Brain Microbleeds Relate to Higher Ambulatory Blood Pressure Levels in First-Ever Lacunar Stroke Patients
Julie Staals, Robert J. van Oostenbrugge, Iris L.H. Knottnerus, Rob P.W. Rouhl, Léon H.G. Henskens and Jan Lodder

Stroke. 2009;40:3264-3268; originally published online August 6, 2009;
doi: 10.1161/STROKEAHA.109.558049

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/40/10/3264

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/
Brain Microbleeds Relate to Higher Ambulatory Blood Pressure Levels in First-Ever Lacunar Stroke Patients

Julie Staals, MD; Robert J. van Oostenbrugge, MD, PhD; Iris L.H. Knottnerus, MD; Rob P.W. Rouhl, MD; Léon H.G. Henskens, MD, PhD; Jan Lodder, MD, PhD

**Background and Purpose**—Hypertension is an important risk factor for brain microbleeds (BMBs) in lacunar stroke patients. However, beyond the qualitative label “hypertension,” little is known about the association with ambulatory blood pressure (BP) levels.

**Methods**—In 123 first-ever lacunar stroke patients we performed 24-hour ambulatory BP monitoring after the acute stroke-phase. We counted BMBs on T2*-weighted gradient-echo MR images. Because a different etiology for BMBs according to location has been suggested, we distinguished between BMBs in deep and lobar location.

**Results**—BMBs were seen in 36 (29.3%) patients. After adjusting for age, sex, number of antihypertensive drugs, asymptomatic lacunar infarcts, and white matter lesions, we found 24-hour, day, and night systolic and diastolic BP levels to be significantly associated with the presence and number of BMBs (odds ratios 1.6 to 2.3 per standard deviation increase in BP). Distinguishing between different locations, various BP characteristics were significantly associated with the presence of deep (or combined deep and lobar) BMBs, but not with purely lobar BMBs.

**Conclusions**—Our results underline the role of a high 24-hour BP load as an important risk factor for BMBs. The association of BP levels with deep but not purely lobar BMBs is in line with the idea that different vasculopathies might be involved. Deep BMBs may be a particular marker of BP-related small vessel disease, but longitudinal and larger studies are now warranted to substantiate these findings. 

(Stroke. 2009;40:3264-3268.)

**Key Words:** brain microbleeds  ambulatory blood pressure monitoring  lacunar stroke

Brain microbleeds (BMBs) are frequently seen on T2*-weighted gradient-echo MR images in lacunar stroke patients. They relate to recurrent stroke and cognitive dysfunction. Hypertension and age are the most important risk factors for BMBs.

Instead of using the qualitative label of “hypertension,” ambulatory blood pressure monitoring (ABPM) allows the quantitative exploration of blood pressure (BP). Additionally, ABPM gives information about circadian BP characteristics. Ambulatory BP is a stronger predictor of hypertension-related organ damage, including brain damage, than single office measurements. In spite of this, data about the association between ambulatory BP and BMBs are limited. Only 1 study reported a relationship between high ambulatory BP levels and BMBs in a stroke-free hypertensive population.

We explored the relationship between ambulatory BP characteristics and BMBs in 123 first-ever lacunar stroke patients. We further distinguished between BMBs in deep and lobar brain locations, because some suggest that the etiology of BMBs differs according to their location, with mainly BMBs in deep locations resulting from hypertension-related small vessel disease (SVD).

**Methods**

**Patients**

We prospectively recruited patients who presented with a first-ever lacunar stroke at Maastricht University Medical Centre and Orbis Medical Centre Sittard, The Netherlands. Lacunar stroke was defined as an acute stroke syndrome with a recent, small, deep infarct on imaging (mostly CT, or MRI when available) compatible with the clinical findings. If no symptomatic lesion was visible, we used established criteria of specific clinical lacunar syndromes. As we aimed to study patients who most likely had their stroke from local SVD, patients with a potential cardioembolic source or ultrasonographically defined carotid artery stenosis >50% were not eligible for inclusion. With informed consent the patients participated in a lacunar stroke research project, which has been approved by the local Medical Ethical Committee. All included patients underwent MRI, which showed a symptomatic lacunar infarct, defined as a T2-weighted hyperintense, sharply marginated subcortical small lesion of <20 mm in diameter compatible with the clinical findings, or no definite recent lesion. Several vascular risk factors were documented: age, sex, diabetes mellitus, current smoking, and total cholesterol level >5 mmol/L. We defined hypertension at time of...
presentation with stroke as known hypertension from medical history (treated or not) or at least 2 BP recordings >140/90 mm Hg before stroke.

MRI Scoring
MR images (1.5T MRI scanner [n=94, 76%] or 3T MRI scanner [n=29, 24%], Philips) were obtained as soon as possible and at least within 6 months after stroke. Besides standard axial T2-weighted fast spin echo and fluid attenuated inversion recovery, we obtained a T2*-weighted gradient echo (GE) sequence (TR shortest; TE 23 ms; flip angle 15°; in-plane resolution 0.9x0.9 mm; field of view 230 mm; matrix 512x512; slice thickness 5 mm and 0.5 mm interslice gap). With knowledge of the clinical syndrome but blinded to other patient characteristics, 2 experienced vascular neurologists assessed the scans by consensus. The interobserver agreement for presence of BMBs, determined before this study, was substantial (κ=0.68).8 BMBs were defined as punctate hypointense lesions on GE-images with a diameter 10 mm. We distinguished between lobar (cortex and white matter), deep (basal ganglia, thalamus and internal, external or extreme capsule), and infratentorial (cerebellum, brain stem) BMBs. Symmetrical hypointensities in the globi pallidi, likely to represent calcification, and sulcal flow voids from cortical vessels were disregarded. Asymptomatic lacunar infarcts were defined as hyperintense lesions on T2-images with a hyperintense rim on FLAIR, diameter of 20 mm and not compatible with clinical findings. Extensive white matter lesions were defined according to Fazekas classification as T2-weighted (early) confluent deep white matter hyperintensities or irregular periventricular hyperintensities extending into the deep white matter.11

BP Measurements
ABPM (using Mobil O Graph equipment, IEM GbmH) was performed after the acute stroke phase, between 1 and 6 months poststroke. Measurements over a 24-hour period were obtained every 15 minutes during day (07.00 to 23.00) and every 30 minutes during night (23.00 to 7.00). Patients continued their prescribed medication, and we registered the use of antihypertensive drugs. Patients kept a record of retining and rising times. We determined day and night periods by excluding a 2-hour transition period around the reported rising and retining times. Valid recordings required a minimum of 15 daytime and 8 nighttime measurements.12 We calculated mean 24-hour, day, and night systolic (SBP), diastolic (DBP), and pulse pressure (PP). Relative nocturnal dipping (SBP/DBP) was calculated as: (day SBP–night SBP)/day SBP×100%.

Statistical Analysis
Analyses were performed using SPSS 15.0. Data are presented as n (%), as mean±SD for parametric data, or as median (range) for nonparametric data. Differences between groups were determined using χ² test, t test, or Mann–Whitney test, where appropriate. We assessed the relationship between the various BP characteristics and BMBs by ordinal regression analyses (classifying the number of BMBs as 0, 1, or >1) or binary logistic regression analyses (presence [−/+] of BMBs in different locations), adjusted for age, gender, and the number of antihypertensive drugs in use by each patient (model 1). We additionally adjusted for the presence of asymptomatic lacunar infaracts and white matter lesions (model 2). Finally we performed some exploratory analyses in which we adjusted for cardiovascular risk factors (diabetes mellitus, hypercholesterolemia, current smoking) by adding them one for one separately to the model. Odds ratios (OR) are given with 95% confidence interval (CI). Statistical significance was considered at P<0.05.

Results
Patient Recruitment
Of 243 eligible first-ever lacunar stroke patients who presented between June 2003 and January 2008 at Maastricht, 112 patients decided not to participate and 34 were excluded because of inadequate MRI or ABPM data, leaving 97 included patients. There were more males among the included than the remaining patients (64.2 versus 47.3% male, P=0.01), whereas age did not differ significantly. Between September 2004 and April 2007 we recruited 26 patients from Sittard, which totals 123 patients. Patients from Maastricht were older than those from Sittard (67.0±10.9 versus 55.8±10.1 years, P<0.001), had higher SBPs (24-hour SBP 141±17 versus 129±12 mm Hg, P<0.001) and PPs (24-hour PP 59±11 versus 50±7 mm Hg, P<0.001), and had more often white matter lesions (43.3 versus 19.2%, P=0.03) and microbleeds (32.0 versus 19.2%, P=0.21). In 24 of 123 patients (19.5%), MRI did not show a symptomatic lacunar infarct.

Patient Characteristics
Microbleeds were present in 36 (29.3%) patients (Table 1). Twenty-five (20.3%) patients had one BMB, and 11 patients (8.9%) had two or more (maximum 19 BMBs). Hypertension was more frequent in patients with BMBs, but the difference was not statistically significant (OR 1.59, P=0.28).

BP Characteristics
Table 2 shows the association between the various BP characteristics and the presence and number of BMBs. Higher SBPs and DBPs were significantly associated with BMBs, after adjustment for age, sex, and number of antihypertensive drugs, and additionally for asymptomatic lacunar infarcts and extensive white matter lesions. Adjusting for diabetes mellitus, hypercholesterolemia or smoking did not change results substantially and no center effect was seen (data not shown). Systolic nocturnal dipping and PP had no significant relationship with presence and number of BMBs.

Different Locations of BMBs
Eleven (8.9%) patients had only lobar BMBs (median number of BMBs 1 [range 1 to 9]). BP levels in these patients were slightly higher than in those without any BMBs, but associations did not reach statistical significance (Table 3). Seventeen (13.8%) patients had only deep BMBs (median 1 [1 to 2]). With exception of night SBP (P=0.061), higher SBPs and DBPs were significantly associated with presence of deep BMBs, after adjustment for age, sex, number of antihypertensive drugs, asymptomatic lacunar infarcts, and extensive white matter lesions. Seven (5.7%) patients had combined (both deep and lobar) BMBs (median 3 [2–19]), and 1 (0.8%) patient had a cerebellar located BMB. ORs on associations between SBP and DBP levels and presence of combined BMBs varied from 1.93 to 2.90, but only reached statistical significance for 24-hour and day DBP. Systolic nocturnal dipping and PP had no significant relationship with presence of BMBs in any location (data not shown).

Discussion
In this study in 123 first-ever lacunar stroke patients the presence and number of BMBs was significantly associated with higher ambulatory day and night SBP and DBP levels, independent of age, sex, and use of antihypertensive medication. Even after additional adjustment for silent lacunar infarcts and white matter lesions, BP levels remained independent predictors for BMBs.
We found a microbleed prevalence of 29.3%, which is lower than the reported 46% to 62% in some other studies. However, lacunar stroke patients in these studies were older, and recurrent strokes were included as well, which both might have biased toward a higher BMBs rate. Studies in first-ever lacunar stroke patients reported a prevalence of 23% to 26%, similar to our percentage.

A systematic review reported an association between hypertension and BMBs in adults with cerebrovascular disease with an overall OR of 2.3. We found a nonsignificant OR of 1.6. However, we documented hypertension by history-taking at the time of presentation with stroke, which might have missed undiagnosed cases. Instead of using the arbitrarily defined and qualitative label of hypertension, generally based on few office measurements, we performed ABPM which gives a better presentation of real BP. Our results comply with a recent study in a stroke-free hypertensive population, in which also day and night ambulatory BP levels predicted the presence of BMBs.

As in other studies, BMBs were strongly associated to the presence of silent lacunar infarcts and white matter lesions, which implies that they are probably all caused by a similar

<table>
<thead>
<tr>
<th>Table 1. Patient Characteristics and MRI Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient Characteristics</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Age, y</td>
</tr>
<tr>
<td>Male sex</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Current smoking</td>
</tr>
<tr>
<td>Cholesterol &gt;5 mmol/L</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Use of antihypertensive drugs</td>
</tr>
<tr>
<td>Diuretics</td>
</tr>
<tr>
<td>Beta-blockers</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
</tr>
<tr>
<td>ACE inhibitors</td>
</tr>
<tr>
<td>Angiotensin receptor blockers</td>
</tr>
<tr>
<td>Alpha-blockers</td>
</tr>
<tr>
<td>No. of drugs</td>
</tr>
<tr>
<td>Asymptomatic lacunar infarcts</td>
</tr>
<tr>
<td>Extensive white matter lesions</td>
</tr>
</tbody>
</table>

Data are missing for current smoking (n=1) and cholesterol (n=2).

<table>
<thead>
<tr>
<th>Table 2. Ambulatory BP Characteristics in Relation to Presence and No. of BMBs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean BP, mm Hg</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>24-hour</td>
</tr>
<tr>
<td>SBP</td>
</tr>
<tr>
<td>DBP</td>
</tr>
<tr>
<td>PP</td>
</tr>
<tr>
<td>Day</td>
</tr>
<tr>
<td>SBP</td>
</tr>
<tr>
<td>DBP</td>
</tr>
<tr>
<td>PP</td>
</tr>
<tr>
<td>Night</td>
</tr>
<tr>
<td>SBP</td>
</tr>
<tr>
<td>DBP</td>
</tr>
<tr>
<td>PP</td>
</tr>
<tr>
<td>SBP dipping (%)</td>
</tr>
</tbody>
</table>

Results of ordinal regression analyses presented as OR with 1 SD increase in the relevant BP (95% CI). The SD of SBP/DBP/PP are 17/11/11 mm Hg for the 24-hour period, 17/12/11 mm Hg for the day period, and 18/11/12 mm Hg for the night period; the SD of dipping is 9%. SBP dipping is defined as (day SBP/night SBP)/day SBP×100%.

Model 1: adjusted for age, gender and No. of antihypertensive drugs; Model 2: additionally adjusted for asymptomatic lacunar infarcts and extensive white matter lesions.

*P<0.05; †P<0.01; ‡P<0.001.
The presence of BMBs, independently of the extent of lacunar infarcts and white matter lesions, relates to the associations between ambulatory BP levels and BMBs remained statistically significant, which suggests that BP is a stronger risk factor for the occurrence of BMBs than for the other BP-related signs of SVD. In CADASIL, a monogenetic cause of SVD, it was also found that office SBP level relates to the presence of BMBs, independently of the extent of lacunar infarcts and white matter lesions.17

Dysfunction of the blood–brain barrier has been suggested as a main initial pathogenetic feature in SVD.18 The blood–brain barrier depends on endothelial integrity, which could be disrupted by BP-related hemodynamic factors. Our findings emphasize the importance of both day and night BP, that is, an increased 24-hour mean BP load. Although increased pulsatile stress is considered to be one cause of small vessel wall damage, brachial PP was not related to BMBs in our study. However, central PP or carotid flow augmentation would be a better measure.19 The phenomenon of reduced nocturnal dipping has also been related to the degree of hypertensive end-organ damage.20 We found no association between the degree of nocturnal dipping and BMBs. However, there is no consensus on how to define nocturnal dipping, its reproducibility is poor, and its clinical relevance needs to confirm our findings concerning BMBs in different locations.

Pathology studies showed that BMBs consist of hemosiderin deposits from blood that presumably leaked from small blood vessels that are affected by lipohyalinosis in most subjects.22–24 In some cases with mainly lobar BMBs, evidence of amyloid angiopathy was found.24 These data, though limited, suggest that deep and lobar BMBs represent different underlying pathologies, analogous to symptomatic intracerebral hemorrhage. Evidence from clinical studies supports this concept. Deep and lobar BMBs were found to associate with the presence of a symptomatic hemorrhage in the corresponding area.25 The APOE ε4 genotype, which is related to amyloid angiopathy, was found to be associated with lobar but not deep BMBs.6 Hypertension and office SBP were more strongly related to deep than lobar BMBs, suggesting that mainly deep BMBs are a marker of BP-related cerebral SVD.5,6 In line with these data, we observed that associations with ambulatory BP levels were stronger for deep than lobar BMBs. However, high BP does not rule out amyloid angiopathy and the vasculopathies may coexist. Furthermore, subgroups in our study were small which withholds us from drawing firm conclusions.

Our study has several limitations. First, it is cross-sectional. Results of ABPM represent the actual BP level without accounting for BP level in the past. We cannot exclude the possibility that BP level changes after stroke or has been influenced by silent cerebral damage. Follow-up studies are needed to confirm a causal relation between BP levels and (progression of) BMBs. Second, more than half of the patients were taking antihypertensive drugs during ABPM, which might have mitigated mean BP levels. We adjusted for the use of antihypertensive drugs but not for the duration of use. However, our approach may have led to an underestimation rather than an overestimation of the strength of the association between BP and BMBs. Third, detection of BMBs strongly depends on MRI characteristics and the detection criteria used.1 This may limit comparability with other studies. Fourth, we were unable to identify a symptomatic lacunar lesion on MRI in 19.5% of our patients. This may relate to short (but at least 24 hours) duration of symptoms in some cases, but also to the rather long (up to 6 months) MRI delay in some other cases, blurring the distinction between recent and possible concomitant asymptomatic lesions. However, we feel that this did not lead to unrightfully included patients as they obviously had a clinical lacunar syndrome. Finally, our sample size was rather small, and most patients with BMBs had only 1 BMB. Larger series are needed to confirm our findings concerning BMBs in different locations and to facilitate stronger analysis of number in addition to presence of BMBs.

In summary, our results underline the role of a high 24-hour BP load as important risk factor for BMBs in lacunar stroke patients. The association between BP and deep but not lobar BMBs is in line with the idea that different vasculopathies might be involved. Deep BMBs may be a particular marker of BP-related SVD. Longitudinal and larger studies are now warranted to substantiate these findings.

**Sources of Funding**

J.S. received a research grant from the “profileringsfonds” Maastricht University Medical Centre. R.R. received a research grant from the Netherlands Heart Foundation (grantnr.2005B022). L.K. received a research grant from the Netherlands Thrombosis Foundation (grantnr.2007-3). L.H. received a research grant of the Novartis Foundation for Cardiovascular Excellence (grantnr.003/07).

---

**Table 3. Ambulatory BP Characteristics in Relation to Presence of BMBs in Different Locations**

<table>
<thead>
<tr>
<th>Mean BP, mm Hg</th>
<th>BMBs + Only deep (n=17)</th>
<th>Model 1 OR</th>
<th>Model 2 OR</th>
<th>BMBs + Only Lobar (n=11)</th>
<th>Model 1 OR</th>
<th>Model 2 OR</th>
<th>BMBs + Combined (n=7)</th>
<th>Model 1 OR</th>
<th>Model 2 OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-hour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>147±17</td>
<td>1.99 (1.12–3.53)*</td>
<td>1.96 (1.05–3.70)*</td>
<td>139±17</td>
<td>1.16 (0.58–2.31)</td>
<td>1.40 (0.58–3.42)</td>
<td>151±23</td>
<td>2.44 (0.98–5.95)</td>
<td>2.56 (0.84–7.68)</td>
</tr>
<tr>
<td>DBP</td>
<td>86±10</td>
<td>2.21 (1.16–4.20)*</td>
<td>2.05 (1.02–4.04)*</td>
<td>84±11</td>
<td>1.87 (0.85–4.12)</td>
<td>2.00 (0.80–5.06)</td>
<td>92±15</td>
<td>3.47 (1.33–9.10)*</td>
<td>2.90 (1.05–7.80)*</td>
</tr>
<tr>
<td>Day</td>
<td>152±18</td>
<td>2.00 (1.15–3.55)*</td>
<td>2.00 (1.07–3.73)*</td>
<td>144±17</td>
<td>1.27 (0.63–2.57)</td>
<td>1.60 (0.63–3.91)</td>
<td>153±26</td>
<td>2.25 (0.97–5.28)</td>
<td>2.29 (0.97–6.57)</td>
</tr>
<tr>
<td>SBP</td>
<td>89±11</td>
<td>2.12 (1.14–3.97)*</td>
<td>1.97 (1.01–3.81)*</td>
<td>87±10</td>
<td>1.95 (0.89–4.23)</td>
<td>1.99 (0.82–4.90)</td>
<td>95±16</td>
<td>3.02 (1.27–7.31)*</td>
<td>2.59 (1.00–6.68)*</td>
</tr>
<tr>
<td>Night</td>
<td>133±14</td>
<td>1.97 (1.08–3.51)*</td>
<td>1.86 (0.96–3.57)</td>
<td>126±23</td>
<td>1.27 (0.56–2.90)</td>
<td>1.36±19</td>
<td>1.90 (0.79–4.60)</td>
<td>1.93 (0.61–6.01)</td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>77±8</td>
<td>2.32 (1.21–4.42)*</td>
<td>2.19 (1.08–4.42)*</td>
<td>76±13</td>
<td>1.76 (0.86–3.57)</td>
<td>1.84 (0.76–4.34)</td>
<td>80±12</td>
<td>2.46 (0.95–6.35)</td>
<td>2.09 (0.72–6.12)</td>
</tr>
</tbody>
</table>

Results of binary logistic regression analyses presented as OR with 1 SD increase in the relevant BP (95%CI). We used the SDs as in Table 2. Models were obtained as in Table 2. *P<0.05.
Disclosures

None.

References


Brain Microbleeds Relate to Higher Ambulatory Blood Pressure Levels in First-Ever Lacunar Stroke Patients
Julie Staals, Robert J. van Oostenbrugge, Iris L.H. Knottnerus, Rob P.W. Rouhl, Léon H.G. Henskens and Jan Lodder

Stroke. 2009;40:3264-3268; originally published online August 6, 2009;
doi: 10.1161/STROKEAHA.109.558049
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/40/10/3264

An erratum has been published regarding this article. Please see the attached page for:
/content/44/10/e139.full.pdf

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/
In the article by Staals et al., “Brain Microbleeds Relate to Higher Ambulatory Blood Pressure Levels in First-Ever Lacunar Stroke Patients,” which was published ahead-of-print on August 6, 2009, and appeared in the October issue of the journal (Stroke. 2009;40:3264–3268) a correction was needed.

Methods: MR images were obtained at an 1,5T MRI scanner (n=94, 76%) or 3T MRI scanner (n=29, 24%).

The authors became aware of the use of different MRI field strengths in their patient population after publication of the results. Several exploring reanalyses (to eliminate possible overrating of microbleeds in patients scanned at 3T compared to 1,5T) on the data showed similar results. The authors conclude that the results and conclusions as published remain valid.