Complexity analysis of beat-to-beat skin-surface laser-Doppler flowmetry signals in stroke patients using acupuncture treatment

使用針刺治療中風病患體表血管搏動之都卜勒血流訊號的複雜分析

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Background 研究背景(1/2)

- Circulation system 循環系統
  - Transport nutrition,O2 and CO2 負責運送養分和氧氣，帶回代謝後的廢物與二氧化碳。
  - System: Heart, artery, capillary and vein 由心臟與動脈、靜脈、微血管完成一套人體循環系統。
  - a greater circulation and pulmonary circulation循環系統分為體循環與肺循環。
- Microcirculation 微循環
  - Basic unit of circulation system人體血液循環最基本的單位[1]。
  - Microcirculation disorder will lead to organic change.如果微循環發生功能異常，使生理代謝無法正常運作，有可能會導致器官病變。

Background 研究背景(2/2)

- Cerebral vascular accident(CVA) is the third cause of death in Taiwan.

Table 5-1 Taiwan's 10 Leading Causes of Death, 2013

	Cause of Death	Number of Deaths	Crude Death Rate (see 1)	Standardized Death Rate (see 2)
1	Malignant neoplasms	44,791	191.9	130.4
2	Heart disease (Hypertension diseases excluded)	17,694	75.8	47.7
3	Cerebrovascular diseases	11,313	48.5	30.3
4	Diabetes mellitus	9,438	40.4	25.8
5	Pneumonia	9,042	38.7	22.5
6	Accidents and adverse effects	6,619	28.4	22.4
7	Chronic lower respiratory disease	5,959	25.5	14.9
8	Hypertensive diseases	5,033	21.6	12.9
9	Chronic liver disease and cirrhosis	4,843	20.7	14.8
10	Nephritis, nephritic syndrome and nephrosis	4,489	19.2	11.9

Note 1: Death rate calculated per 100,000 people

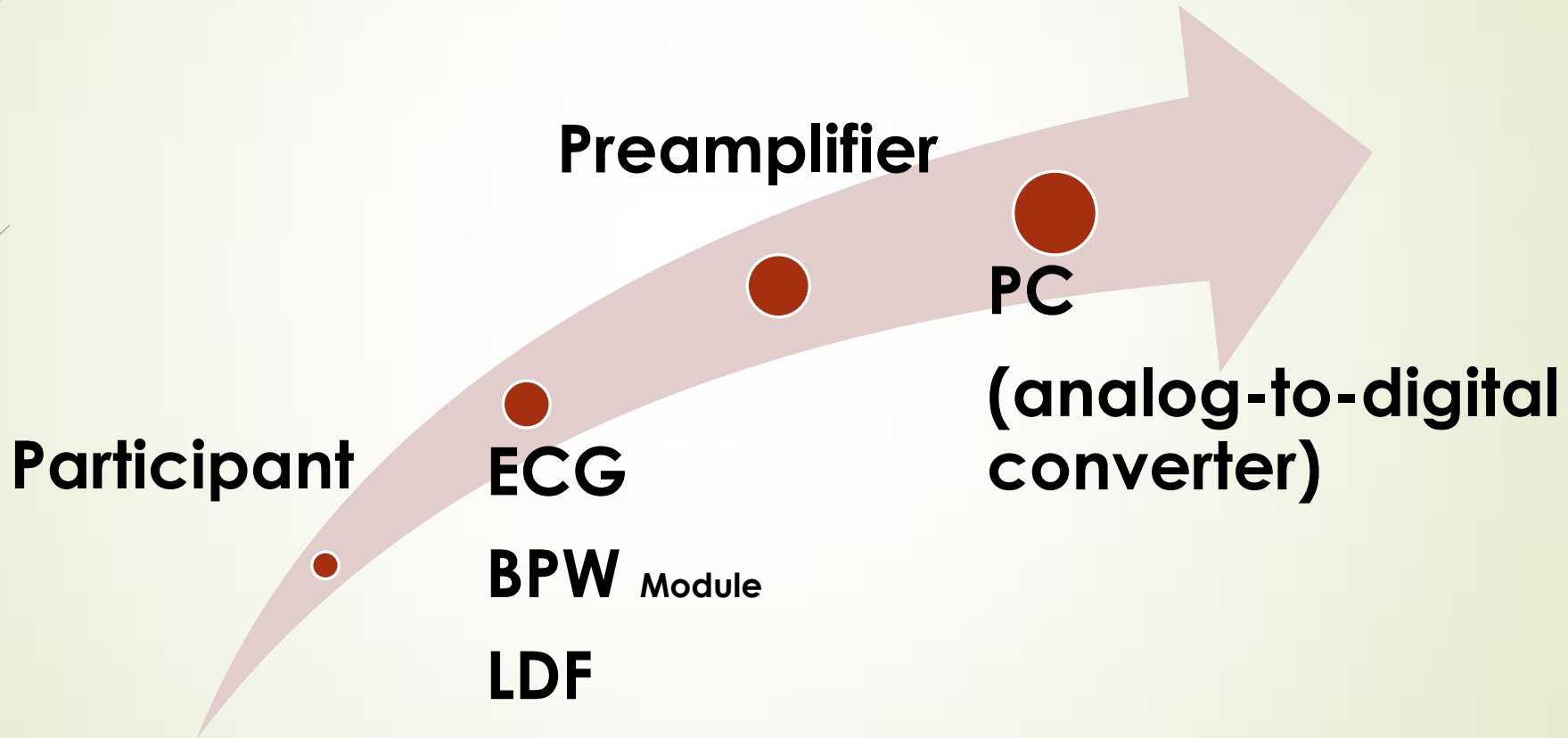
Note 2: Calculated on the basis of the standard world population defined by the WHO in 2000.

Source: Statistics on Cause of Death, Ministry of Health and Welfare

Motivation 動機

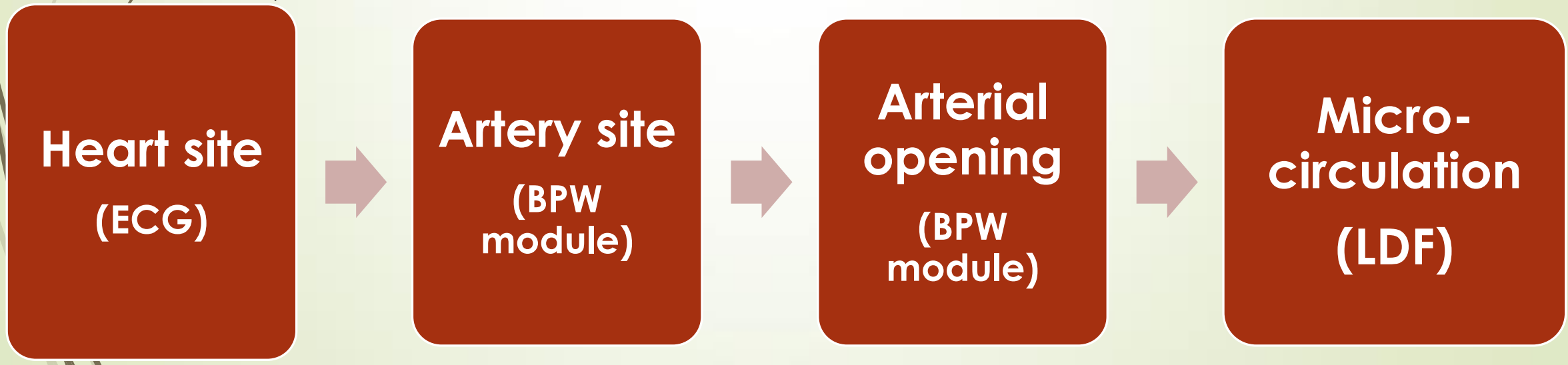
- This study performed skin-surface laser-Doppler flowmetry (LDF) measurements and sample entropy (SampEn) analysis with the aims of
  - (1) capturing the temporal complexity of cerebral hemodynamics in stroke patients
  - (2) discriminating stroke patients from healthy control subjects.
- We also investigated the response induced by acupuncture stimulation (AS) in beat-to-beat SampEn indexes of LDF signals.

Design 研究設計(1/2)
The framework of measurement system



Design 研究設計(2/2)

- **The framework of measurement**
  - The propelling force of pulse pressure, which is generated by heartbeat and transmitted along the artery, plays an important role in driving the MBF(microcirculation blood flow) into capillaries through precapillary openings on the arteriole walls.
  - Signals心電訊號:**上游**量測心臟端狀態，**中游**量測動脈管壁的傳遞過程，**下游**量測微循環端供血狀態。

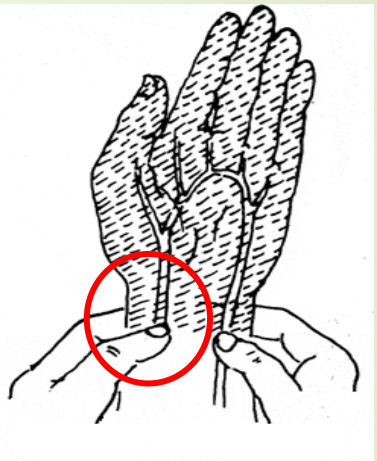


Hardware 實驗設備(1/3)-心臟端(Heart site)

- ECG-100C：BIOPAC
- Purpose目的
  - 擷取R波訊號以進行切波，並計算參數(Ex:heart rate and HRV)來評估病患的生理狀態
- Specification 規格
  - Frequency：250Hz
  - Minimum CMRR: 110 dB (50/60 Hz)
  - Max output voltage:±10V
- 本實驗採用第一導程

Hardware 實驗設備(2/3)-
動脈端(Artery site)

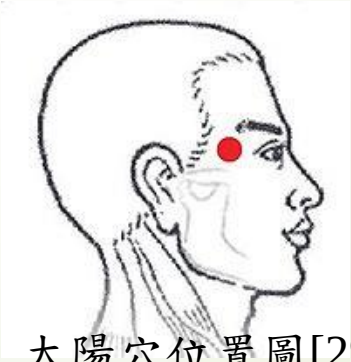
- **BP waveforms measurement module**血壓波形量測模組
  - UV-10 Amplifier(訊號放大器):Honeywell
  - 應變計FCA-3-350-11-03LJU
- Purpose 目的
  - Take radial artery BPW signals.擷取橈動脈血壓波形訊號
- Specification 規格
  - 350歐母雙軸應變計
  - 阻抗差：350±1%
- Location of measurement 量測位置
  - 2cm above wrist on the surface of radial pulse 手腕往上臂方向約在2cm的皮膚表面



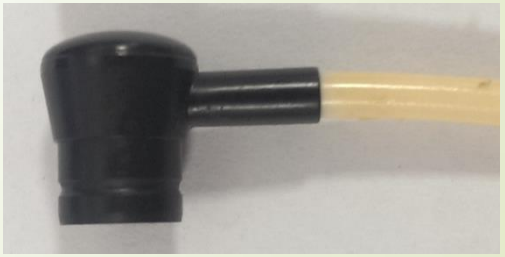
橈動脈位置圖[28]

Hardware實驗設備(3/3)-微循環端(Pulse site)

- laser-Doppler flowmetry
  雷射都卜勒血流儀
- Purpose 目的
  - Detect microcirculation on skin surface 偵測體表微循環的血流量
- Specification 規格
  - 取樣頻率(Frequency)為40Hz
  - 量測深度(Deepth)為1-2mm
  - 波長(Wavelength)為780±10nm
- Sites量測位置
  - 兩側太陽穴(taiyangxue)

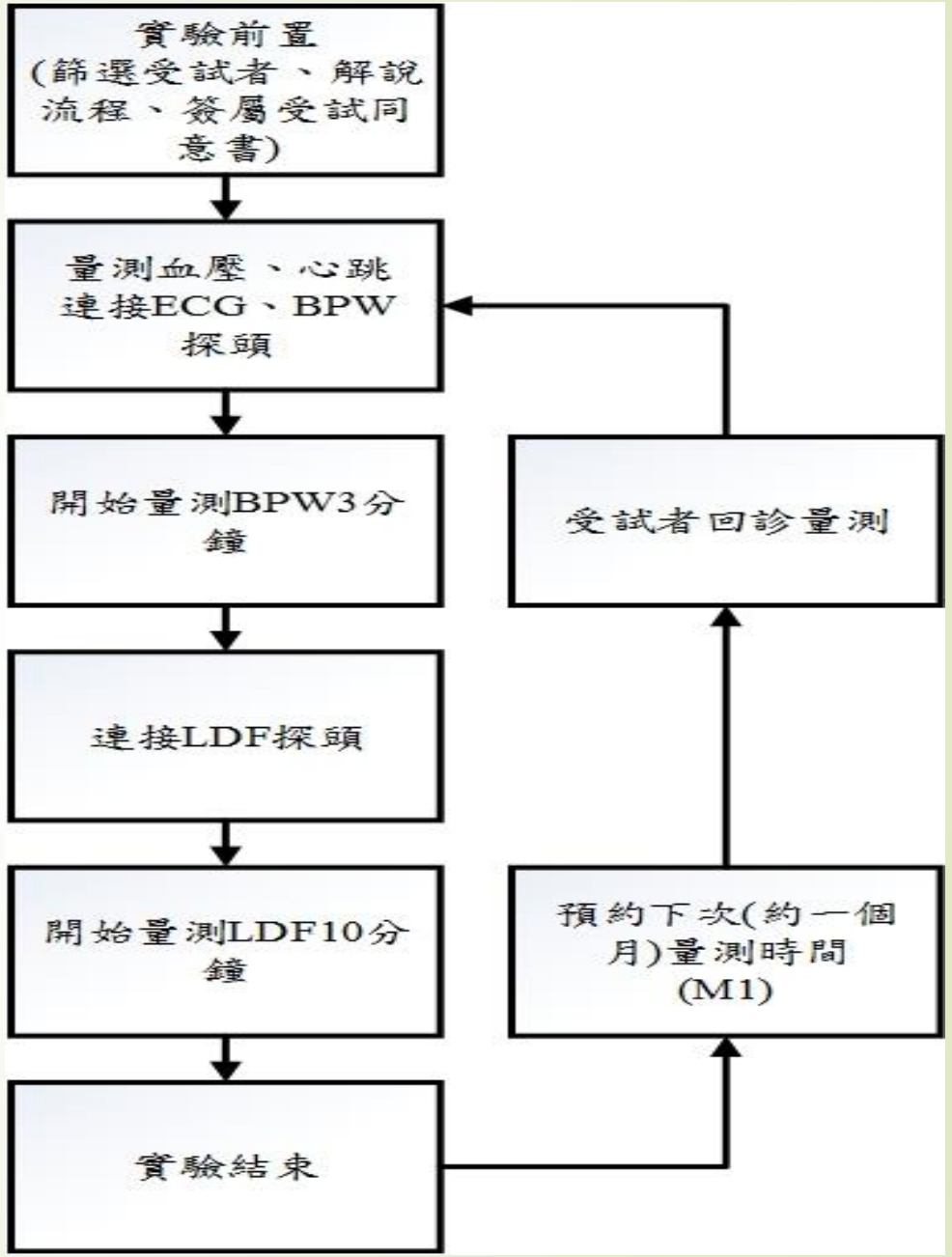


太陽穴位置圖[29]



Materials and Methods: Procedure實驗流程

- Participant recruitment受試者招募
- Patient recruitment was coordinated by the Department of TCM and Rehabilitation of the RenAi Branch of Taipei City Hospital.由中醫科相關醫師，招募有意願之中風病患，並進行相關說明
- Excluding Participant 受試者篩選
  - Age<20 or >75, chronic disease,(Ex: hypertension,heart disease and cancer etc).
  - Poor control post-operation or drug
  - Fear acupuncture
  - post-operation and skull unrepaired
  - Others. 其他導致受試者無法合作狀況，如篩選後不適合、不簽署受試者同意書等。



Materials and Methods:Grouping (1)

- Experiments were performed in the following **two groups:**
  - **Group S** (stroke patients **52**; **38** males and **14** females; aged 63.7±17.7 years old)
  - **Group C** (healthy controls; **21** males aged 23.2±3.9 years). The subjects in Group C were nonsmokers, had no apparent illness, had not taken any medication for 3 days before experiments, and had a body mass index of lower than 26.0 kg/m2

Table 1 Detailed characteristics of the included stroke patients.

Stroke characteristics	Number of patients
stroke location	Putamen: 9 Middle cerebral artery: 7 Brain stem: 4 Thalamus: 11 Basal ganglia: 7 Temporal lobe: 2 Frontal lobe: 2 Other: 10
Stroke side	Left : 27 Right: 25
Hemorrhage or infarction	Infarction: 16 Hemorrhage:36
Operation performed	Cardiac operation: 4 Cerebral operation: 10
Weeks from onset of stroke	8.78±2.76 weeks
Walking ability	Can walk independently with cane or walker: 28 cannot walk independently: 24

Table 2: Multiple regression analysis of the baseline (M0) LDF parameters.

	Adjustment type	*p* values	Unadjusted/ adjusted R^2
SampEn_FRT	age	0.179	0.045/0.009
	gender	0.902	
SampEn_FDT	age	0.527	0.032/0.019
	gender	0.546	
SampEn_PW	age	0.785	0.001/0.041
	gender	0.831	

Materials and Methods: Intervention(1/6)

24 traditional acup points

- The acupuncture protocol consisted of the following combination of several traditional acupuncture points on the body surface:

1.Scalp acupuncture : one needle penetrating through the trembling control region, the motor region, and the sensory region (S).（一針透三區，靳三針）

2.Around or on the head: PaiHui(百會) Du-Channel-20 (SC), TungTien (通天) Bladder-7 (SC),, FengChih (風池) Gallbladder-20 (SC), YingTang (印堂) (SC), RenZhong (人中) Du-Channel-26 (SC), and LienChuan (廉泉) Ren-Channel-22 (SC).

(S:Stroke leision ,C: Contralateral side , SC: both)

Materials and Methods: Intervention(2/6)

24 traditional acup points

3. On the upper extremity: ChuhChih(曲池) Large-Intestine-11(S) , Hogu (合谷) Large-Intestine-4(SC) , Neiguan (內關) (C) Pericardium-6 , SanYanLo (三陽絡) Sanjiao-8 (S), and ChiZe (尺澤) Lung-5(C).

4. On the lower extremity: YinLingQuan(陰陵泉) Spleen-9 (C), YangLingQuan (陽陵泉) Gallbladder-34 (S), ZuSanLi (足三里) Stomach-36 (S), SanYinJiao (三陰交) Spleen-6(C), XuanZhong (懸鍾) Gallbladder-39 (S), and TaiChong (太衝) Liver-3 (SC).

- (S:Stroke leision ,C: Contralateral side , SC: both)

Materials and Methods: Intervention(3/6)

24 traditional acup points

- Manual stimulation was applied to the body acupoints, and was applied to the body acupoints until the De-qi(得氣) feeling was perceived.
- Acupuncture using stainless steel, 1-inch, 30-gauge acupuncture needles (for the scalp and TaiChong acupoints) or stainless steel,1.5-inch, 30-gauge acupuncture needles (other acupoints), which were inserted and left in place for 20 min.

Materials and Methods: Intervention(4/6)

24 traditional acup points

- The scalp acupuncture was applied on the stroke side. The body acupuncture around the head were applied on both sides.
- For the acupoints located on the extremities,
  - Stroke side: Yang-side meridians (such as the Stomach, Gallbladder, Bladder, Sanjiao, and the Large-Intestine meridians)
  - Contralateral side: Ying-side meridians (such as the Spleen, Liver, and the Lung meridians)
- Bilateral Hogu (合谷) and TaiChong (太衝) form a set of acupoints, which may help to activate the brain function.

Materials and Methods: Intervention(5/6)

24 traditional acup points

- For post-stroke weakness and hemiplegia, acupuncture treatment plan involves choosing the contralateral scalp points on the healthy side, mainly the three Yang meridians on the diseased side, and the three Yin meridians on the healthy side as support. Since a wind disorder mostly affects Yang meridians, the emphasis is on the acupoints on the three Yang meridians. According to TCM theory, the Yang Ming meridian has abundant Qi and Blood. When the Qi and Blood of Yang Ming meridian are flowing smoothly, it can nourish the healthy Qi of the entire body and allow the gradual recovery of bodily functions.
- 中風後半側偏癱無力，針刺的方案為選取對側頭皮針與患側三陽經為主，健側三陰經為輔。因風病多犯陽經，故以三陽經腧穴為主。以中醫理論來說，陽明為多氣多血之經，陽明經氣血通暢，可以補充全身正氣，使身體功能逐漸恢復。

Materials and Methods: Intervention(2-6)

24 traditional acup points

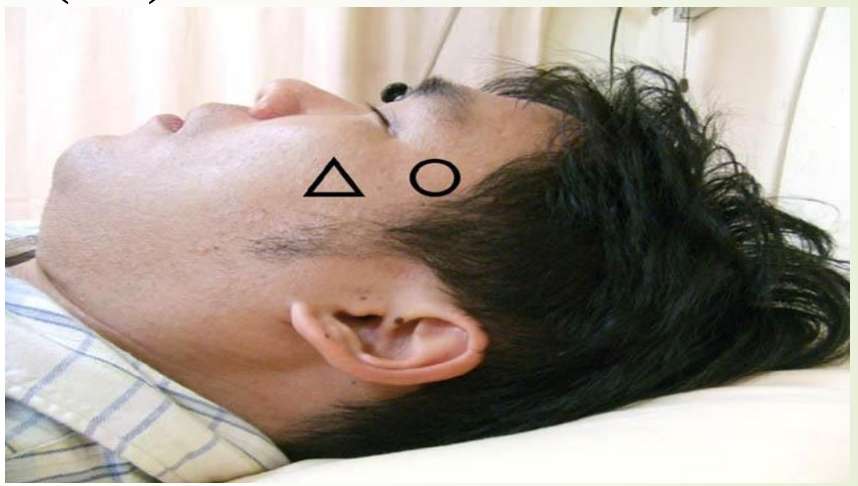
- Each assessment involved performing the following procedures (in the indicated order):
  - 1. Recording during a 20-min **baseline period (M0**) prior to AS.
  - 2. Acupuncture using stainless steel, 1-inch, 30-gauge acupuncture needles (for the scalp and TaiChong acupoints) or stainless steel, 1.5-inch, 30-gauge acupuncture needles (other acupoints), which were inserted and left in place for 20 min.
  - 3. Removal of the acupuncture needles to stop AS; **effect-period (M1)** data were recorded for a further 20 min from this point.

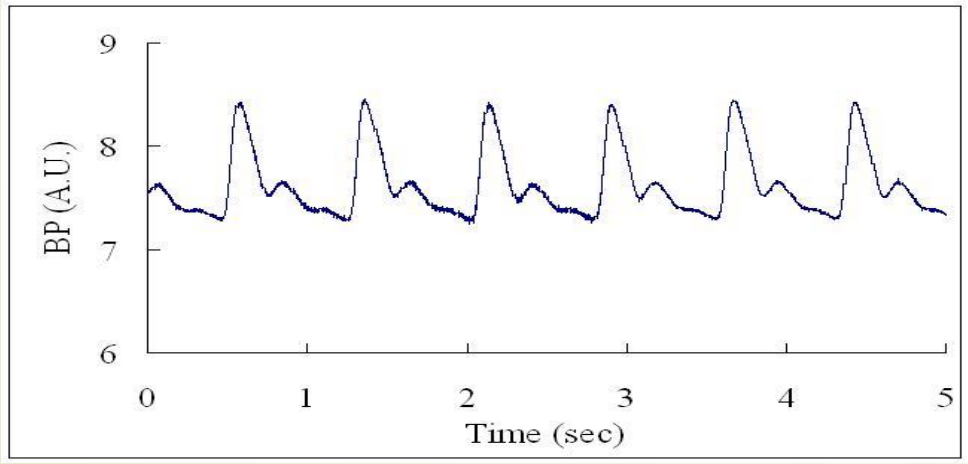
Materials and Methods: Measurements (1/2)

- LDF signals were obtained at bilateral TaiYang acupoints in 52 stroke patients.
- Each assessment involved a 20-min baseline recording, a 20-min AS, and a subsequent 20-min recording.
- The foot delay time(FDT), flow rising time (FRT), and pulse width(PW) were calculated for each pulse of the LDF signals, and then their SampEn values were calculated.

Materials and Methods: Measurements (2/2)

- Two BP waveform(BPW) and two LDF signals were obtained simultaneously and noninvasively by disk-shaped pressure transducers (KFG-2-120-D1-11, Kyowa) with 1-mm thickness and 4-mm diameter and Moor Instruments (UK)





the LDF measurement site (the LDF measurement sites are the bilateral **TaiYang** acupoints

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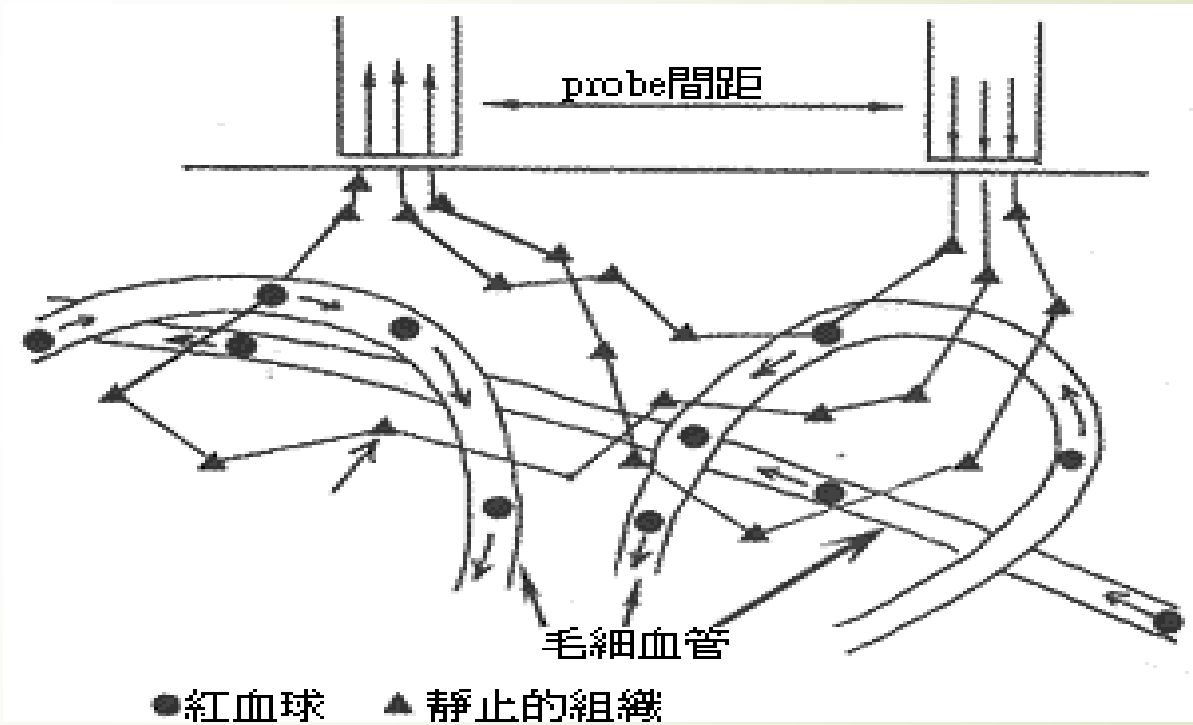
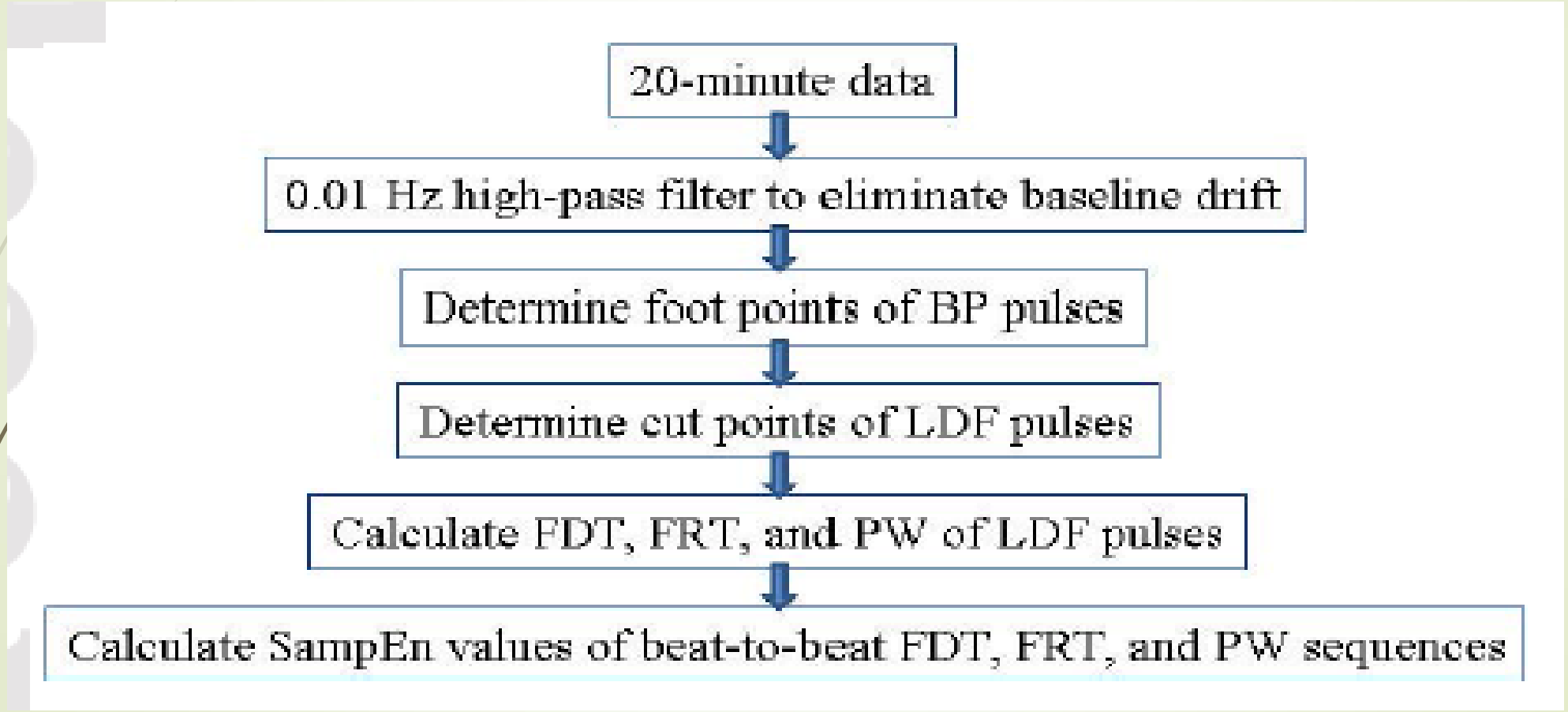
LDF：Laser-Doppler Flowmetry



LDF量測原理

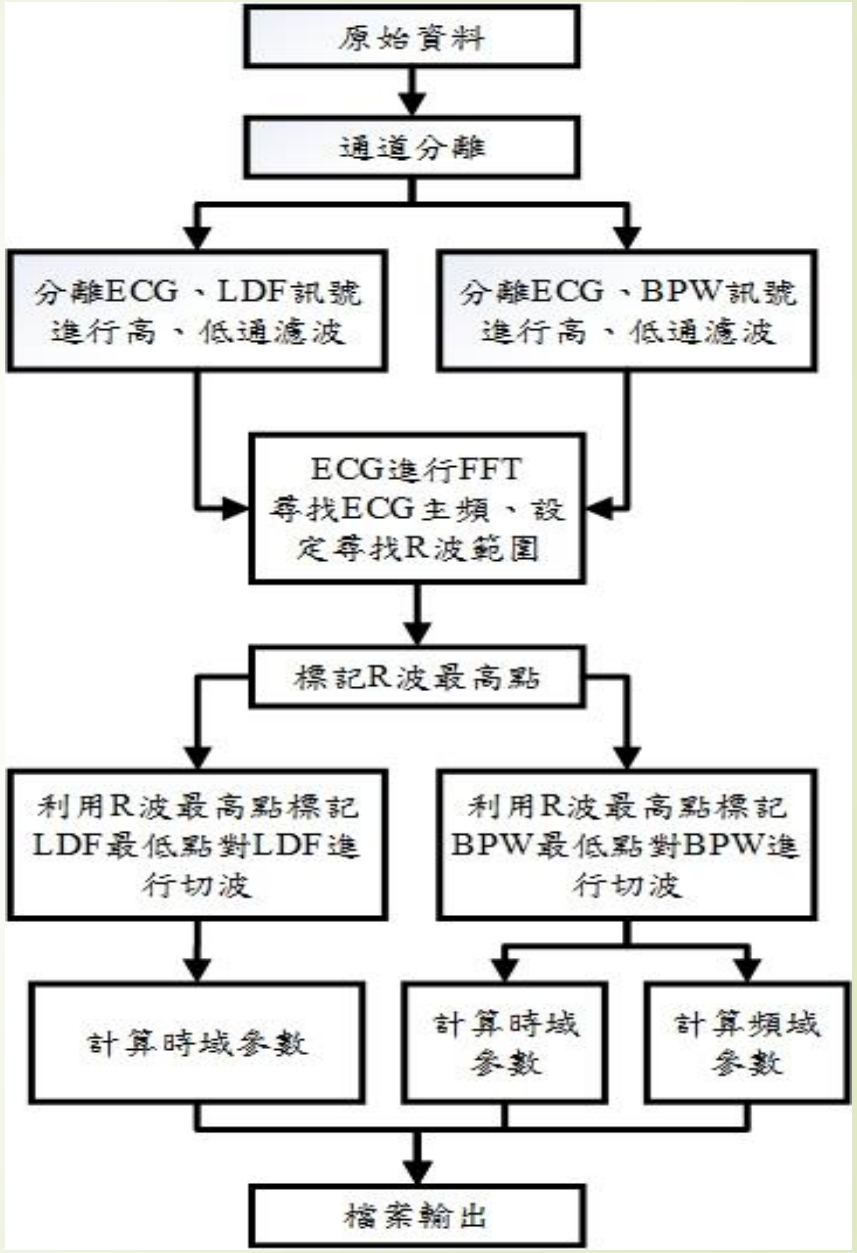
- 由光源產生單色光透過探頭進入生物介質
- 在測量深度內的活動顆粒表面發生散射而返回
  - 活動顆粒指的就是在血管內移動的紅血球
- 此時經過散射返回的光頻率已經改變了，此現象稱為Doppler shift
  - Doppler shift的幅度和強度分別與紅血球的速度和數量有關，與紅血球移動方向無關。
- 可藉由model估算出血流量與流速

Fig.2: Flowchart of LDF indexes calculation.



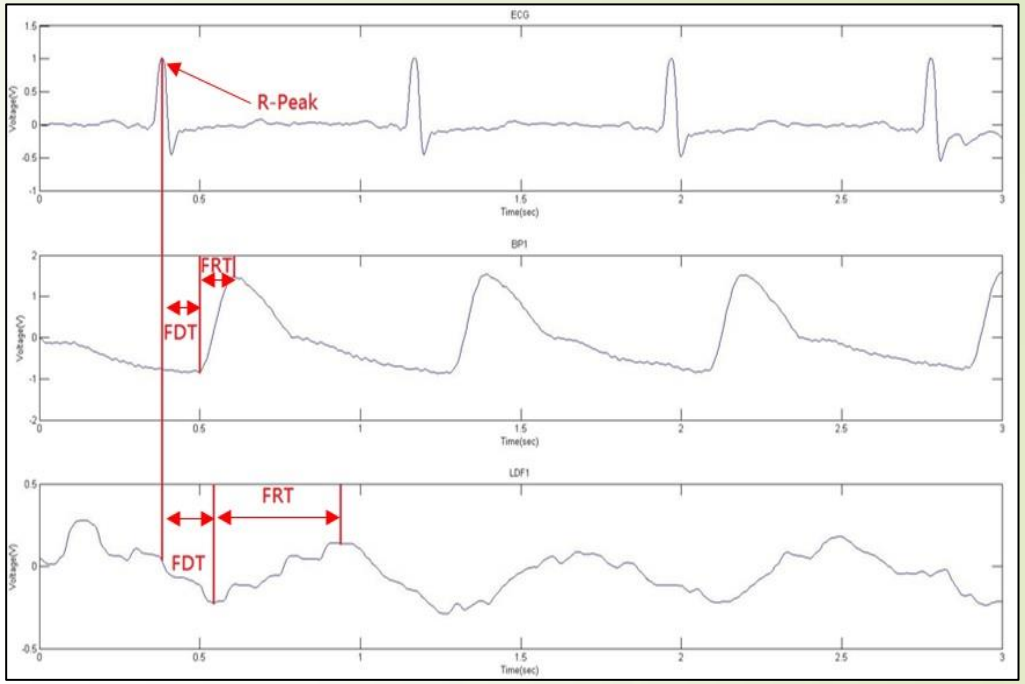
Data analysis分析流程(1/3)

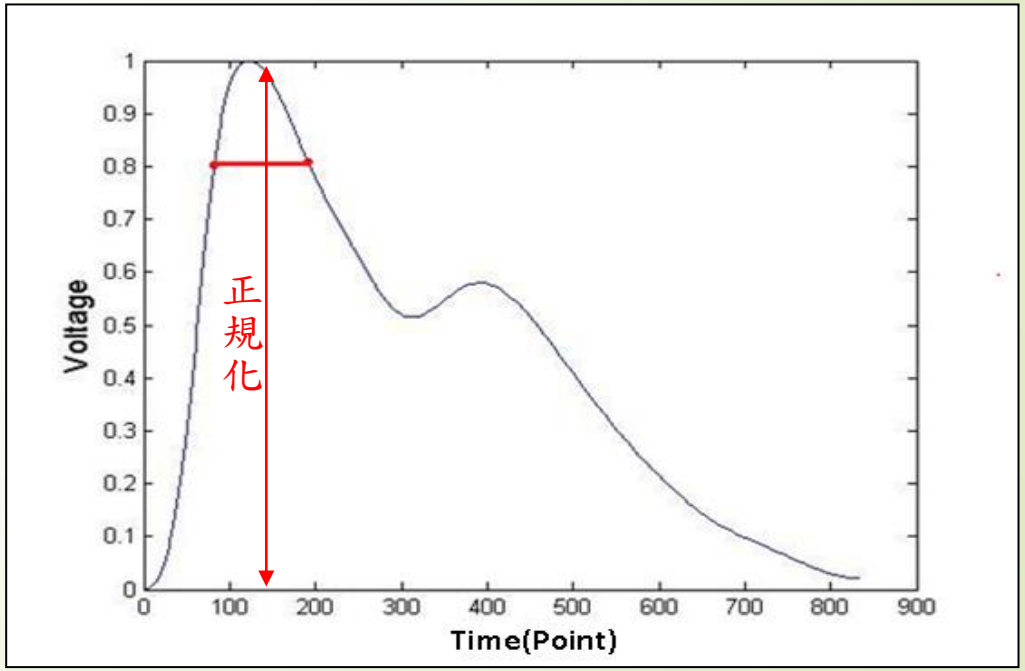
- LDF signals
  - Time domain parameter 時域參數
- BPW signals
  - Time domain parameter時域參數
  - Frequency domain parameter頻域參數(諧波參數)
- Stastics
  - using SPSS, version 13.0.
  - one-way ANOVA.
  - The Tukey-HSD method was used for post-hoc analysis.



Data analysis
分析流程(2/3)

- LDF Time domain parameter時域參數
  - FDT(Foot Delay Time)： FDT was defined as the time interval between the BPW foot point and the LDF foot point. 從心臟供血到微血管的延遲時間。
  - FRT(Foot Raising Time)：FRT was defined as the time interval between the foot point and the maximal point of the LDF flux signa l微血管血流從低點灌流到最高峰的爬升時間。
  - PW(Pulse Width)：PW was defined as defined as the time interval between the points at which the flux value was 80% of the pulse peak on the rising and falling edges 血流供應維持時間或動脈管壁撐開的時間。
  - 以上所有參數也一併計算對應變異係數CV之結果。





Data analysis
分析流程(3/3)

- SampEn analysis, which quantifies the unpredictability of fluctuations in a time series, was then performed on each 20-minute sequence of FDT (SampEn_FDT), FRT (SampEn_FRT), and PW (SampEn_PW) of the beat-to-beat LDF data sequence. For a sequence $u(N)=\{x_1, x_2, ..., x_N\}$, the SampEn (we used pattern length m=2; criterion of similarity r=0.2×SD) can be calculated as followed:

SampEn=-log(A/B)

where

A= number of template vector pairs having $d[X_{m+1}(i), X_{m+1}(j)] < r$ of length m+1,

B= number of template vector pairs having $d[X_m(i), X_m(j)] < r$ of length m,

$X_m(i) = \{x_i, x_{i+1}, ..., x_{i+m-1}\}$,

$d[X_m(i), X_m(j)]_{(i \neq j)}$ represents the Chebyshev distance.

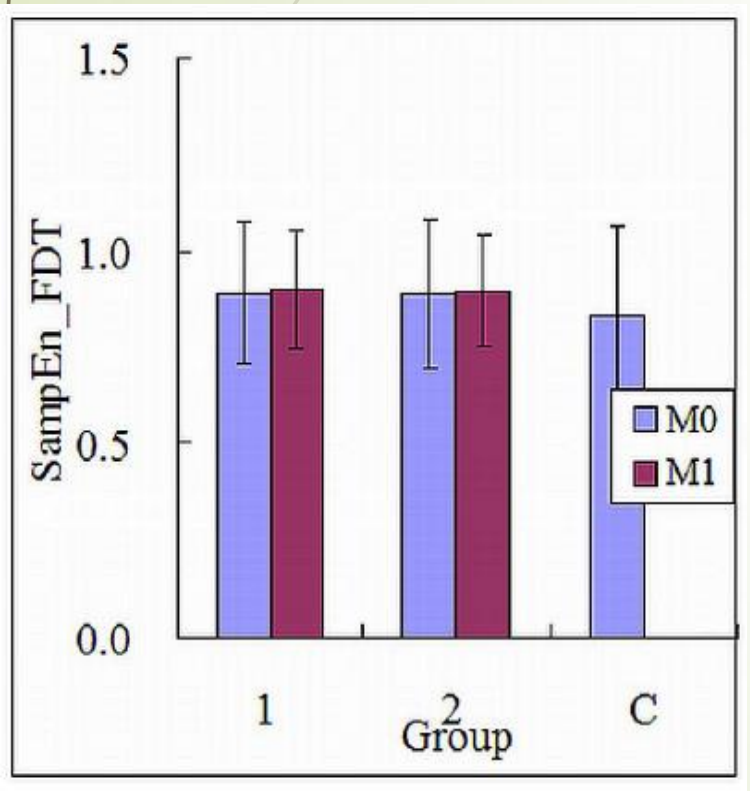
Results

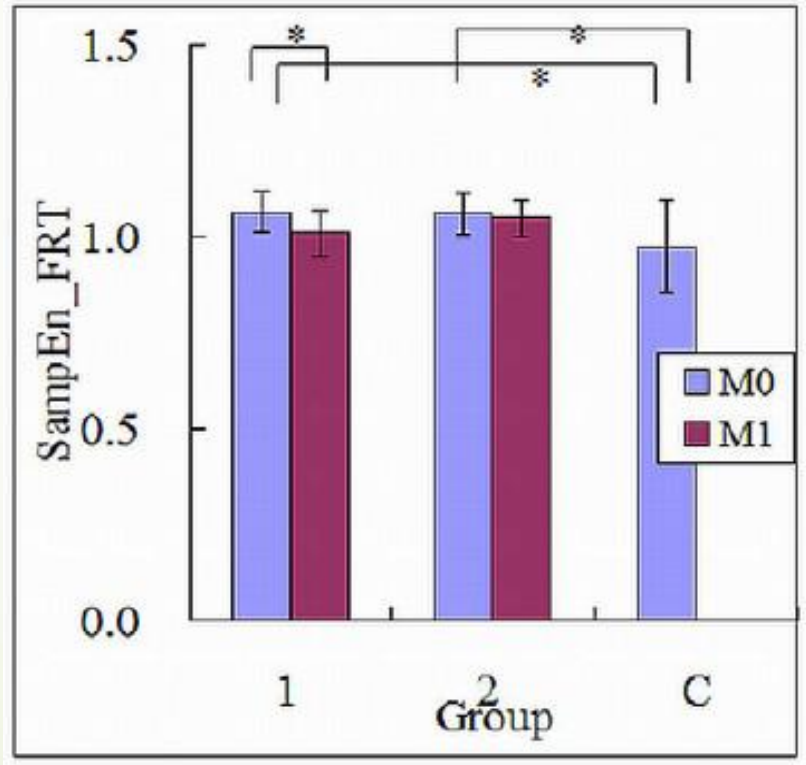
- The SampEn values of FRT were significantly larger in the stroke group (1.064±0.052 and p=0.013 at the stroke side; 1.059±0.055 and p=0.017 at the controlateral side) than in the control group (0.975±0.120).
- On the stroke side, the SampEn of value of FRT was significantly decreased following AS (1.064±0.052 to 1.008±0.060;p=0.027).

Fig.4: Comparison of SampEn (sample entropy) values among groups and following AS.

(a) SampEn values of FDT (foot delay time); (b) SampEn values of FRT (flow rising time); (c) SampEn values of PW (pulse width). '1' and '2' denote Sites 1 and 2 in the stroke group, and 'C' denotes the control group. '*' indicates $p<0.05$. Data are mean and SD values. M0: baseline period; M1: after AS.

Site 1: stroke site: **Site 2: contralateral site**





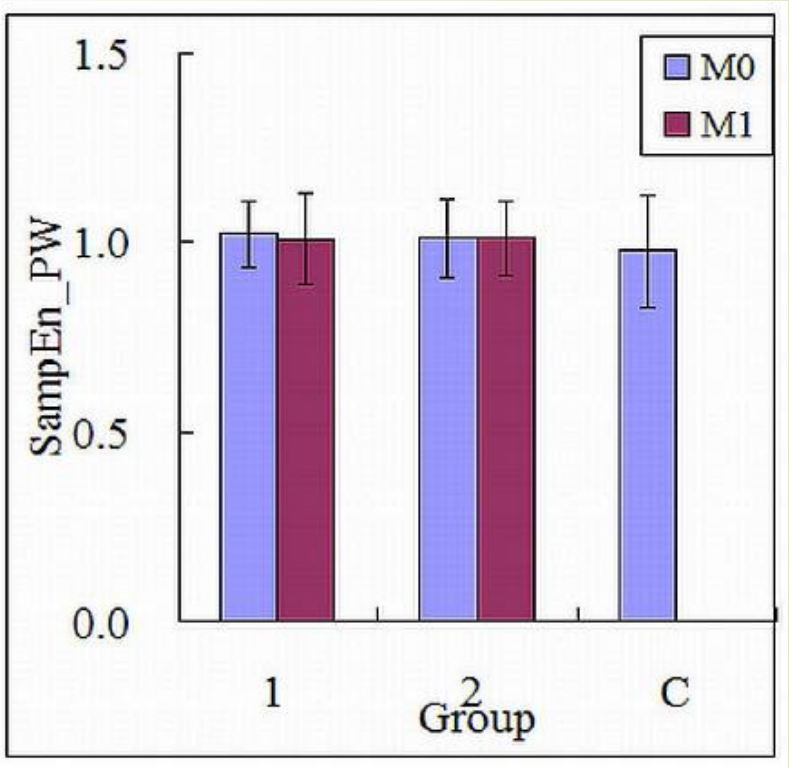


Fig. 1: Typical LDF (laser-Doppler flowmetry) waveforms passing the high-pass filter: (a) during the baseline period in a stroke patient, (b) during the effect period in a stroke patient, and (c) in a control subject. M0: baseline period; M1: after AS.

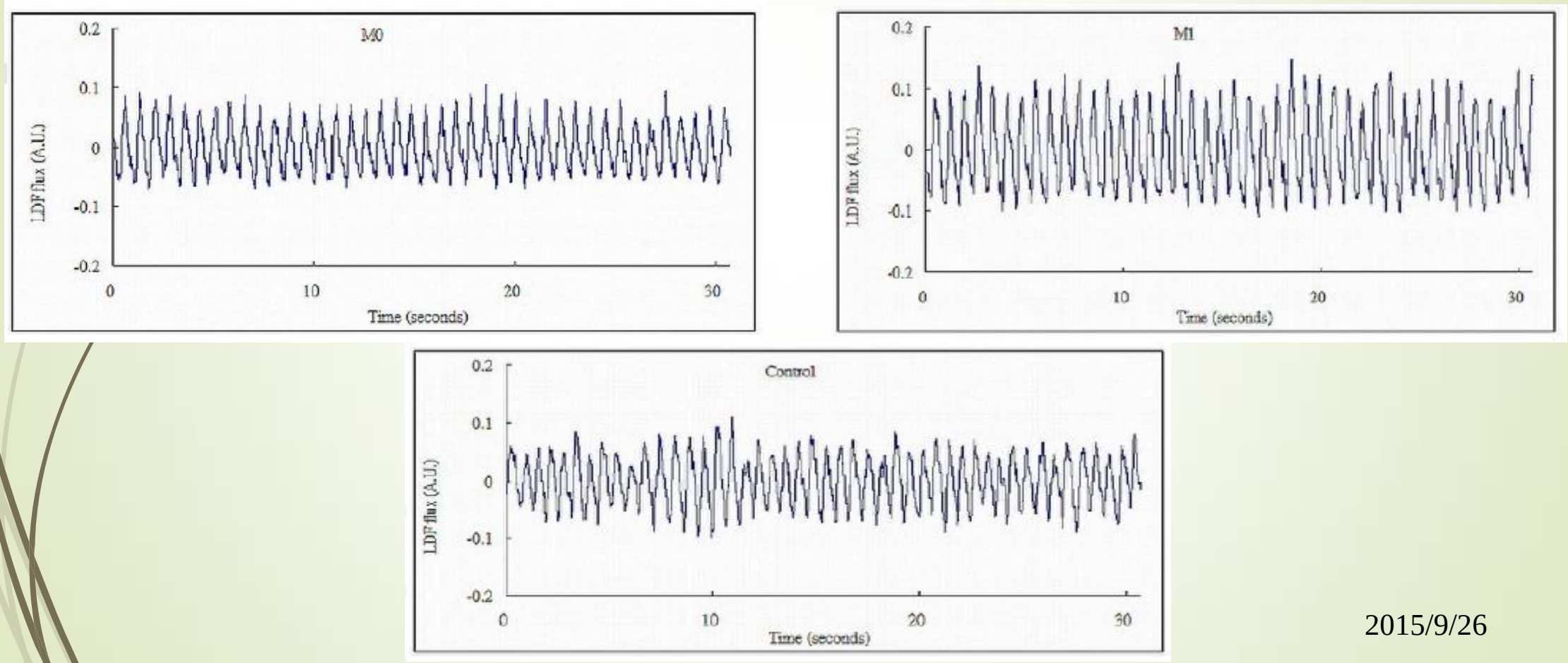
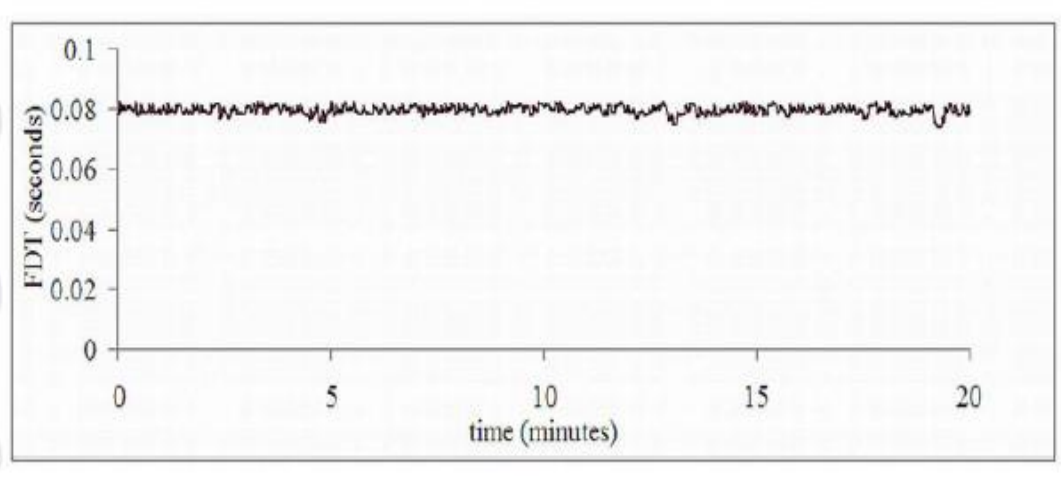
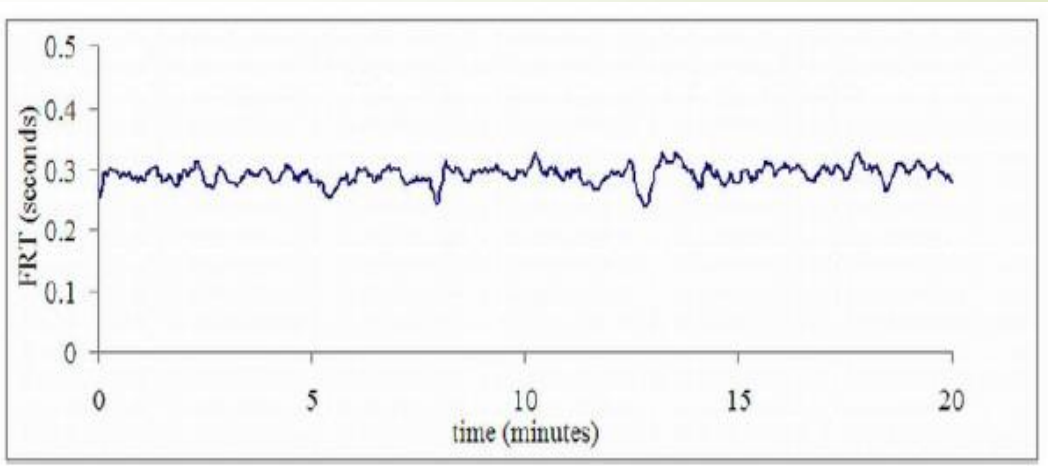


Fig.3: Typical time series of the beat-to-beat LDF parameters during the baseline period in control subject. (a) FDT; (b) FRT; (c) PW.





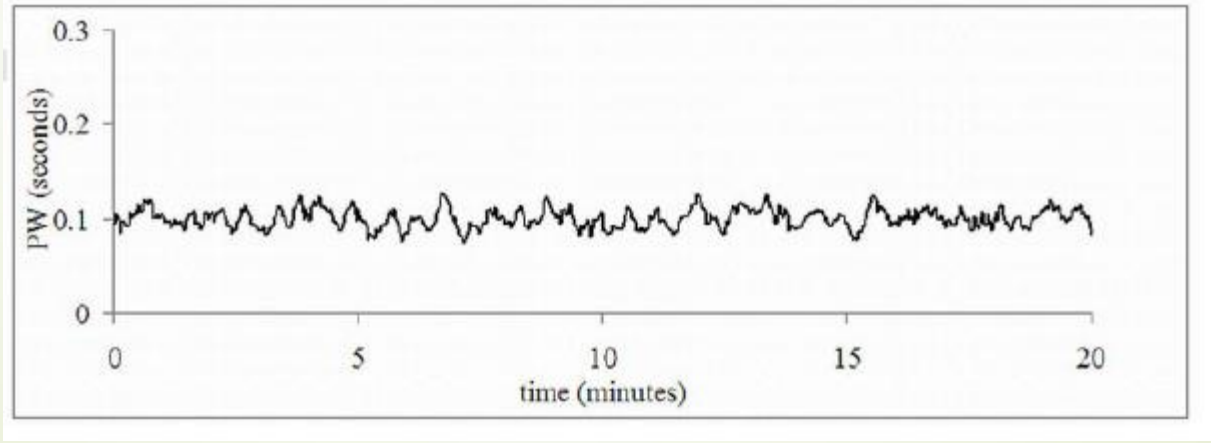
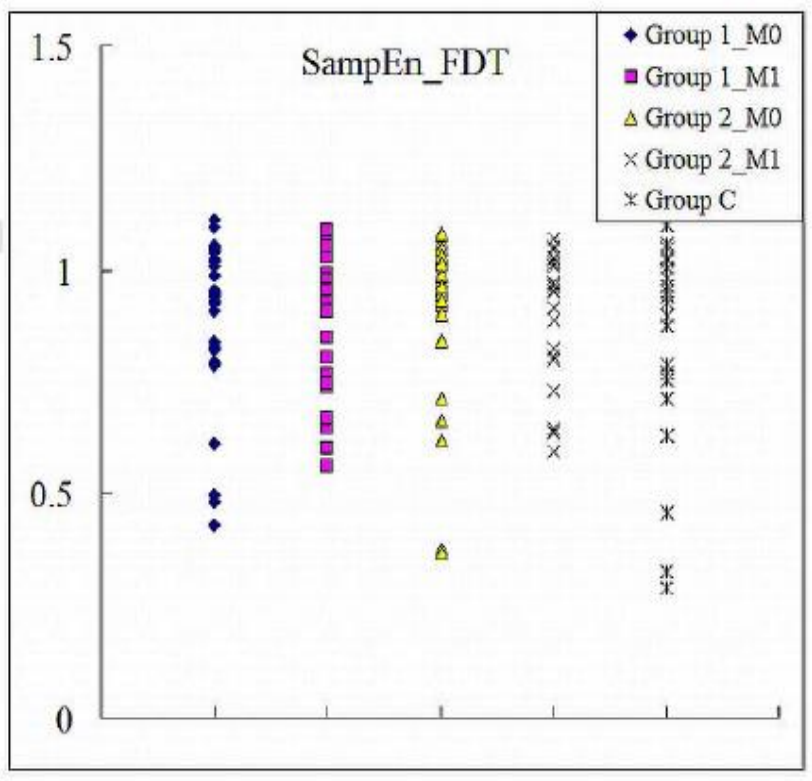
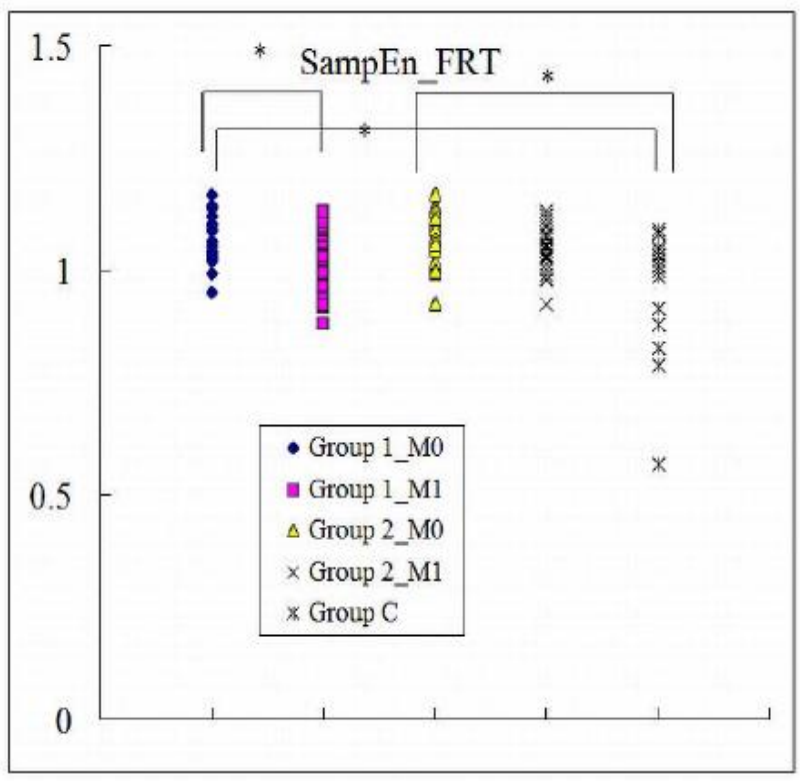
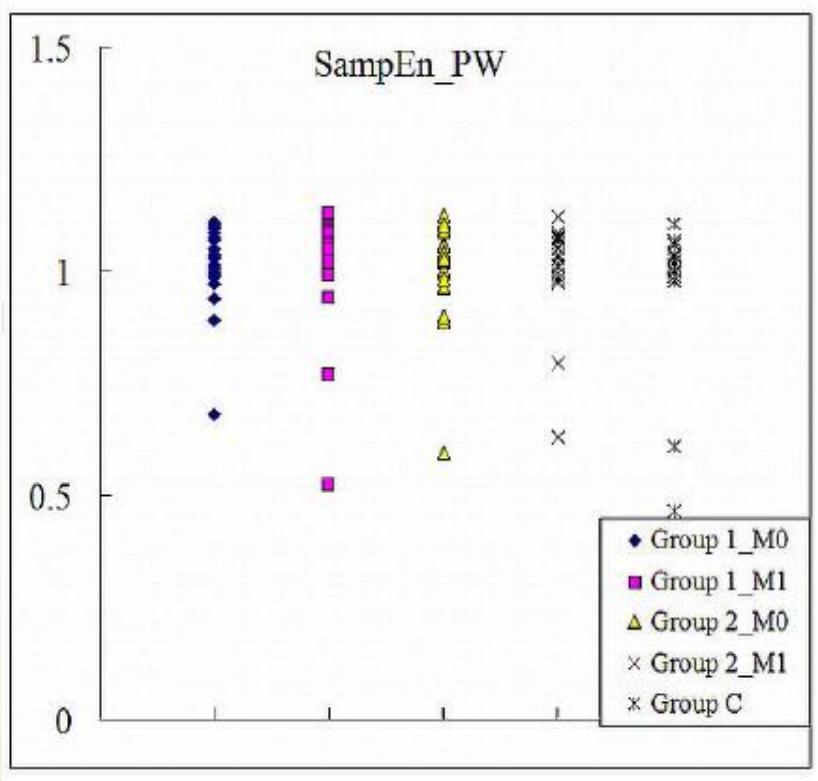


Fig.5: Detailed distribution of SampEn values of LDF parameters.

(a) SampEn values of FDT (foot delay time); (b) SampEn values of FRT (flow rising time); (c) SampEn values of PW (pulse width). 1' and '2' denote Sites 1 and 2 in the stroke group, and 'C' denotes the control group. '*' indicates $p<0.05$. M0: baseline period; M1: after AS.







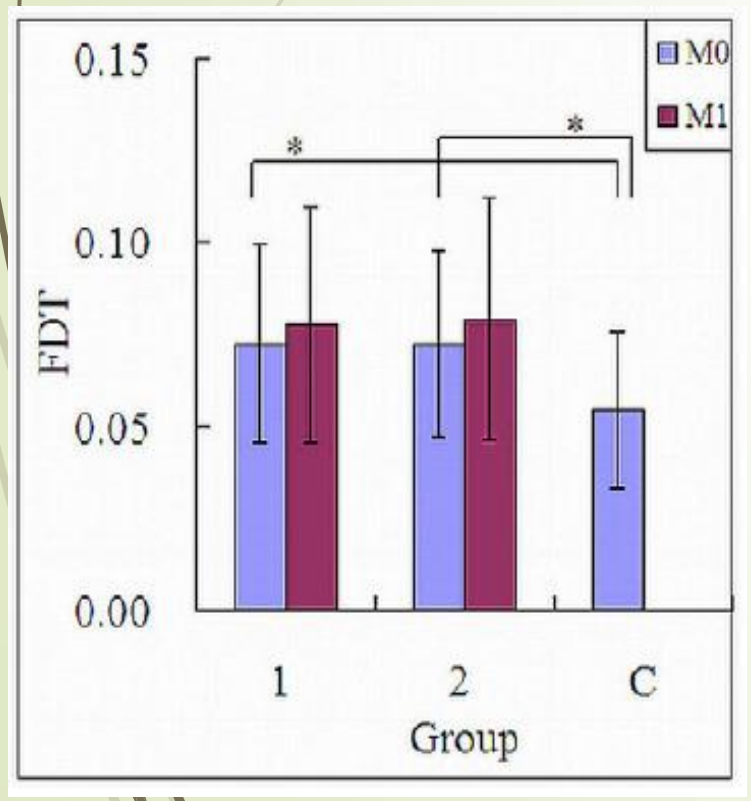
Site 1: stroke site: **Site 2: contralateral site**

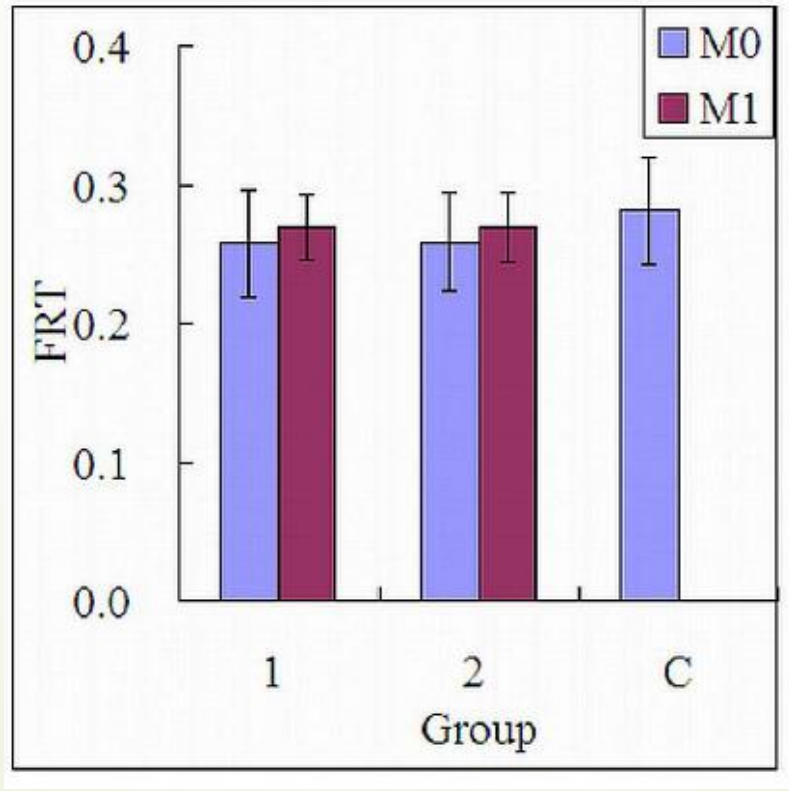
- Among SampEn values of the beat-to-beat LDF index, the only significant difference between the stroke and control groups was in the SampEn value of FRT, and this was present despite there being no significant difference for FRT itself. The SampEn values of FRT on both sides were significantly larger in stroke patients than in control subjects. It is possible that the induced abnormal vascular conditions and blood flow perfusion resistance in the cerebral arterial system result in more types of mechanisms participating in the local regulation in stroke patients, possibly with the aim of adjusting the perfusion function of the AO, maintaining the homeostasis of CBF supply, and meeting the tissue blood-flow requirements. The complexity of the local regulatory activities on the stroke side may therefore increase, thus leading to an increase in the SampEn value of FRT.

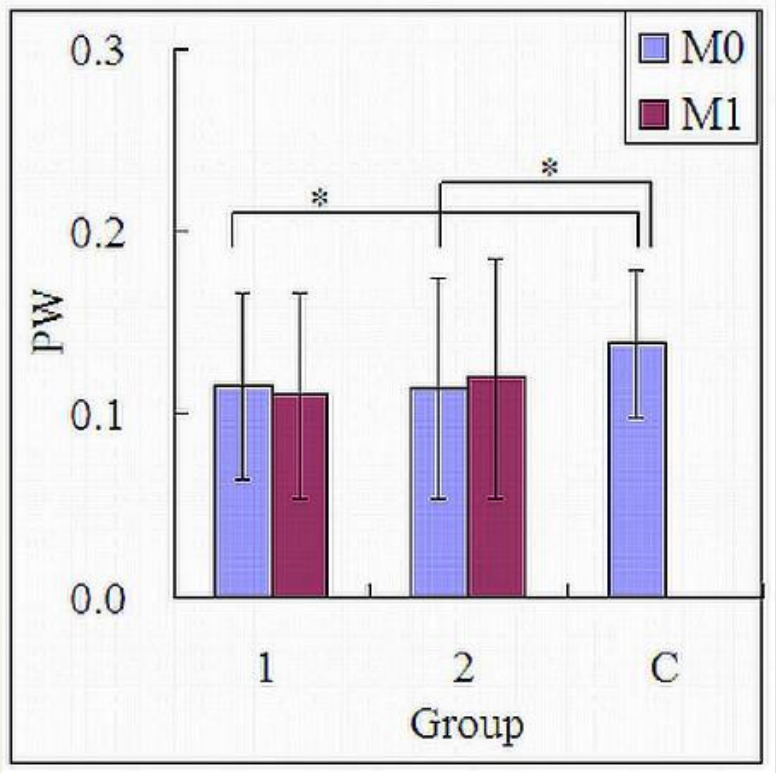
Fig.6: Changes in beat-to-beat LDF parameters (mean values of each time series for each subject).

(a) FDT (foot delay time); (b) FRT (flow rising time); (c) PW (pulse width). FDT, FRT, and PW are in seconds. '1' and '2' denote Sites 1 and 2 in the stroke group, and 'C' denotes the control group. '*' indicates $p<0.05$. Data are mean and SD values. M0: baseline period; M1: after AS.

Site 1: stroke site: **Site 2: contralateral site**

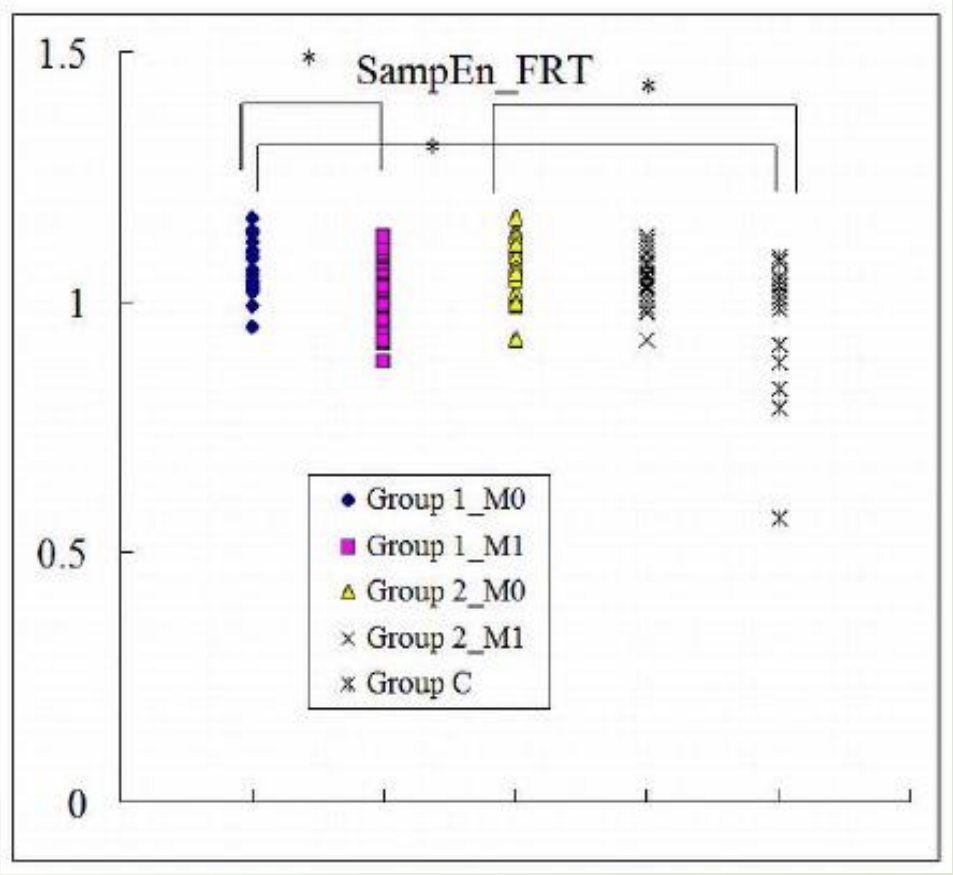






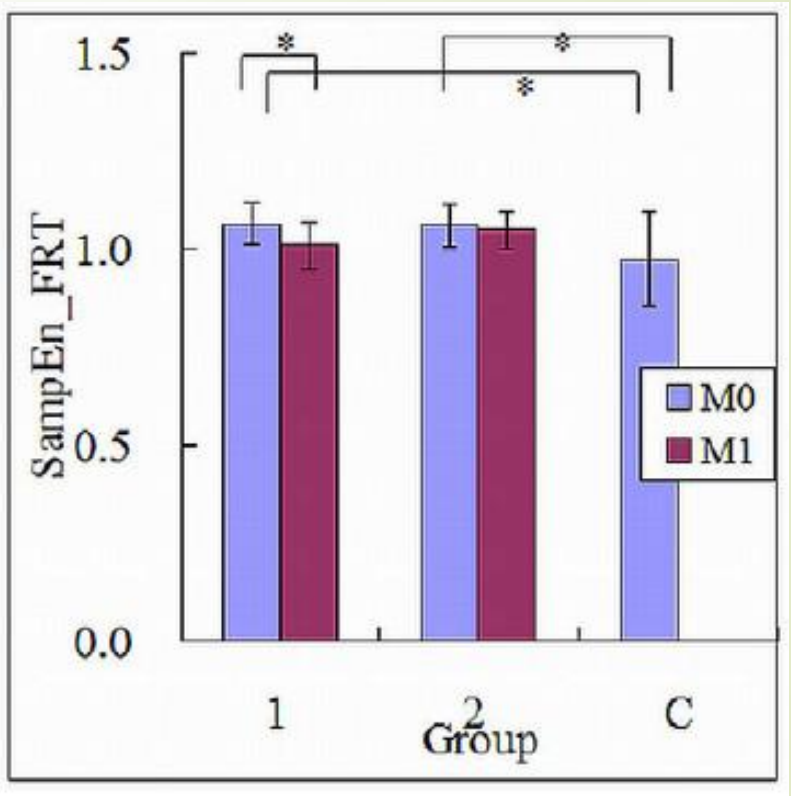
Discussion 討論(1/5)

- **Fig 5b :** The SampEn values of FRT on both sides were **significantly larger** in stroke patients than in control subjects. It is possible that the induced abnormal vascular conditions and blood flow perfusion resistance in the cerebral arterial system result in more types of mechanisms participating in the local regulation in stroke patients, possibly with the aim of adjusting the perfusion function of the AO(Aterior openings), maintaining the homeostasis of CBF supply, and meeting the tissue blood-flow requirements. The complexity of the local regulatory activities **on the stroke** side may therefore **increase**, thus leading to an increase in the SampEn value of FRT.



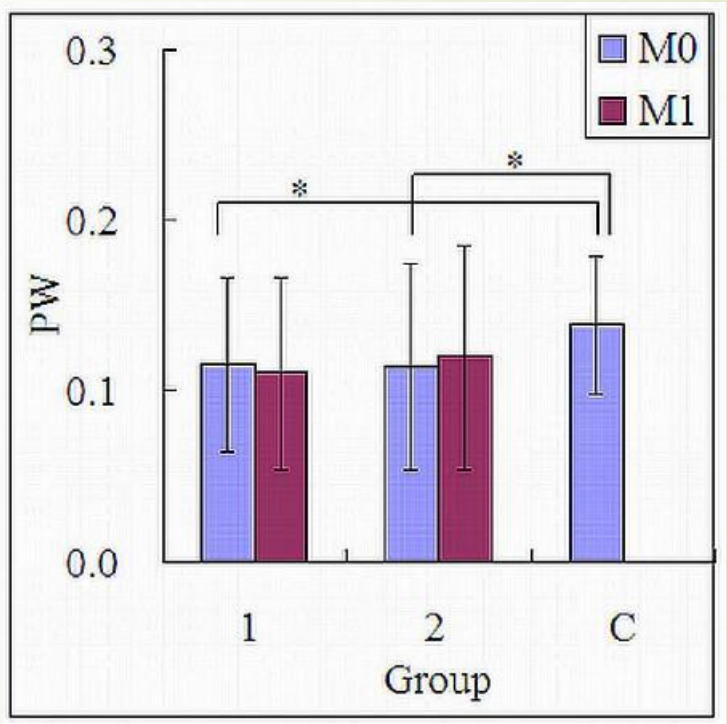
Discussion 討論(2/5)

- **Fig 4b** reveals that AS induced significant decreases in SampEn values of FRT. Although there were no significant differences following AS in the present results, previous studies have found that AS can increase the local MBF around the stimulated site. It has been suggested that this is partly attributable to the vasodilation induced by a decrease in the sympathetic neural activity.
- An improvement in the MBF supply condition of the local obstruction in the cerebral vascular beds will reduce the need for the local regulatory activities to deal with the hemodynamic abnormality. The microcirculatory regulatory activities can return to their normal states, and thus the SampEn values will decrease.



Discussion 討論 (3/5)

- Fig.6c reveals that PW was smaller in stroke patients than in control subjects, which can be associated with a lower blood flow through **AO(Arteriolar openings).** It can also be partly attributed to the stroke-induced stiffening of the vessel, since the AO can be more difficult to distend, resulting in a smaller blood flow through them. Changes in these beat-to-beat LDF indexes may be correlated with impaired CBF perfusion efficiency in stroke patients, and may therefore help to noninvasively discriminate the different CBF perfusion conditions in stroke patients and control subjects.



Discussion 討論 (4/5)

- The present results revealed significant differences in SampEn value of FRT, but not in those of FDT and PW. The present three beat-to-beat LDF indexes could be used to aid the monitoring of local MBF perfusion condition for different period of AO opening.
- For example:
  - FRT is defined as the rise time of the LDF pulse, and hence could be correlated with the perfusion resistance for the MBF through AO in the rising edge;
  - PW can be correlated with the time proportion at the peak period of the AO's opening
  - ;FDT can be correlated with accumulation time before AO's opening

Discussion 討論 (5/5)

- **Table 2** indicates that there were **no** significant associations between age and SampEn index. It has been noted that complexity of cardiac regulation decreases with age at rest.
- In the present study, SampEn values of FRT sequence is revealed to be larger in stroke subjects (also with older age) than in healthy control, which is opposite to the changing trend in Porta's work, although the analyzed cardiovascular signals were different between the present and Porta's studies. It implies that the present findings could still have substantial meanings despite of the interference effect of subject age.

Table 2: Multiple regression analysis of the baseline (M0) LDF parameters.

	Adjustment type	*p* values	Unadjusted/ adjusted R^2
SampEn_FRT	age	0.179	0.045/0.009
	gender	0.902	
SampEn_FDT	age	0.527	0.032/0.019
	gender	0.546	
SampEn_PW	age	0.785	0.001/0.041
	gender	0.831	

Conclusion 結論

- Larger SampEn values of FRT can be partly attributed to the local regulatory activities that are present in the stroke subjects when facing the induced abnormal vascular conditions and blood flow perfusion resistance.
- The present findings could aid the development of a noninvasive monitoring technique that will enable discrimination of the different microcirculatory responses in stroke patients.

Thanks for your attention
Q&A