Letters to the Editor

Cholesterol and Carotid Stenosis

To the Editor:

In a case-control study, Dr Mathiesen and colleagues1 compared the blood levels of high-density lipoprotein cholesterol (HDL-C) in 216 patients with carotid stenosis with those in 223 subjects who were free from the disease. They found an inverse association between HDL-C levels and the risk of carotid stenosis, a precursor of ischemic stroke.1 The authors acknowledged that the main problem with their study was its use of cross-sectional data—the HDL-C levels among the cases were measured at the time of the diagnosis of carotid stenosis. Consequently, the postdiasease levels of HDL-C in cases were compared with the prediasease levels in control subjects. If the study risk factor had a value that could not change, such as a blood group type, the case-control comparisons would not have been biased. However, blood cholesterol is a variable whose value can change due to medications or it can physiologically change over time. This case-control study could not establish that the HDL-C levels in cases at the time of diagnosis represented their HDL-C levels before atherosclerotic disease of carotid arteries had developed. Consequently, the time lag between measuring the baseline attributes and ascertaining the disease was not possible.

It could be argued that a relative increase or decrease in blood cholesterol among cases in this study could have been caused by the disease. This problem with cross-sectional data was addressed by an analysis of the data on cholesterol in the Framingham Heart Study. Although atherosclerotic carotid stenosis was associated with total serum cholesterol and HDL-C levels 8 years prior to the diagnosis,2,3 there was no association between carotid stenosis and total blood cholesterol at the time of the diagnosis.3 These data clearly show that the total cholesterol levels at the time of the diagnosis were not representative of the levels 8 years prior to the diagnosis.

The HDL-C very likely protects against carotid artery disease and ischemic stroke. However, this association should be established in more than 1 population. Existing data sets of large cohort studies, such as the Framingham Heart Study, would be an ideal setting for this investigation. Expensive experimental studies, as called for by Mathiesen et al,1 are not necessary at this stage.

We agree with Dr. Sheikh that inference about causality cannot be done in a cross-sectional study. However, we doubt that data from the Framingham study can be used to study the issue of concern in our article, namely the relationship between risk factors and plaque echogenicity in carotid stenosis. As far as we know, the Framingham study has not published data concerning plaque echogenicity. Apart from our own study, we are not aware of any population-based, prospective studies on plaque echogenicity that have collected data on cardiovascular risk factors several years prior to the ultrasound examination. The Tromsø study is a prospective study on cardiovascular disease, with repeated population-based health surveys. The first survey was done in 1974, and ultrasound examination of the carotid arteries was included for the first time at the fourth survey, in 1994 to 1995. In 95 of the 216 subjects with carotid stenosis, we have information on HDL cholesterol levels and other risk factors collected 8 years prior to the ultrasound examination. We have therefore examined the association between ultrasound plaque morphology and risk factors measured 8 years prior to the ultrasound examination in these subjects. We found that subjects with echolucent and predominantly echolucent plaques had significantly lower mean level of HDL cholesterol than subjects with echogenic and predominantly echogenic plaques (1.38 mmol/L versus 1.56 mmol/L, P=0.04). In multivariate analysis with 4 categories of plaque echogenicity and adjustment for age, sex, degree of stenosis, triglycerides, and systolic blood pressure, the odds ratio for being in a lower plaque echogenicity category was 0.53 (95% CI 0.33 to 0.87; P=0.01) for a 1-SD increase in HDL (SD=0.39 mmol/L). We think these results add evidence to the assumption that HDL is an important risk factor for echolucent, rupture-prone stenotic plaques.

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References

Diurnal Variation in the Intensity of Anticoagulation in Atrial Fibrillation

To the Editor:

We read with interest the letter by Lip et al1 about the diurnal variation in the onset of stroke in atrial fibrillation (AF), which reported the presence of a circadian rhythm in stroke onset among these patients, with a higher number of strokes taking place between 6 AM and 6 PM and fewer between midnight and 6 AM. Although it is not specified if this variation occurs in both anticoagulated patients and those who are not, it seems that it is also present in patients who were receiving anticoagulation in the moment the stroke took place.

It is known that the majority of cardiovascular events (myocardial infarction, sudden death, etc.) follow a circadian pattern,2,3 with most events occurring early in the morning. We also know that AF increases the risk of systemic embolism and that AF has been associated with the presence of thrombus in the left atrial appendage.4 However, there has been little scientific interest in the study of a possible circadian pattern in the hypercoagulable state seen in AF. Li-Saw-Hee et al4 analyzed the circadian rhythm in some hemostatic factors in patients with chronic AF; they concluded that there was no significant diurnal variation in plasma levels of any of these factors and, thus, that the prothrombotic state in AF was persistent. Decousus et al10 suggested that there is a diurnal variation in the intensity of anticoagulation, even if intravenous heparin is administered in a constant infusion. This variation in the intensity of anticoagulation together with the persistent hypercoagulable state in AF could be responsible for a different thromboembolic risk during the day, existing periods when protection against stroke would be insufficient.

To study the presence of circadian variations in the degree of anticoagulation, we measured the international normalized ratio (INR) in 21 patients with chronic AF at four different moments of the day (noon, 6 PM, 11 PM, and 8 AM). All these patients had received controlled anticoagulation therapy with acenocoumarol (once daily, at 6 PM), maintaining a consistent INR between therapeutic limits (INR = 2.00 to 3.00) for at least 4 weeks. Oral anticoagulant therapy was monitored using an automated assay (ACL Futura, Instrumentation Laboratory Co.) with a bovine calcium thromboplastin (Pro-IL-Complex, ISI 1.02). Factor VII is one of the proteins inactivated by oral anticoagulants—as it has the shortest half-life of all of them (6 hours), its diurnal variation in response to anticoagulant therapy can be seen in a 24-hour period. We determined levels of factor VII using a clotting assay with factor VII deficient plasma and its activated form (factor VIIa; STACLOT VIIa-rTF, Diagnostica STAGO), both in an automated coagulometer (STA, Roche).

There was a diurnal variation in INR measurements in patients with AF, with lower levels at 11 PM and 8 AM compared with those at noon and 6 PM (paired-samples t test, P = 0.046). The majority of our patients were anticoagulated correctly, with only 2 of the measurements (2.4%) under therapeutic range. There was also a circadian rhythm in factor VII and factor VIIa levels (see Table). Based on these results, it seems to be a period (between 11 PM and 8 AM) when the intensity of anticoagulation is lower (reflected by lower INR and higher levels of factor VII).

To our knowledge, no studies have analyzed circadian variations in anticoagulation intensity in patients with AF. Our study demonstrates the existence of a circadian rhythm in the intensity of anticoagulation in these patients, with lower INR values at 11 PM and 8 AM; moreover, the levels of factor VII and factor VIIa (inactivated by oral anticoagulants) were higher in the same period. The lower intensity of anticoagulation during the night and in early morning together with the lack of diurnal variation in hemostatic factors that has been reported could explain a clustering in stroke occurrence in the morning and early evening, as shown by Lip et al.

We agree with the second aspect of their letter, which points to the underutilization of antithrombotic therapy in patients with AF. The efficacy of this therapy to prevent embolic events in high-risk patients has been demonstrated (advanced age, high blood pressure, heart failure, history of stroke, etc.),4,11–13 with nearly 65% reduction in the incidence of stroke.14 In spite of this, several studies have showed that it is scarcely used.15–18 To analyze the use of this therapy in our community, we collected data about the use of antithrombotic therapy in 57 patients with chronic AF of 1506 consecutive patients who came to our emergency service on 8 aleatory days. Although all of them had at least one clinical or echocardiographic risk factor for stroke, only 47% were taking anticoagulation therapy (acenocoumarol) and 23% were taking aspirin; none of them were taking both acenocoumarol and aspirin. In the multivariate analysis, only 2 variables were associated with the use of anticoagulation: age (the use of acenocoumarol was significantly higher in younger patients) and the fact that the patient was visited by a cardiologist rather than by a general internist or a family physician. Risk factors such as hypertension, heart failure, history of stroke, and diabetes showed no relationship to the use of antithrombotic therapy, nor did other aspects studied such as sex, comorbidity (CIRS scale), left atrial diameter, and left ventricle ejection fraction.

To conclude, our study shows the presence of a diurnal variation in the intensity of anticoagulation in patients with AF who were under oral anticoagulation with acenocoumarol, with lower levels during the night and early morning. This could explain the higher number of strokes taking place during the morning and afternoon. On the other hand, and in accordance with other studies, we have found that antithrombotic therapy is scarcely used in patients with AF, even if they have risk factors for stroke.

Values of International Randomized Ratio, Factor VII, and Its Activated Form (FVIIc and FVIIa) at 4 Different Moments of the Day

<table>
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<tr>
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<th>Noon</th>
<th>6 PM</th>
<th>11 PM</th>
<th>8 AM</th>
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</thead>
<tbody>
<tr>
<td>INR</td>
<td>2.95±0.74</td>
<td>2.80±0.61</td>
<td>2.74±0.61</td>
<td>2.73±0.71</td>
</tr>
<tr>
<td>FVIIc (%)</td>
<td>35.38±10.68</td>
<td>42.71±12.51</td>
<td>45.57±14.59</td>
<td>41.21±15.82</td>
</tr>
<tr>
<td>FVIIa (U/mL)</td>
<td>7.84±5.5</td>
<td>7.25±4.46</td>
<td>11.03±7.39</td>
<td>10.05±6.20</td>
</tr>
</tbody>
</table>

Analysis of variance for repetitive measures was performed (MANOVA). INR, international normalized ratio; FVIIc, factor VIIc; FVIIa, factor VIIa.
INR levels at 11 PM and 8 AM, and higher factor VII levels, when compared with levels at noon and 6 PM, would be consistent with our observations.

We would also agree with their second point that antithrombotic therapy is underutilized in patients with AF, despite the presence of stroke risk factors. While the evidence is there, and “high-risk” groups can be identified who would benefit most from warfarin,1 there still remains considerable variation in the management of AF among consultant physicians2 and more recently, among accident and emergency (A&E) department clinicians.3

Observations from the latter survey of 124 A&E consultants in England2 are important, as A&E consultants were more likely to initiate treatment only if the patient with AF had signs of shock or heart failure, but when patients had other associated medical problems, they were more likely to be referred directly to the “on-call” medical team. There was also a general reluctance to start anticoagulation in the A&E department, this being seen as something best handled by the medical team, and differences in opinion over how long should AF have persisted for anticoagulation to be necessary in the context of electrical cardioversion. Given the current view of AF as the “front line” of medical management, it makes it even more important that A&E clinicians should at least initiate management of patients with AF and be prepared to manage them for some time in the A&E department.

A final point relates to the patients’ perceptions of AF, their understanding of the disease, and the risks/benefits of antithrombotic therapy. In the West Birmingham Atrial Fibrillation Project, we have recently shown that many patients with AF possess very limited knowledge of AF as well as its consequences and therapy; furthermore there were significant differences between the different ethnic groups (Caucasian, Afro-Caribbean, Indo-Asian) in terms of their knowledge of the risks, actions, and benefits of warfarin as well as AF itself.4

We recently extended this study to a small pilot survey of 36 patients (11 men; mean age 73 years, SD 6) with AF in Kuala Lumpur, Malaysia; 75% were Chinese, 19% Malay, and 6% Indian. The commonest comorbid factors were hypertension (31%), heart failure (19%), and ischemic heart disease (14%). When these AF patients were asked whether they knew what condition they had, 72% reported “don’t know,” while other responses included “heart condition” (17%), and “fast/irregular heartbeat” in 8%. Only 1 patient could specify that the condition was “atrial fibrillation.” When asked about the seriousness of their condition, only 30% regarded AF as “serious,” with 36% considering AF as “not serious,” and 34% “did not know.” Only 8% of respondents knew that AF led to an increased risk of stroke, and 36% said that warfarin use did not pose any risk(s). The majority (58%) considered that fate/God had the most control over their health. Only 16 patients (44%) were taking warfarin (despite risk factors in most of them), and 81% of these did so “because their doctor told them to.” Given the paucity of literature from non-Caucasian populations with AF, these observations from a Malaysian AF patient population are broadly in keeping with our observations from Birmingham, England—highlighting the need for greater patient education and improvement in their understanding of this common but potentially serious cardiac arrhythmia.

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Response
We thank Garcia et al for their interest in our letter on diurnal variation in stroke among patients with atrial fibrillation (AF). Their proposal on a diurnal variation in INR intensity, with lower

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Reasons Why Stroke Trials Underestimate the Neuroprotective Effects of Drugs

To the Editor:

Despite the fact that stroke is the third highest cause of mortality and the second highest cause of morbidity, there are no effective drug treatments for acute stroke, with the possible exception of thrombolytic therapy. The persistent failure of neuroprotective agents to show benefit in acute stroke trials has absorbed immense resources for little tangible benefit or even adverse effects.1,2 More than 20 drugs have been discontinued after trials in acute stroke, and >100 000 patients may have been screened for entry into these trials without a favorable outcome being reported. Multiple mechanisms of action (antagonism of calcium and sodium channels and of N-methyl-D-aspartate [NMDA] and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid [AMPA] receptors, inhibition of glutamate release, scavenging free radicals) have been tried to reduce cell damage, all apparently without beneficial effects, despite remarkable efficacy in animal models. These paradoxes are discouraging pharmaceutical research in a critical area for therapeutic benefit.

I have been associated with the discovery and development of drugs for stroke and consider that experimental and clinical flaws in trial design result in drugs being tested under conditions in which they cannot show a positive outcome. Obviously not all drugs are uniformly neuroprotective, and it is important to ensure that a drug will work in a wide range of animal models, with good activity when administered several hours after damage. It is relatively easy to show apparently good effects for a neuroprotective drug by juggling the experimental conditions in one test, but criteria within the field are now established that should prevent the clinical testing of agents that are not robustly active.

Several flaws in preclinical testing have been noted. Preclinical testing is usually performed in young, healthy animals with low blood sugar. Cerebral vessels therefore have intact endotherelium and low levels of circulating cytokines. In contrast, the clinical situation relates to an aged population with atherosclerotic lesions, circulating cytokines, and frequently elevated blood sugar levels. Elevated blood sugar levels can negate the protective effects of some classes of neuroprotective drugs.

Flaws in clinical testing have also been observed.3 The demonstration of a penumbra and the concept of a therapeutic time window for intervention have resulted in clinical trials being focused on rapid treatment of patients after an ischemic event, with inclusion criteria being reduced from 1 to 2 days to 6 or even 3 hours to obtain positive results. However, rapid inclusion induces a major difficulty in that peak mortality is at 3 days. Neuroprotective drugs displace insult–lesion size relationships to the right (Figure) in parallel. However, this leads to 2 problems.

First, patients with very slight strokes will have very little damage, and protective effects in this population will be difficult to measure, eliminating the benefit seen in the upper portion of the Figure.

Second, patients with very serious strokes would be expected to die during the time course of the study, but a neuroprotective drug may save some of these patients, leaving them with considerable neurological deficit (lower triangle in the Figure).

However, early phase stroke trials are almost never powered to show effects on mortality, so that it is invalid to subtract the poor scores seen in the survivors. Consequently, these scores detract from the effects of the drug unless an effect on mortality is shown, which the trial is not designed to show.

Consequently, the parallel shift to the right of the insult–lesion size relationship merely results in the same score of neurological deficit, despite a powerful neuroprotective effect. The trials cannot show therapeutic benefit under these conditions.

Is this a real phenomenon? Consider the results with lifarazine, a sodium-calcium channel inhibitor, with use-dependent kinetics at sodium channels that favored ischemic conditions (time constant of 70 ms, favoring sodium channels that are depolarized for long periods of time in ischemic conditions; the drug was without effect on normal neuronal functioning or on animal behavior).4,5 The drug was active in a wide range of focal and global models of ischemia.4,5 A clinical trial was set up with rigorous inclusion criteria (maximum 6-hour delay after stroke, with CT scan; 1 in 20 patients accepted). In the control group 13 patients died, and in the treated group only 9 died, which just missed statistical significance (by 1 patient). Consequently, the neuropathology score (Barthel Index, 16% benefit at 3 months) just missed being significant, presumably because of inclusion of severely damaged patients who would have died without treatment. A powerful significant effect (35% benefit) was seen if patients who had shown a hypotensive effect were excluded (see below). Thus, this drug was classified as ineffective despite just missing positive effects on mortality in a clinical trial of only 147 patients.6 The Figure explains the reason for this.

A second issue that may cause difficulty is that elderly patients may have chronic carotid obstructions and be borderline for an ischemic event caused by hemodynamic events. Global ischemia in such patients may be caused by any prolonged fall in blood pressure7 or by ethanol consumption. Patients of this type are particularly at risk because they usually have already adapted their lifestyle to compensate for the occlusion, and the appearance of stroke damage is the end point. It is illusory to imagine that neuroprotective therapy can be applied chronically to this type of patient, and the likely outcome is that any mild hemodynamic effects of a drug will exacerbate damage.8 Stroke trials including this type of patient are liable to include exacerbated mortality or offset the benefits in mortality that may be expected in other patient populations.

Several possible solutions are worth considering. Powering studies to show beneficial effects on mortality, and then adjusting the neuropathological score to take into account severely compromised patients, is the simplest method. If appropriate inclusion criteria and trial design are used9–11 with a Kaplan-Meier survival analysis, then this strategy may not require very large numbers of patients to show benefit. Severely compromised patients could be excluded from the trials. Methodology is also...
needed for the exclusion (or posttreatment reassessment) of patients with almost complete carotid obstruction. However, this approach also raises ethical issues of whether severely damaged patients should be treated. These issues are common to all neuroprotective therapies, and unless they are addressed correctly, neuroprotection will remain a preclinical curiosity, and stroke therapies based on this concept will not progress.

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