Limited Value of Laser-Doppler Flowmetry for Intraluminal Middle Cerebral Artery Occlusion in Wistar Rats

Hideki Taninishi, Jin Yong Jung, Huaxin Sheng, Miwa Izutsu, Zhengfeng Wang, David S. Warner

Multidisciplinary Neuroprotection Laboratories, Department of Anesthesiology, Surgery and Neurobiology, Duke University Medical Center, Durham, NC, USA

Introduction

Intraluminal middle cerebral artery occlusion (MCAO) is widely employed because of less surgical expertise, less interruption of muscles of mastication and less peri-ocular edema than transcranial approach. Positioning of the filament is blind, so MCA occlusion can be difficult to confirm. Laser Doppler flowmetry (LDF) use to obtain a consistent magnitude of cerebral blood flow decrease (CBF) has been advocated to confirm proper filament placement. However, no investigation has examined LDF effectiveness in standardizing the severity of ischemic injury. It is important to define this because the LDF system is expensive and requires an additional incision in the scalp and creation of a skull burr hole in animals potentially destined for long-term recovery.

This study was designed to examine behavioral or histologic outcome as a function of LDF measurement. Surgeons were randomly blinded to or assisted with LDF values obtained during intraluminal MCAO. Neurological and histological outcome were compared 7 days post-MCAO. As a secondary measure, we considered effects of surgeon experience with the MCAO model on LDF efficacy.

Methods

Four surgeons with various levels experience (novice ~ 15 year experience) in animal surgery participated. Male Wister rats (250 to 300g) were subjected 90 min intraluminal MCAO. A burr hole was made and a LDF probe was inserted in all animals. Rats were assigned to either LDF assisted (Assisted) or blinded groups (Blinded) (12-17 rats in each group) for each surgeon. In the Blinded group, the surgeon was not allowed to see values displayed on the LDF monitor during MCAO. In the Assisted group, LDF values were available to the surgeon throughout the procedure.

Neurological examination was performed at 1h (simple scoring system of 4 grades [1]) and 7 days (comprehensive 48-point scoring system [2]) post - MCAO. Then cerebral infarct volume was measured using a subtraction method to control for edema.

LDF change was analyzed by repeated measures ANOVA. Other variables were tested by t-test (Student or Welch) or Mann-Whitney U-test dictated by normality of distribution and homogeneity of variance. $P < 0.05$ was considered to be significant.

Table 1: Immediate neuroscore

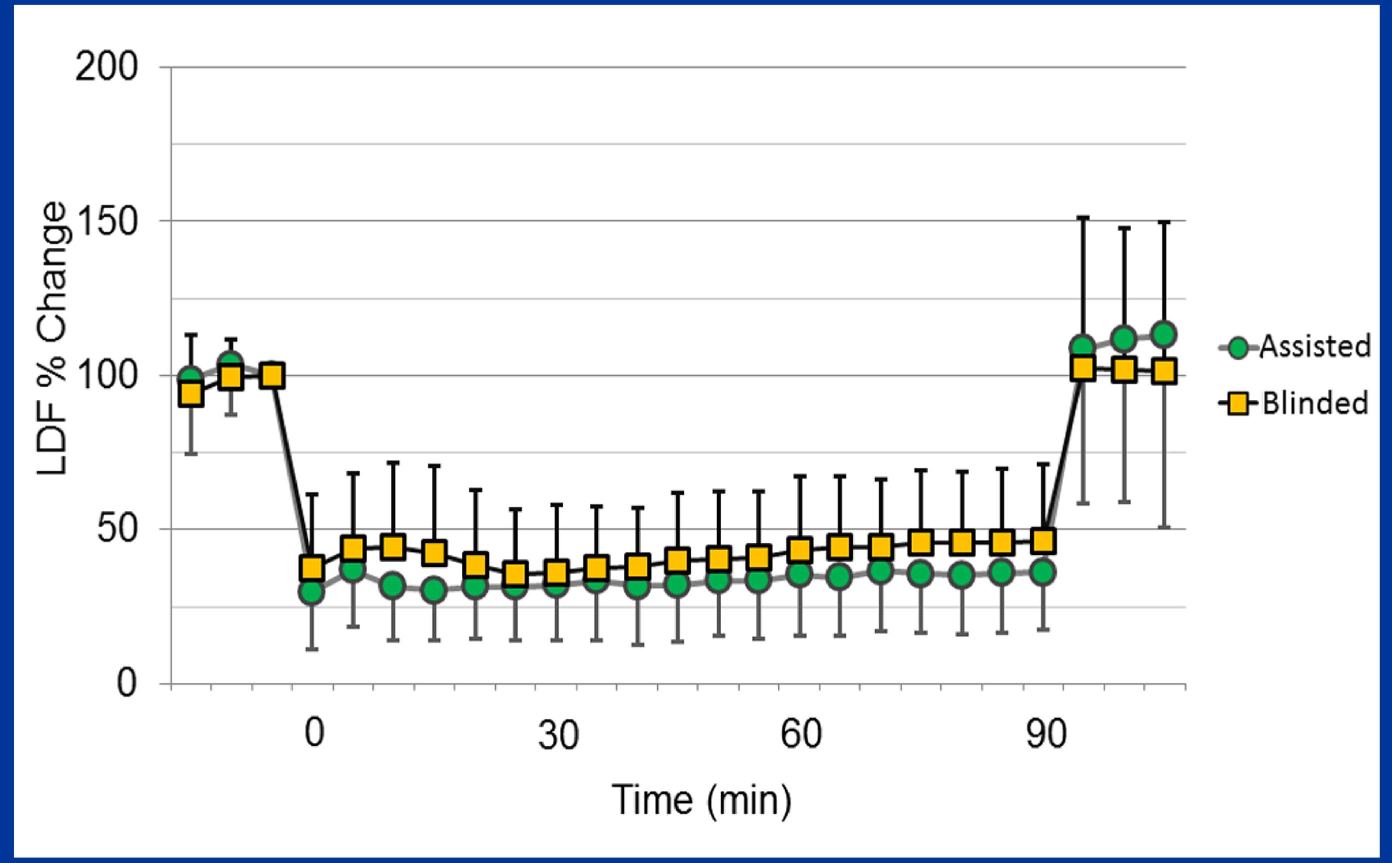
	Assisted				Blinded			
Bederson`s Score	0	1	2	3	0	1	2	3
Number of animals								
Surgeon #1	1	0	0	11	0	0	0	12
Surgeon #2	0	0	0	16	0	0	0	17
Surgeon #3 *	0	0	0	12	1	1	3	7
Surgeon #4	0	1	0	12	0	0	0	13
All surgeons (Total)	1	1	0	51	1	1	3	49

* indicates $p < 0.05$ between Assisted and Blinded groups.

Bederson`s score ;0: no observable deficit, 1: forelimb flexion,
2: decreased resistance to lateral push (and forelimb flexion) without circling,
3: same behavior as grade 2, with circling.

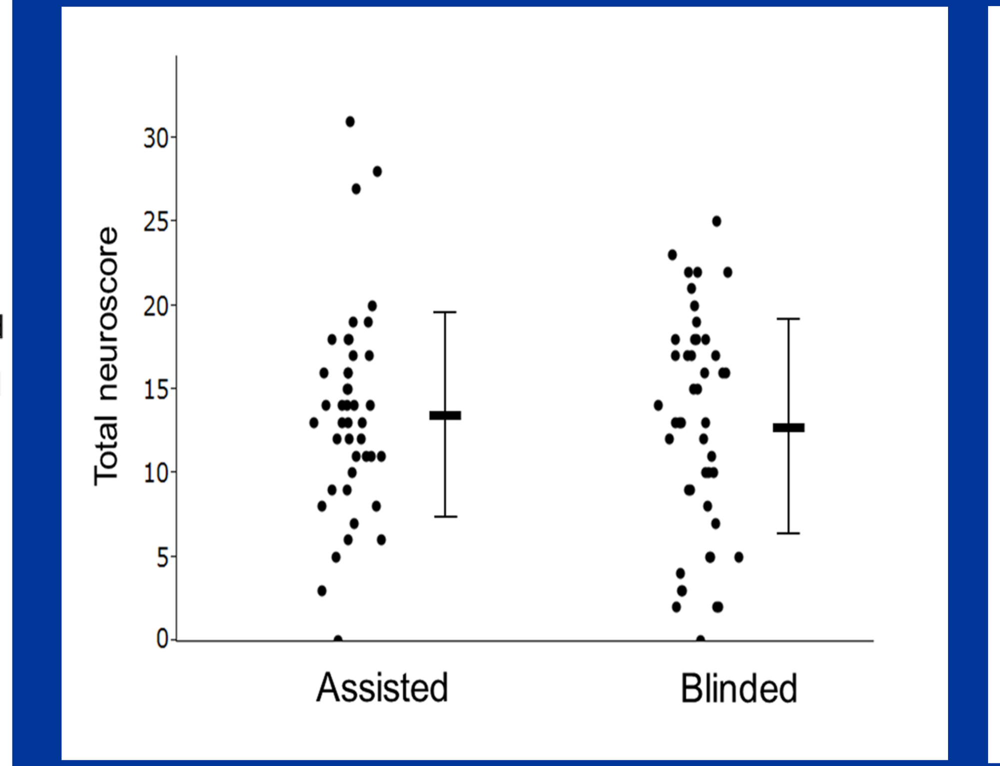
Results (Pooled across surgeons)

Figure 1: LDF change during MCAO



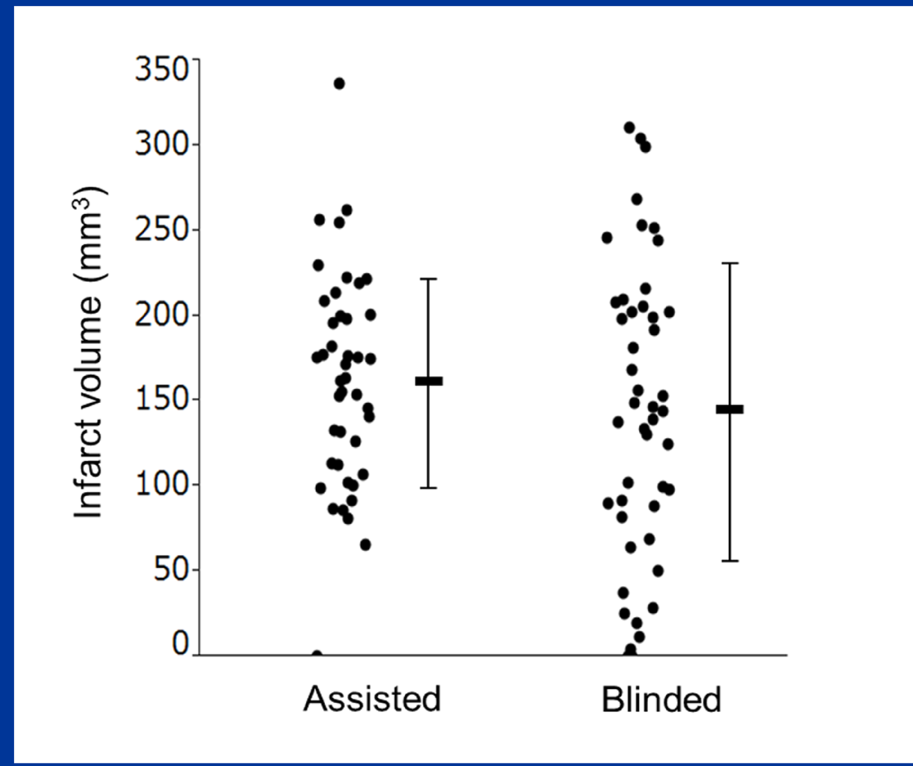
Values over 5 min immediately prior to ischemia were averaged to establish baseline (100%). Subsequent values are depicted as % change from baseline. There was a main effect for time ($P < 0.0001$), but an effect for LDF use was not detected ($P = 0.12$).

Figure 3: Total neuroscore (7 days post-MCAO)



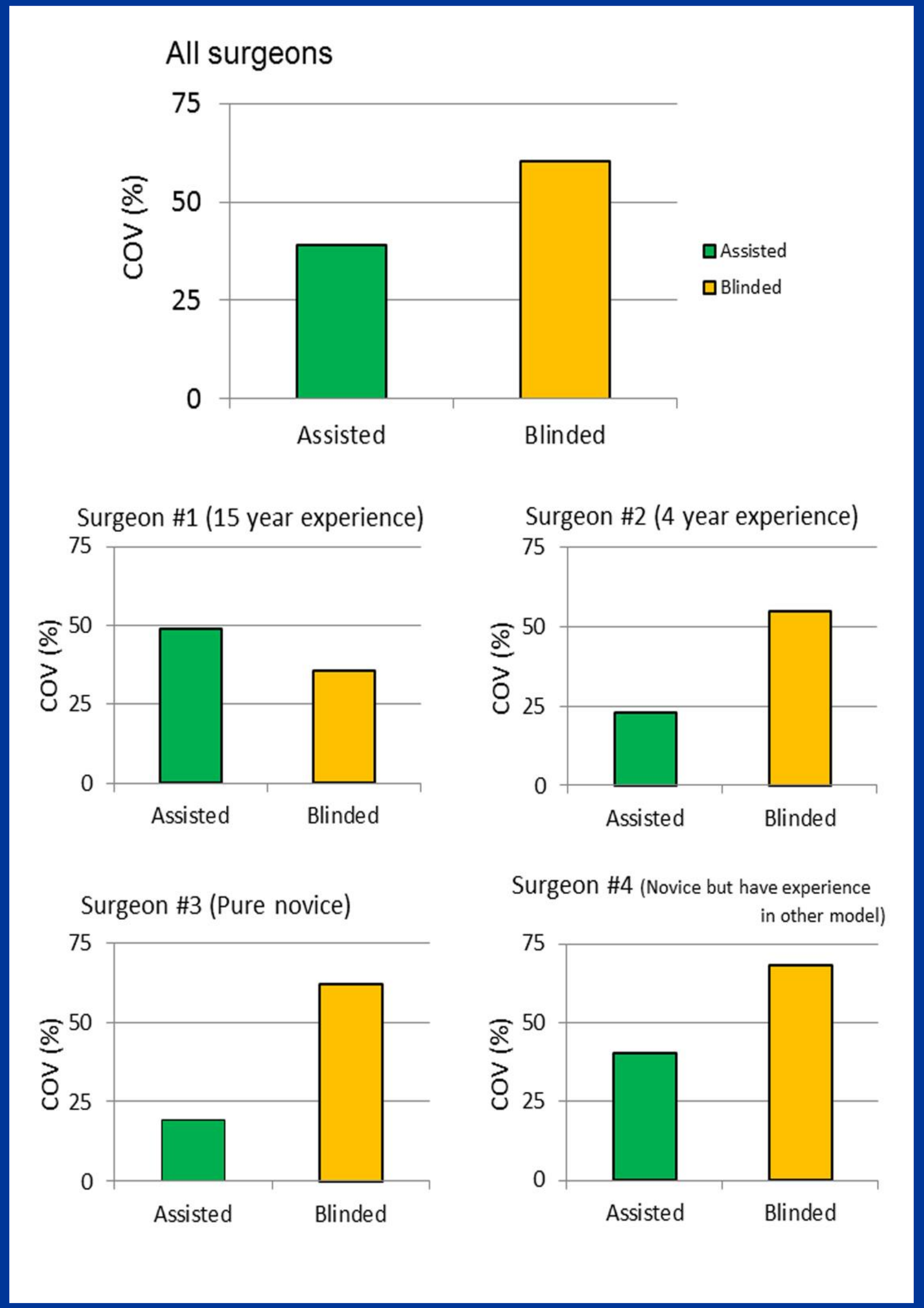
There was no difference (Assisted *vs.* Blinded = 14 ± 6 *vs.* 13 ± 7, P = 0.61)

Figure 5: Infarct volume



There was no difference (162 ± 63 mm^3 *vs*. 143 ± 86 mm^3, P = 0.24). Standard deviation in the Blinded group was greater than in the Assisted group.

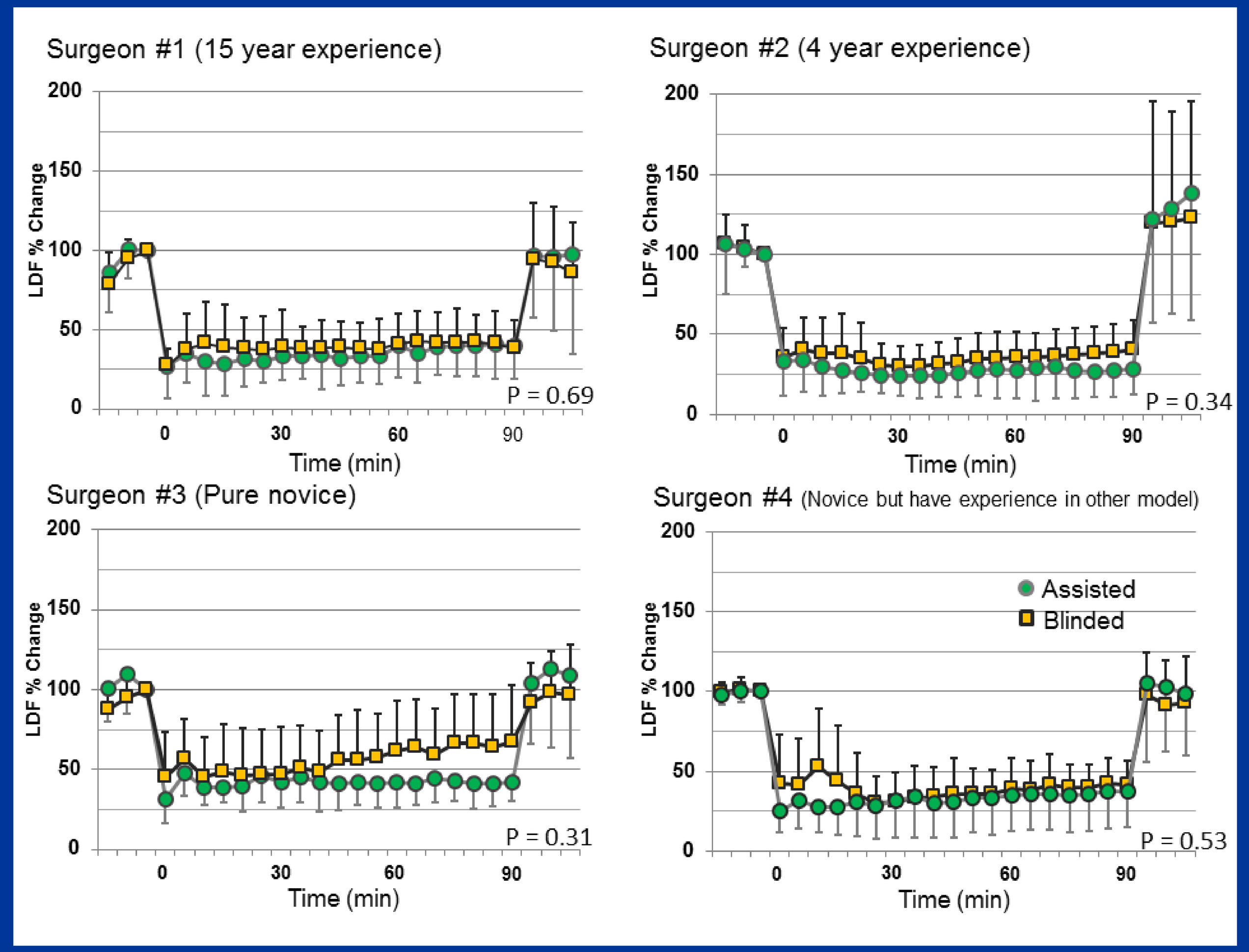
Figure 7: Coefficient of variation



Laser Doppler flowmetry use decreased COV from 60% to 39% (35% decrease), pooling values across surgeons. For surgeons #2, #3 and #4, COV in the Assisted group were smaller by 59%, 69%, and 41% than those in the Blinded group, while COV for surgeon #1 was increased by 37% in the Assisted group.

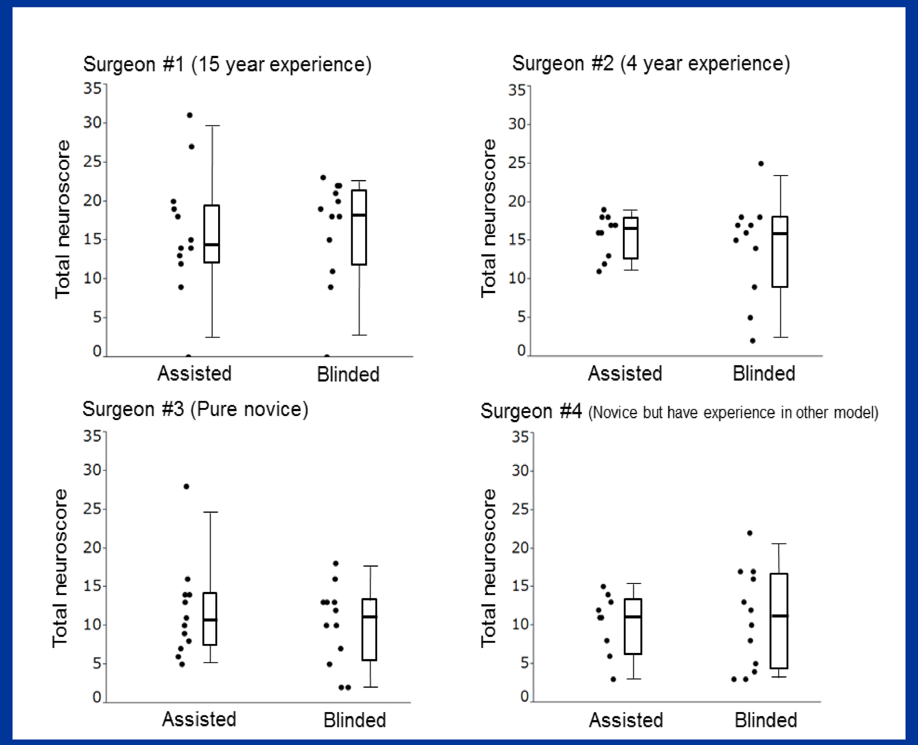
(Individual surgeons)

Figure 2: LDF change during MCAO



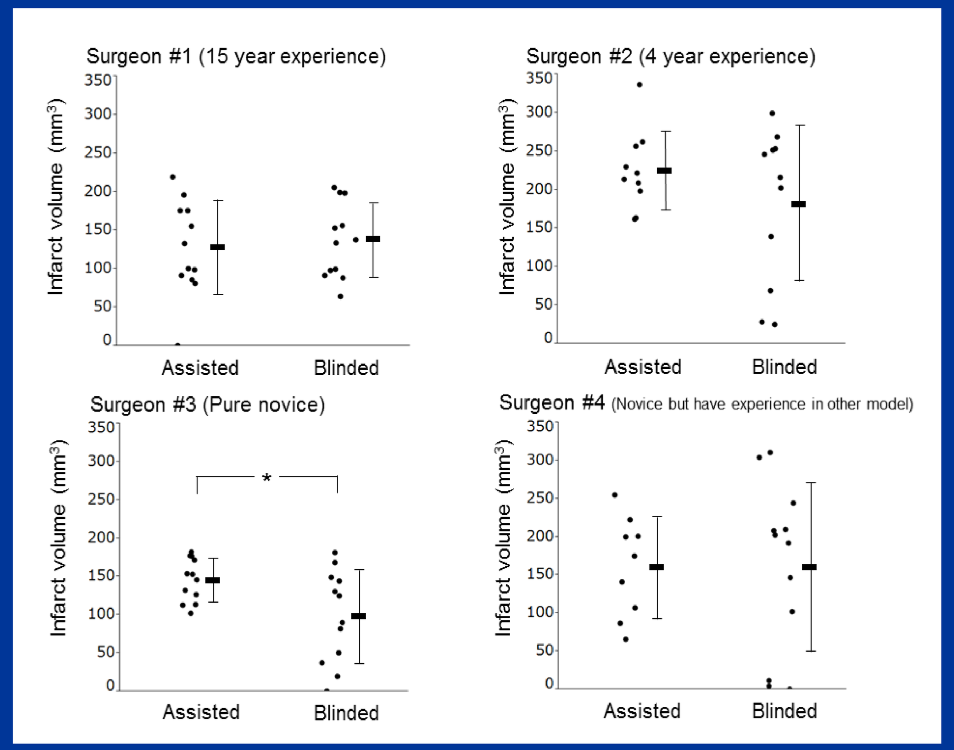
Repeated measures ANOVA found no significant differences between the Assisted and Blinded groups for any surgeon.

Figure 4: Total neuroscore (7 days post-MCAO)



There were no significant differences between the Assisted and Blinded groups for any surgeon.

Figure 6: Infarct volume



Only for surgeon #3 there was a difference between Assisted and Blinded groups (p = 0.03).

Conclusion

We could not provide evidence to support LDF use as a requisite tool for performing intra-luminal MCAO. This held true across a range of surgical expertise when applied to both 7-day neurologic and histologic outcome. However, LDF did decrease coefficient of variation, which might allow use of fewer animals to test hypotheses when the primary dependent variable is cerebral infarct size.

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References [1] Bederson JB, *et al*. 1986. [2] Yokoo N, *et al*. 2004