interpret these findings. Obviously, even experts in neurology are puzzled when anatomic information gets lost on CT because of gray matter hypopattenuation. The old prejudice that CT remains negative within the first 24 to 48 hours after ischemic stroke is still prevalent in review articles,8 although many studies have reported positive findings even within the first 3 to 6 hours of stroke onset.9 The specificity of positive CT findings for irreversible lesions is still widely ignored. Moreover, the diagnostic significance of a normal CT in a patient with stroke is underestimated by many stroke physicians and explained by a low sensitivity of the method. A normal CT in acute stroke means, however, that the patient will soon recover or the ischemic edema is delayed and appears after 6 hours, which occurs in about one third of the patients.7 This observation is really important for treatment strategies and the discussion about the therapeutic time window.10

Key to recognizing early ischemic changes on CT is understanding the concept of how stroke pathophysiology may appear on CT. Dr Grotta and his 20 coauthors like to remain vague in this regard: “The pathological significance of such findings, in particular hypopattenuation of the x-ray signal, is uncertain, but it probably represents increased tissue water content caused by early cytotoxic edema, which may or may not signal irreversible injury” (page 1529).2 Their uncertainty is reflected by the categories applied in this study: The authors differentiated between “loss of gray-white distinction” and “hypodensity,” although both mean x-ray hypopattenuation due to tissue water uptake. Unfortunately, the authors do not explain how they interpret the decreasing x-ray attenuation after arterial occlusion and do not discuss why CT enabled Dr Patel to predict irreversible ischemic damage with high specificity within 3 hours of stroke onset.2 The authors discuss abnormalities on diffusion-weighted MRI that may be used instead to predict the irreversible damage, ignoring the relatively small database and a recent report on spontaneous reversibility of disturbed diffusion in TIA patients.11

Maybe white spots on MRI can be more easily detected than subtle changes of the gray scale on CT. Agreement among physicians seems not a problem exclusively with regard to radiological findings. We would very much like to know to what extent there is agreement among the NINDS investigators about the clinical categories “large- and small-vessel occlusion” on which they based their important conclusion that rt-PA is beneficial even in patients without large vessel occlusion.

We fully agree with the authors that improved methods of recognizing early CT changes are needed not only for the NINDS rt-PA Stroke Trial Group. These methods should include formal training in reading CT for all physicians dealing with acute stroke and who cannot rely on a 24-hour neuroradiological service. Moreover, our scientific journals should further foster the comprehension of imaging by taking care of optimal image quality and interpretation. It should not happen again that an article about the need for an improvement in CT interpretation is accompanied by a CT printed upside down12 or that this journal publishes a CT with an easily visible ischemic lesion as a normal CT.13

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**Response**

Our study should not be interpreted as discounting the importance of CT in early stroke diagnosis and the pioneering work of the ECASS group in general and Drs von Kummer and Hacke in particular in drawing attention to early changes of ischemia on CT. However, we stand by our findings that disagreement about the presence and extent of such findings is likely in everyday practice. Our study was not powered to detect the effect of method of film viewing, image quality, and different training and experience of raters. However, as stated, we found no consistent differences in performance between specialties, nor did we see improved agreement later in our study when newer CT scans had been introduced. Nevertheless, we agree with Hacke and von Kummer that these are important factors in CT interpretation. The variability in these factors in everyday practice, in which CT scans may be interpreted by general radiologists on their home computer using telemetry techniques or experienced neuroradiologists viewing the films on the CT console, only emphasizes the fact that variability in interpretation is likely.

We also agree with Hacke and von Kummer that “loss of gray-white distinction” and “hypodensity” are continuums of the same process, namely, hypotension due to tissue water uptake. In fact this is already clearly stated in the paper. Nevertheless, partly as a response to concerns about identifying very subtle changes, clinicians commonly subdivide early CT changes into microcategories, such as “insular ribbon sign,” etc. However, even if all of these are collapsed into a single category of “any change occurring in more than one third of the MCA territory,” only fair agreement was found.

Although we mention MRI in our paper, we did not advocate MRI over CT, since there are even less data evaluating variability in MRI interpretation and correlating early MRI changes with response to therapy.

In conclusion, the ideal test, or how to best interpret existing tests such as CT, has not yet been determined. CT is currently the gold standard, but our data suggest that we need more precision in its interpretation. Most importantly, clinicians should recognize the pitfalls inherent in overreliance on subtle CT changes in acute stroke patients, particularly since at present there are still no data to support exclusion of patients on the basis of early ischemic changes on CT who would otherwise qualify for thrombolytic therapy within 3 hours.

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**Letters to the Editor**

**Measuring Outcome in Acute Stroke Trials**

To the Editor:

We welcome the article of Sulter and colleagues in the August edition of your journal, in which they investigate the use of the Barthel Index (BI) and Modified Rankin scale (MRS) in acute stroke trials. The lack of objectivity in defining what is a favorable outcome lends itself to redefining the primary end point should the results of a clinical trial not meet with expectations. The temptation to move the goal posts (MRS <1 to <2) is a clear indication of the ambiguity of the end point.

In the event of a safe, simple, and effective treatment for stroke being discovered, it is likely that this will be given to patients with significant levels of comorbidity and preexisting disability—stroke related or otherwise. In this situation it will be much more difficult to evaluate the clinical effectiveness of the treatment, and use of “poor outcome” as a clinical end point becomes much more relevant. It could be further argued that since the principal objective of stroke treatment is to reduce disability and dependency, it is the avoidance of this poor outcome that is of paramount importance.

The clinical relevance of a stroke treatment trial inevitably reflects the trial population; if a highly selected group of patients with no previous disability is selected (MRS=0), it is impossible to be sure what a favourable outcome for such an individual would be. Conversely, we believe there would be little doubt among patients and their carers what a poor outcome would be: death, institutionalization, MRS >3, or BI <60. Dichotomizing outcome to a 3-month MRS of <1 or <2 is even more subjective. In a more pragmatic acute stroke trial (for example, trials of acute stroke unit care or intervention trials for raised glucose or temperature), the inclusion of patients with prior disability (Rankin Scores <3) makes the “good outcome” even less appropriate and emphasizes the practical relevance of the poor outcome. Interestingly, in designing the Glucose Insulin in Stroke Trial (GIST), we sought to determine the effectiveness of maintaining euglycemia in acute stroke in a representative population of patients who might present to any acute stroke unit. To recruit patients with prior disability (Rankin <3), we recognized the inappropriateness of the good outcome and opted for poor outcome, defined as death or MRS >3.

In the United Kingdom the results of the Stroke Unit Trialist’s Collaboration (reduced dependency, institutionalization, and mortality) have led to widespread introduction of organized stroke care. We believe that the use of a poor outcome in stroke trials deserves wider recognition for its clinical, practical, scientific, and ethical relevance.

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Disturbed Diffusion and X-Ray Hypoattenuation in Acute Stroke

To the Editor:

Barber and colleagues[1] are the first to study the important question of whether diffusion-weighted MRI (DWI) is superior to CT in predicting irreversible ischemic tissue damage. They presented the MRI and CT findings obtained from 17 patients within the first 6 hours of symptom onset and used a T2-weighted image at 90 days as gold standard for the final infarct size. According to their table, 15 patients had an infarct on the follow-up MRI. One of 2 patients without infarct on the follow-up MRI was later identified as a nonstroke patient (patient 13). This patient, however, had a positive DWI but a negative CT at baseline, whereas the other had a negative DWI and CT at baseline. I cannot follow the authors saying that “hyperintense lesions on DWI consistent with acute ischemia were seen in all 16 patients with a final diagnosis of stroke, giving a sensitivity and positive predictive value for DWI of 100%.” The predictive values for a ischemic lesion on follow-up imaging in this study are presented in the Table.

Based on these data, it is premature to conclude that “DWI is able to identify the presence of early infarction with greater sensitivity than CT.” Moreover, CT was obtained earlier than MRI in 10 patients, with a difference of >3 hours in 2 patients. Although disturbed diffusion is more easily depicted on DWI than slight hypoattenuation on CT, it is an open question which finding is more specific for irreversible tissue damage.[2,3]

I conclude from this study that we need more data obtained in a manner similar to that used by Barber et al.[1] I would like to encourage the authors to continue their efforts in imaging acute stroke patients with these two modalities. We would like to know whether disturbed diffusion and x-ray hypoattenuation show the same pathophysiology, eg, early ischemic edema. We need to know at which time after symptom onset or at what degree these phenomena are specific for irreversible tissue damage. And, finally, we should carefully study patients with stroke who have a negative early DWI or CT. Stroke in these patients may be different compared to patients with positive early imaging. Irreversible tissue damage may not occur in these patients or may be delayed.

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Response

We appreciate the opportunity address the points raised by Prof von Kummer. On page 2061 of our study,[1] we mistakenly identified patient 12 in the table as patient 13 in the text. Thus, the only patient without a final diagnosis of stroke was patient 12. He was included in this analysis because his presentation resulted in initial treatment as a stroke patient. However, normal acute CT and DWI studies prompted a search for an alternative diagnosis, which resulted in a final diagnosis of a brachial plexopathy. This oversight was in the misidentification of the patient in the body of the text, not in the calculation of the sensitivity and positive predictive value for DWI. We apologize for the confusion that this mistake may have caused.

The second issue is that Prof von Kummer has reinterpreted the results using day 90 T2-weighted images as the gold standard for a final diagnosis of stroke. While T2-weighted imaging at day 90 was used to measure final infarct size, a final diagnosis of stroke was determined on the basis of both standard clinical criteria and imaging results, a distinction noted in both the Methods and Results sections. Therefore, in patient 13, an investigator blinded to the clinical data and the results of earlier imaging studies was unable to detect evidence of a relatively small acute DWI lesion on the day 90 T2-weighted images. Previous studies have found that chronic infarct size may be smaller than DWI lesions in the first days following stroke.[2–4] Possible explanations for this observation have included chronic cerebral atrophy or reversal of the DWI lesions at the margins of the hyperacute lesion.[3,4] In contrast, the attending physicians arrived at a final diagnosis with full knowledge of a patients’ clinical history and course and the unblinded interpretation of all investigations. These included magnetic resonance perfusion imaging (PI) and subacute MRI studies at day 3, as well as other more conventional poststroke investigations not reported in this study. Thus, hyperintense lesions consistent with ischemia were indeed seen in the acute DWI studies of all 16 patients with a final diagnosis of stroke. Our results and conclusions remain the same.

We agree that the question of whether DWI or CT is more specific in the early identification of irreversible change is open. This and the other important questions raised by Prof von Kummer require more animal or larger human studies. Furthermore, the full potential of DWI to identify individual patients most likely to benefit from acute interventional stroke therapies, either alone or in combination with other MRI sequences such as PI, needs investigation. To this end we are recruiting and imaging acute hemispheric stroke patients with both CT and DWI before treatment with tissue plasminogen activator.

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**TCD in Acute Stroke**

*To the Editor:*

We read with great interest the *Stroke* article by Alexandrov and colleagues.1 This study stresses the importance of transcranial Doppler ultrasonography (TCD) for the rapid assessment of patients with acute cerebral ischemia. Expedient measurement of cerebral hemodynamics with TCD in the emergency room offers new insight into the process of acute stroke and provides guidance for and monitoring of therapeutic interventions. Regarding their findings and interpretation, we would like to make the following comments.

(1) It is our understanding that the authors used digital subtraction angiography (DSA), MR angiography (MRA), and CT angiography (CTA) for calculation of sensitivity, specificity, and overall accuracy of TCD findings. However, the “angiographic studies” (DSA, MRA, and CTA) were performed within 48 hours after admission. We think that the use of delayed angiographic studies to determine accuracy of TCD is questionable. The time difference and sequence between TCD and angiographic studies may change the sensitivity of early TCD if recanalization occurred between TCD completion and angiographic studies. Although spontaneous recanalization of thrombosed extracranial and intracranial vessels is known to occur, the timing of spontaneous recanalization is unknown. The cumulative experience suggests a spontaneous recanalization frequency between 14% and 24% during the first 24 hours after onset of cerebral ischemia.2–4 The timing of MRA and TCD investigations could influence the findings of Alexandrov and his coworkers.1 Ideally, angiographic studies and TCD comparisons should be made using studies performed within 2 to 4 hours of each other to limit discrepancies caused by the natural history of thrombosis.

(2) Another consideration is the fact that the authors used 3 different angiographic methods (DSA, MRA, and CTA) to evaluate specificity and sensitivity of TCD. TCD is likely to have different specificity and sensitivity when compared with each of these 3 angiographic methods. The use of the combination for this comparison is therefore arguable.

(3) It is accepted that the specificity and sensitivity of TCD varies from one segment to another.5,6 We have shown that in the first 24 hours after acute stroke, overall TCD specificity in detecting abnormal cerebral blood flow velocity (CBFV) in all affected vessels (anterior and posterior circulation) is 33%.7 When we analyzed only the middle cerebral artery territory lesions, the specificity increased to 100%, which is in keeping with the higher values noted by Camerlingo et al8 (92%) and Alexandrov et al1 (88.6%). We would be interested to know whether the results of Alexandrov et al1 showed any difference in specificity according to the location of the arterial lesion. Finally, we had significant difficulty obtaining methodological information (the Appendix with the description of fast-track protocol and diagnostic criteria for the location of arterial obstruction) using the Internet (http://www.strokeakha.org). We would suggest that important information pertinent to the paper should be published in the body of work.

We agree with Alexandrov et al1 that CBFV quantitative measurements using TCD will provide a powerful tool for the selection of patients for reperfusion therapy.

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**Response**

We appreciate the comments of Drs Razumovsky and Oppenheimer and would like to reply to the points made.

(1) We had to use all available angiographic imaging modalities (ie, DSA, MRA, or CTA), since these tests are often used to judge TCD performance in clinical practice and time delays between TCD and angiography are unavoidable outside of a rigorous trial. We agree that arterial recanalization, clot propagation, or reocclusion may have occurred after TCD was performed, thereby decreasing its accuracy compared with that of angiography. We provide additional data regarding time delays from TCD to angiographic studies: 19% of the angiograms were performed within 2 hours after TCD; 29% within 2 to 6 hours; 19% within 6 to 24 hours; and 33% were delayed by more than 24 hours after TCD.

(2) Despite these shortcomings, we demonstrated that TCD accurately reflects arterial patency in 88% of patients when compared with a combination of DSA, MRA, and CTA. Although this combination may not be an adequate standard, individual management decisions are often based on an angiographic test obtainable or when the risks associated with DSA were justified. An overall good agreement between TCD and various angiographic modalities indicates that TCD can be used as a reliable screening or complimentary test when emergent angiography cannot be performed or serial angiography is impossible.

(3) Drs Razumovsky and Oppenheimer correctly pointed out that TCD accuracy varies with different arterial segments involved. In our decisions drawn from bedside TCD studies, we consider that TCD accuracy is the highest for the proximal anterior circulation lesions and is less for the posterior circulation. So far, we have analyzed 190 patients with variable duration of symptoms of cerebral ischemia who had TCD and DSA or MRA and have calculated the accuracy parameters for TCD in identifying arterial occlusions at different segments.1 For the middle cerebral artery occlusions, TCD had sensitivity of 93%, specificity 98%, positive predictive value 93%, and negative predictive value 98%. For occlusions located at other arterial segments, the accuracy parameters were as follows: distal internal carotid artery 81%, 96%, 81%, 96%; proximal internal carotid artery 94%, 97%, 94%, 97%; basilar artery 60%, 96%, 60%, 96%; and vertebral artery 55%, 96%, 71%, 92%.1 Although sensitivity for posterior circulation occlusions was low, a normal
TCD examination excluded major arterial occlusion at any level with at least 92% certainty. The high specificity values achieved in our study resulted from a large number of consecutive patients who had patent vessels on DSA or MRA (75%) and may also be attributed to the use of detailed diagnostic criteria for TCD developed for the assessment of stroke patients.\(^1\) We also appreciate the comment regarding the access to the Web site that contains the Appendix to our article. It had been suggested that we shorten the article by placing the fast-track insonation protocol and diagnostic criteria on the Internet. Since the article we shorten the article by placing the fast-track insonation contains the Appendix to our article. It had been suggested that we shorten the article by placing the fast-track insonation protocol and diagnostic criteria on the Internet. Since the article has been published, several people have had difficulty accessing this web location, which apparently requires an online subscription to Stroke. We hope that this issue will be resolved and online publications in the future will have an easy and free access.

We are also glad that Drs Razumovsky and Oppenheimer are on the same wavelength with us regarding the usefulness of TCD in acute stroke management.

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Editor's Note: We apologize to readers for any difficulty they had while trying to access the appendix. The website problem has been repaired and we hope access will now be simple and direct. This appendix can now be accessed in several ways. One way is to click on the title of the article. When the article appears on your screen, a box on the right hand side of the title will list various links, including “Appendix.” Click on “Appendix” and it will appear on your screen.


Duplex Scanning of the Ophthalmic Artery and Carotid Endarterectomy

To the Editor:

Nuzzaci and colleagues presented in their article, “Duplex Scanning Exploration of the Ophthalmic Artery for the Detection of the Hemodynamically Significant ICA Stenosis,” data from 3 groups of patients (351 in total, with 548 internal carotid arteries [ICAs]) to support their argument that adding Doppler sonographic study of the ophthalmic artery (OA) to color duplex scanning of the extracranial ICA would improve the detection of critical ICA stenosis. I wish to make the following comments.

First, the benefit of carotid endarterectomy in secondary stroke prevention has been proved among highly selected patients with high-grade ICA stenosis defined by angiography plus a stringent method of measurement.\(^2,3\) The benefit in primary stroke prevention is small but statistically significant in patients with high-grade ICA stenosis defined by angiography, although the ICA stenosis of many medically treated patients was defined by Doppler sonography of the extracranial ICA.\(^4\) Thus, results of the carotid endarterectomy trials\(^2-4\) may not apply when measurements of the ICA stenosis are made with other methods. It is imperative to compare the results of noninvasive studies with angiography at individual centers and to validate the noninvasive studies against angiography before results of noninvasive studies can be used to select the appropriate patients for carotid endarterectomy.\(^1\) In addition, results of carotid endarterectomy trials\(^2-4\) are tied with the particular method of measuring the ICA stenosis on angiography, although 30%, 40%, 50%, 60%, 70%, 80%, and 90% stenosis in the North American Symptomatic Carotid Endarterectomy Trial corresponds to stenosis of 65%, 70%, 75%, 80%, 85%, 92%, and 97%, respectively, in the European Carotid Surgery Trial.\(^5,6\) Therefore, disagree with the authors in their selection of the residual lumen of the ICA plaque as the “gold standard” in comparing the “accuracy” of different diagnostic methods (see their Table 1).\(^1\) Instead, all other methods should be compared with the angiographic findings so that carotid endarterectomy can be performed in appropriately selected patients without angiography in the future.

Second, it is important to note that the 351 patients were selected by a referral for possible carotid endarterectomy, and other patients with severe contralateral stenosis or intracranial artery stenosis were excluded from the study.\(^1\) The selection bias would limit the application and usefulness of Doppler sonography of the OA in the general population of patients. I also wonder whether duplex sonography, MR angiography, or angiography was performed in any of the 351 patients before being referred to the authors.

Third, the reasons of dividing the patients into 3 groups were not mentioned by the authors.\(^1\) It appears that some of the patients in the second and third groups did not undergo carotid endarterectomy because their ICAs were occluded on the imaging studies.

Finally, I agree with the authors that a differentiation between occlusion and high-grade stenosis of the ICA is clinically important.\(^1\) Nevertheless, none of the authors’ criteria from Doppler sonography of the OA can achieve this differentiation.

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Response

We are grateful for the opportunity to reply to the comments of Dr Raymond T. F. Cheung on our study.\(^1\)

Regarding his first point, we would like to quote from a recent Stroke editorial. “The actual degree of stenosis that constitutes the necessary environment to initiate the stroke process remains unknown. It is possible that it will be less than a 70% stenosis; current ongoing studies should tell us. However, it is also possible that only anatomically tighter stenoses are clinically relevant; new studies of different design may be required to clarify this issue. Of the two possibilities, our experience favors the latter, and the issue might only be resolved by using residual lumen diameter measurements, rather than percent stenosis...
calculations, to determine degree of stenosis. . . Following the lead of C. Miller Fisher we routinely measure residual diameters. We rarely see a stroke in association with a carotid artery lesion that has a residual lumen diameter greater than 1.5 mm, and we uncommonly see a stroke when it is greater than 1.0 mm. . . By carotid Doppler frequency and velocity measurements we find that internal carotid artery flow begins to fall when the residual lumen diameter is 0.5 to 0.7 mm. This is a long way from a 70% stenosis.2

The evaluation of the residual lumen of ICA performed on the plaque removed en bloc at the time of carotid endarterectomy is presently considered the best gold standard for comparing the accuracy of different diagnostic methods.3

Although angiography has allowed us to achieve very important diagnostic information about the ethiopathogenesis and the prevention of stroke, this modality is, nevertheless, not the most accurate one for the detection of the hemodynamically significant artery stenosis. All of us know that the pathognomonic feature of this type of stenosis consists of the fall of the blood pressure downstream from the stenosis. An approach that provides us with the measurement of this parameter appears therefore to be the best one. Although we are presently not able to achieve this parameter non invasively, nevertheless we can overcome this drawback if we keep in mind that the fall of the blood pressure downstream the stenosis is necessarily associated with the activation of the collateral supply to the vascular territory.4

Even if the detection of this activation is an indirect test yet it represents the most reliable index presently available, on a non invasive way, of the hemodynamically significant arterial stenosis. With the OA being the first branch of ICA and therefore the nearest to the carotid bulb where the stenosis is located, it is very suitable to reveal the supply activation, as our results pointed out. Angiography is by nature a morphological and not physiological diagnostic modality. Moreover, angiography cannot provide us with any information on the vessel wall, and without this parameter we cannot accurately measure the diameter of the residual lumen of the artery.

Regarding the second point: The aim of our study was to check the accuracy of OA duplex sonography for the detection of ICA hemodynamically stenosis. The application of our approach in the general population did not concern the target of this study. We agree that it should be done. No patients underwent duplex sonography, MR angiography, or angiography before being referred to our study.

Third point: According to the aim of our study, we checked in the first group of patients the agreement between our method and the gold standard. On the basis of the obtained results we afterward studied, in the patients of the second group, the screening capacity of each of the 5 categories of the OA duplex sonography signals. These results pointed out that only one, low positive (LP) signal, was not reliable for the screening. Finally, to increase the specificity of this signal, we studied the third group of patients whose OA duplex sonography showed the LP signal in one eye.

Fourth point: Our results suggest that by performing duplex examination at both the ICA bifurcation and the ipsilateral OA, we can easily know, according to our criteria, whether the ICA stenosis is hemodynamically significant or not; moreover, we can also achieve the differentiation between stenosis and occlusion of the ICA.

Finally, we agree that it is important to differentiate between a high of degree stenosis and occlusion of the ICA; however, we also agree that the detection of the former carotid pathological condition is much more clinically relevant.

Letters to the Editor

Akira Hara, MD, PhD
Hideki Mori, MD, PhD
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the maturation rate but only postponed the neuronal damage. While we agree with these results, we concur with the concerns expressed in the Editorial Comment on this article regarding their conclusions that the delayed neuronal death (DND) is not apoptosis, but is in fact necrosis. This conclusion was made on the basis of the lack of the morphological evidence for apoptosis by ultrastructural examination. However, morphological change during apoptosis occurs dynamically, with initial steps occurring within the first few hours. It is, therefore, very important to observe the early phase of apoptotic process to obtain the morphological evidence for apoptosis.

The authors performed the ultrastructural study 7 days after 15 minutes of ischemic insult. We think that this time point is too long after the initiating insult. In their time course data using light microscopic examination, 74% of CA1 neurons subjected to 15 minutes of ischemia exhibited damage as early as 3 days after ischemia. It is speculated that electron microscopic evidence of apoptosis has disappeared by 7 days after the ischemia. In fact, an electron microscopic study by Nitatori et al,2 which was performed in accordance with the original method of DND,3 demonstrated the apoptotic evidences at 3 days after ischemia.

Morphological characteristics themselves do not provide an unambiguous definition of apoptosis. Other studies, such as DNA fluorescence assay, in situ terminal deoxynucleotidyl transferase–mediated dUTP nick-end-labeling (TUNEL) method,4 gel electrophoresis,5 and caspase activation,6 indicate that DND is a form of programmed cell death, or apoptosis. It remains unknown, however, whether DND is necrosis or apoptosis.

Recently, we reported a sequential TUNEL technique as a method for tract tracing to demonstrate novel apoptotic evidence for DND in hippocampal CA1 pyramidal cells after transient ischemia.7 In this study, we demonstrated the migration of the fragmented DNA from the nuclei into the apical dendrite in the CA1 pyramidal cells after ischemia. The migration of the fragmented DNA into apical dendrites in stratum radiatum of the hippocampus was first observed 66 hours after the ischemic insult. The fragmented DNA localized in the dendrites appeared most prominently at 96 hours (see arrowheads in the Figure). Pooling of the fragmented DNA around the dendrite terminal end in the stratum lacunosum molecular of the hippocampus was observed at this time (see arrows in the Figure). The localization of fragmented DNA in apical dendrites was confirmed by DNA fluorescence staining.8

The characteristic morphological changes such as chromatin condensation, its movement to the nuclear periphery, and apoptotic body formation in the typical apoptotic cells require ATP-dependent energy support. Interestingly, ATP is essential for morphological changes occurring in nuclei during apoptosis but not for DNA fragmentation.9 Axonal or dendritic transport also requires ATP-dependent energy support. Yasumoto et al10 have reported that the ATP levels in the stratum radiatum were 118% and 43% of those of control at 2 and 4 days after ischemia, respectively. It is speculated that the dendritic DNA flow based on ATP metabolism is over within 4 days.

Necrosis is defined, in other words, as a failure of cellular metabolism. Our results reveal ATP-dependent transport system of CA1 pyramidal neurons after transient ischemia is still active after nuclear DNA fragmentation, which is considered to be a late event in the apoptotic process. This finding strongly supports the hypothesis that DND is indeed apoptotic cell death. We also wish to emphasize that the dynamic events of apoptotic process of DND are transient and are complete within 4 days.

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Response
Our study1 examined the light and ultrastructural characteristics of CA1 neuronal death following 5 minutes of ischemia (14-day survival) or 15 minutes of ischemia (7-day survival). We did not find any morphological evidence classically described as apoptosis (eg, apoptotic bodies). It is possible that we simply missed these features, which may have occurred earlier. However, we were specifically interested in comparing two different rates of DND in CA1, which certainly did occur at those times sampled. Furthermore, in a comparable study2, in which CA1 DND was examined at 4, 14, and 60 days in gerbils subjected to ischemia with and without delayed hypothermic neuroprotection, we still did not observe any morphological signs of apoptosis. Again, CA1 neuronal death occurred throughout this period, because some CA1 loss was postponed by the delayed hypothermic treatment. Many others have similarly failed to see morphological evidence for apoptosis (eg, References 3 through 5) at these and earlier survival times.

It is perhaps true that morphological findings cannot rule out biochemical events such as occur with programmed cell death. Indeed, as stated in our article3 and as noted in both the editorial comment by Clemens4 and the above letter by Hara et al, there are several events that occur in CA1 neurons destined to die which also occur in programmed cell death (eg, caspase activation). However, it has yet to be clearly proved whether these events are causal or merely coincidental with CA1 DND. Furthermore, some of this evidence, such as TUNEL staining, is nonspecific and occurs with a necrotic-type cell death.5 Other evidence, such as use of caspase inhibitors to reduce CA1 loss, is also suspect, because the observations have not always been replicated6 and, importantly (as we made clear in our study), the survival times have been inadequate (eg, 4-day survival time7).
C-Reactive Protein and Outcome After First-Ever Ischemic Stroke

To the Editor:

We have carefully read the study of Keith Muir and colleagues published in Stroke regarding the putative role of C-reactive protein (CRP) as outcome predictor after ischemic stroke and would like to add further observations to their data. Despite the theoretical importance of CRP in ischemic stroke, very little direct evidence exists to implicate CRP in stroke. In their study, Muir and colleagues had not only investigated the CRP role but also discussed the causative role of inflammation in acute ischemic stroke. They found an intriguing association between CRP levels within 72 hours of stroke and an increased risk of death with an excess of cardiovascular mortality. The authors suggest three possible explanations to support the CRP role as predictor of outcome. They state that (1) CRP concentration may reflect the degree of stroke severity correlating with the degree of inflammation directly consequent to cerebral infarction, (2) CRP concentration may indicate underlying unstable atherosclerotic lesions, and (3) CRP may be raised as a consequence of secondary complications of stroke at the time of sampling. Although the mechanism responsible for this increased risk was unclear, the authors’ recommendation to stratify patients with ischemic stroke into relatively high-risk and low-risk groups according to inflammation level sounds appropriate, considering that the relevance of inflammation in cerebrovascular disease is not completely established.2

Their data are in agreement with the preliminary results of our study on CRP in ischemic stroke.3,4 We found increased CRP levels in our stroke patients, with a notable difference in mean level of CRP between patients and healthy control subjects. Higher CRP levels were correlated with a significant neurological deficit and a relevant disability.3,4 To shed some light on these issues, we would like to present our recent results in a larger series of stroke patients (n=72; 30 men and 42 women; mean±SD age 73±9 years) included in the Villa Pini Stroke Data Bank, Chieti, Italy. In this series we investigated whether CRP levels remain elevated after stroke event and whether persistent elevation is associated with new vascular events or death. We measured plasma levels (within 24 hours) of CRP, fibrinogen, total serum C3c (C3) and C4 (C4) complement fractions, ferritin, and total cholesterol after stroke and at discharge (mean±SD 12±5 days). Patients were followed for 6 months. To avoid confounding factors, we excluded patients with history of recent clinical infection; concurrent major renal, hepatic, and cancerous diseases; and obvious signs and clinical evidence of acquired infection within 2 weeks after stroke. At discharge, CRP was elevated (≥10 mg/L) in 57 patients (79%); of these, 26% had elevated levels within the first 24 hours after stroke. Only 1 patient (7%) with discharge levels of CRP ≤10 mg/L died, but 35% of those with elevated CRP (n=20; P=0.04, log-rank test) died or had a new vascular event. New vascular events or death occurred in 12.5% of patients in the lower tertile of CRP (≤14 mg/L), in 25% of those in the intermediate tertile (15–28 mg/L), and 50% of those in the upper tertile (≥29 mg/L; $P=0.01$, log-rank test; $P=0.005$ $\chi^2$ for trend). In conclusion, our data suggest that CRP was increased in patients with cerebral ischemia, may remain elevated after stroke, and is associated with the prognosis of such patients.

We believe that the role of CRP after ischemic stroke is far more complicated than perhaps we realize. CRP may be primarily an indicator of other vascular risk factors that are themselves related to prognosis. In our patients, CRP levels were positively correlated with serum ferritin levels (r=0.6; $P<0.001$; Pearson correlation coefficient), which suggests that the elevation of CRP may be an epiphenomenon. Iron overload may elevate the risk of atherosclerotic disease by promoting the oxidation of LDL.
cholesterol and has been identified as risk factor and outcome predictor in recent studies on vascular diseases.\textsuperscript{5,6} CRP displays both anti-inflammatory and proinflammatory effects, including the ability of ligand-bound CRP to activate the complement system.\textsuperscript{7} Interestingly, patients with activated complement system, detected by total C3 and C4 serum levels, had a significantly higher occurrence of new vascular events or death (40\% versus 7\%; \(P=0.02,\) log-rank test). Probably, CRP may reflect something fundamental about the patient’s inflammation system. Some patients might be predisposed to intense activation of inflammation in response to a variety of stimuli such as stroke. We speculate that stroke patients in whom the inflammation system reacts most intensely may be at greater risk for subsequent events. In this way a stroke may show the abnormal reactivity of the inflammation system. CRP levels would identify those patients whose inflammation system responds most actively to stimuli. These might be the patients at highest risk for subsequent new vascular events or death, in whom more aggressive therapy and clinical surveillance might be appropriate.

If confirmed in larger studies, our findings may have relevant practical implications because low serum levels of CRP at the time of hospital discharge identify a group of patients at low risk. Conversely, elevated CRP levels at discharge identify patients at high risk who therefore may benefit from a more careful clinical follow-up and appropriate antithrombotic (and probably anti-inflammatory) treatment. Precise knowledge of the possible triggers of the inflammation and the determinants of its individual response may open novel therapeutic avenues.

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Novel Apoptotic Evidence for Delayed Neuronal Death in the Hippocampal CA1 Pyramidal Cells After Transient Ischemia
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