# The Hemodynamic Effects of External Counterpulsation in Patients with Recent Stroke

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# DECLARATION OF ORIGINATION

I hereby declare that the studies contained in this thesis are completely original, which are carried out in the Department of Medicine and Therapeutics, Faculty of Medicine, the Chinese University of Hong Kong, starting from August 2008 to May 2011. None of the work has been submitted to any other universities or institutions for a degree, diploma or other qualification.

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LIN, Wenhua

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# ABSTRACT

Abstract of thesis titled with:

*The hemodynamic effects of external counterpulsation in patients with recent stroke*

Submitted by LIN WENHUA

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External counterpulsation (ECP) is a noninvasive method used to improve the perfusion of vital organs, which may increase cerebral blood flow and/or its collateral circulation resulting in possible benefits to ischemic stroke patients as shown in our previous studies. ECP is a safe and established effective treatment for ischemic heart disease (IHD). It is triggered by electrocardiogram (ECG) to inflate pressure during diastole on lower extremities through three pairs of pneumatic cuffs and deflate pressure before the start of systole. Diastolic augmentation induced by ECP also exerts effects on cerebral hemodynamics.

However, as to ECP on cerebral circulation, there are little available data and many unknown questions, especially on the aspect of treatment for ischemic stroke patients. We aim to explore in details the hemodynamic effects of ECP on the cerebral circulation in patients with recent ischemic stroke. First, we investigated the changes in flow velocities in both the infarction affected side as well as the contralateral side during ECP, which may be regarded as part of the collateral circulation. Second, we studied the dynamic

augmentation effects of ECP compared with the well established vasomotor reactivity. Third, we used external counterpulsation to assess the cerebral autoregulation on patients receiving cerebral angioplasty of stenting. Then we proceeded to determine the optimal treatment pressure to achieve the maximal hemodynamic effect on the cerebral blood flow velocities. As the ultimate goal of ECP treatment is to improve neurological outcome after stroke, we analyzed our database in the Prince of Wales Hospital to identify any promising hemodynamic parameters or independent factors to predict better functional outcome for ECP-treated ischemic stroke patients. Finally, to overcome the problem of insufficient or absent of acoustic temporal bone windows for MCA monitoring, we explored the use of cervical internal carotid artery monitoring with a newly developed probe holder.

In this study, we recruited recent ischemic stroke patients with large artery occlusive disease receiving ECP treatment. We monitored their cerebral blood flow velocities of bilateral MCAs using transcranial Doppler (TCD). We started ECP treatment pressure on the lower body from 150mmHg (0.02MPa), then gradually increased to 187.5mmHg (0.025MPa), 225mmHg (0.03MPa) and 262.5mmHg (0.035MPa). TCD parameters, including peak systolic velocity PSV, peak diastolic augmentation velocity PDAV, end diastolic velocity EDV and mean flow velocity MV were recorded for 3 minutes before and during each pressure increment. Monitoring data was analyzed based on whether it was ipsilateral or contralateral to the cerebral infarct.

We found the mean flow velocities during ECP (150mmHg) increased significantly on ipsilateral side and contralateral side when compared with baseline velocities, but there was no increase difference between the two sides when compared with each other. Peak diastolic velocities significantly increased on ipsilateral side (78.70%, $p<0.001$) and contralateral side (83.10%, $p<0.001$), compared with baseline end diastolic velocity. No difference was found between two sides on the increase of peak diastolic augmentation.

The mean flow velocities on the symptomatic side under different ECP pressures, increased 18.49% (150mmHg), 19.33% (187.5mmHg), 19.16% (225mmHg) and 18.46% (262.5mmHg). All were significantly higher than baseline but did not differ among various pressures. Under increasing pressures, PDAV gradually increased (78.70%, 88.24%, 96.66%, 103.02%, respectively compared with baseline EDV, $p<0.001$) while PSV decreased (3.60%, 2.64%, 1.02%, -1.88%, compared with baseline PSV, $p=0.001$) and EDV decreased (-1.35%, -4.18%, -9.28%, -11.87%, compared with baseline EDV, $p=0.002$). Contralateral MCA velocity change tendency showed similar picture.

The hemodynamic effect during ECP on cerebral blood has not been quantified. We proposed a measurement of cerebral augmentation index (CAI) to evaluate the augmentation effect induced by ECP. It may be a measure of how the brain accommodates to elevated blood pressure and flow diversion. It may also be considered as a form of vaso-reaction. We compared CAI to a well-established measurement of vasomotor reactivity, breathholding index (BHI). We performed ECP treatment and breathholding test combined with TCD monitoring on ischemic stroke patients with large

artery occlusive disease and good temporal window. We aimed to explore the correlation between the augmented hemodynamic effect of ECP and cerebral vasomotor reactivity.

The MCA mean flow velocities in stroke group significantly increased after ECP (CAI ipsilateral 8.11±9.79, contralateral 7.74±8.99) but not in the controls, (CAI -0.47± 2.89). BHIs were smaller in the stroke group, (ipsilateral 0.79±0.51, contralateral 0.93± 0.53) than that of the controls (1.40±0.45). CAI did not correlate with BHI on the ipsilateral or contralateral side of stroke group as well as in controls. BHI was significantly lower on the ipsilateral side than the contralateral side, p= 0.011, but CAI showed no difference. CAI of stroke patients on the ipsilateral side was strongly related to the systolic and diastolic blood pressure change.

In a subgroup study of one randomized controlled study of intracranial stenting, we assessed 18 ischemic stroke patients (10 patients received stenting) 2 years after randomization, who had symptomatic high-grade (≥70%) intracranial internal carotid artery or middle cerebral artery (MCA) stenosis. We measured the CAI of both MCAs. MCA mean blood flow velocities before and during ECP were recorded for 3 minutes. CAI was evaluated and compared based on the presence of infarction (symptomatic versus contralateral side).

The subgroup analysis of stenting trial showed baseline NIHSS and demographics were comparable between stenting and non-stenting groups. All patients had no stroke recurrence after randomization. MCA mean flow velocities significantly increased during

ECP in both groups ($p<0.05$). CAIs of stenting group were lower than that of non-stenting groups (symptomatic 3.89 ± 2.90 versus 7.47 + 3.42, $p=0.051$; contralateral 3.00 ± 4.03 versus 7.04 ± 2.82, $p=0.013$). CAI was not different between symptomatic and contralateral sides in both groups.

We recorded the demographics and medical history of ECP-treated stroke patients , as well as the stroke outcome assessed at 3 months using the modified Rankin Scale (mRS), categorizing good outcome as mRS 0~2 and poor outcome as mRS 3~6. Then we investigated the association between cerebral augmented flow and outcome after ECP treatment.

Among 36 patients with TCD monitoring and 3-month follow-up completed, twenty-one patients (58.3%) had good outcome as defined by mRS 0~2 and 41.7% patients had poor outcome (mRS 3~6). Statistic analysis showed that NIHSS on admission was significantly lower in good outcome group. During ECP, baseline mean flow velocity and augmented mean flow velocity on the ipsilateral side were relatively higher in good outcome group without significance. We did not find specific hemodynamic parameters to predict outcome of ECP treatment based on current data.

We analyzed our ECP registry of acute ischemic stroke patients with cerebral large artery stenosis who underwent ECP therapy at the Prince of Wales Hospital. A standard treatment protocol consisted of 35 daily ECP sessions (each session one hour). We included 155 patients who completed at least 10 sessions of ECP and had 3 months

follow up data. Patients were divided into different outcome groups according to mRS. We compared the differences in the two groups in terms of demographics, medical history and ECP treatment duration time.

Among 155 patients in ECP registry, who finished at least 10 ECP sessions and 3-month follow-up, ninety-nine (63.9%) patients had a good outcome at 3 month after stroke. Compared with poor outcome group, patients in good outcome group were younger, with lower admission NIHSS, lower total cholesterol, higher admission systolic blood pressure, and longer ECP therapy duration. Patients with good outcome were more likely to have the history of transient ischemic attack (TIA) and a longer interval from stroke onset to the start of ECP. Multivariate logistic regression showed that ECP duration (OR 1.073, 95%CI 1.012~1.137, p=0.018), admission NIHSS (OR 0.744, 95%CI 0.658~0.841, p<0.001) and admission systolic blood pressure (OR 1.024, 95%CI 1.008~1.041, p=0.003) were independent predictors for a favorable outcome.

We did a pilot study to research the augmentation effect of ECP on internal carotid artery (ICA) from carotid TCD monitoring with a novel neck frame. Comparing the CAI of MCA with that of ICA, we aimed to investigate if any correlations exist between augmentations observed from two vessels. Further test the feasibility of ICA monitoring.

We recruited 14 cases into the preliminary study of ICA monitoring. Mean age of these patients was 60.93. Median of admission NIHSS for these patients was 3. Mean flow velocities of MCA and ICA both decreased after ECP in this study. Median of CAI on

MCA was -5.08 and median of CAI on ICA was -4.79. There was not significant correlation between cerebral augmentation of MCA and ICA induced by ECP. Thus, the use of ICA to substitute MCA in patients without good temporal window cannot be supported.

In summary, the extent of cerebral blood flow augmentation during ECP in patients with recent stroke appears to be the same in the infarct territories when compared with contralateral side. These findings suggest that potentially circulation may be enhanced to improve the collateral blood supply of ischemic territories both from the infarct ipsilateral side and contralateral side. Dynamic augmentation effects as measured by CAI were different from the well established vasomotor reactivity. CAI is a measure of how well the brain accommodates blood flow augmentation, independent of vasomotor reactivity. Evaluated by CAI, stenting of intracranial atherosclerosis may improve the cerebral autoregulation and ability for the ischemic brain to accommodate flow augmentation in long term. 150mmHg appears to be the optimal and safe pressure to be used to inflate the cuff in the ECP in order to increase cerebral blood flow. Further increase in pressure does not increase cerebral blood flow velocity. The duration of ECP therapy is found to be an important predictor for stroke recovery on ECP-treated acute ischemic stroke patients, in addition to the well-known prognostic factors such as admission NIHSS and blood pressure. This series of studies elucidate the important mechanisms of the potential benefits of ECP on stroke patients.

## 中文摘要

體外反博 （External counterpulsation，ECP）是一種無創傷性用以增加重要器官血流灌注的方法。正如我們之前的研究表明，它可以增加腦血流和/或者側枝循環血流，從而幫助缺血性腦卒中的患者。ECP 是一種安全高效的治療方法，廣泛應用于冠狀動脈缺血性心臟病。它是通過心電導聯觸發，在舒張期對附於下肢的一對氣囊充氣，并在收縮期之前釋放壓力。由 ECP 引起的舒張期血流增加影響腦血流動力學。

然而 ECP 對於腦循環的作用，特別是在治療缺血性腦卒中患者方面，相關數據和認識很少。此文目的是爲了研究 ECP 對於急性缺血性卒中患者腦循環的血流動力學作用。首先，我們研究了患者大腦梗死灶側及對側在 ECP 作用下血流速度的改變。其次我們比較 ECP 的血流增加效應與已被廣泛認可的血管運動反應性。第三，我們運用 ECP 來評價接受支架腦血管成形治療患者的腦循環自主調節功能。然後我們進一步研究 ECP 作用下達到最大腦血流速度作用時的最佳反博壓力。由於 ECP 治療的最終目的是促進卒中事件后神經系統缺損的康復，我們分析了威爾斯親王醫院的 ECP 數據庫，尋找預示 ECP 對於缺血性腦卒中良好治療效果的血管動力學指標或者獨立因素。最后我们嘗試应用一种新型颈部探头监测颈内动脉颅内段，以克服颞窗不良影响大脑中动脉血流监测的难题。

在這個實驗中，我們研究具有大血管堵塞并接受 ECP 治療的急性缺血性腦卒中患者。我们使用經顱多普勒監測他們頭顱兩側的大腦中動脈血流。作用於下肢的 ECP 治療壓力由 150mmHg（0.02MPa），逐渐上升到 187.5mmHg（0.025MPa），225mmHg（0.03MPa），262.5mmHg（0.035MPa）。記錄基線值及各個壓力下的經

顱多普勒超聲指數，包括收縮期峰值 PSV、舒張期增加峰值 PDAV、舒張末期值 EDV 及平均血流速度 MV，各個時段分別記錄 3 分鐘。監測數據根據位於大腦梗死灶的同側或對側進行分析。

我們發現 ECP 作用下（150mmHg）大腦兩側的平均血流速度顯著增加。但兩側相比增加幅度沒有差別。與基線舒張末期值比較，舒張期峰值明顯增加，同側增加 78.70%，p<0.001；對側 83.10%, p<0.001。二者的舒張期增加幅度也沒有顯著區別。

在不同的 ECP 壓力下，梗死灶同側的平均血流速度增加 18.49%（150mmHg），19.33%（187.5mmHg），19.16%（225mmHg）及 18.46%（262.5mmHg）。所有壓力下均顯著高於基線值，但各壓力之間沒有差別。隨著壓力增加，PDAV 也逐漸增加（78.70%，88.24%，96.66%，103.02%，分別與基線 EDV 比較，p<0.001）；然而 PSV 減少（3.60%，2.64%，1.02%，-1.88%，與基線 PSV 比較，p=0.001），EDV 也減少（-1.35%，-4.18%，-9.28%，-11.87% 與基線 EDV 比較，p=0.002）。對側 MCA 流速也呈現相似的變化趨勢。

ECP 對於腦血流的動力學作用仍沒有量化。我們提出腦血流增大指數 CAI 來評價由 ECP 引起的腦血流增加作用。它是一個衡量大腦調節上升血壓和血流轉移的指標。它也可以認為是一種血管反應性的形式。我們將 CAI 與廣泛認可的衡量腦血管運動反應性的指標閉氣指數 BHI 進行比較。我們結合了經顱多普勒超聲監測，對有大血管阻塞的缺血性腦卒中及良好顱窗的患者進行了 ECP 治療及閉氣試驗，從而研究 ECP 血流增大效應與腦血管運動反應性的關係。

ECP 作用下卒中患者的大腦中動脈平均血流速度顯著增加（CAI 同側 8.11±9.79, 對側 7.74±8.99），然而對照組血流速度則下降（CAI -0.47±2.89）。卒中患者的閉氣指數（同側 0.79±0.51，對側 0.93±0.53）明顯小於對照組（1.40±0.45）。CAI 無論在患病組或對照組均與 BHI 沒有相關性。梗死灶同側的 BHI 明顯低於對側，p=0.011，但 CAI 在兩側沒有明顯區別。卒中患者梗死灶同側的 CAI 與收縮期及舒張期的血壓改變顯著相關。

在一項顱內支架治療隨機對照試驗中，我們納入 18 例具有症狀性高程度顱內動脈或大腦中動脈狹窄的缺血性卒中患者（10 例接受支架治療）在隨機分組后 2 年進行試驗。我們測量雙側大腦中動脈 CAI。ECP 之間及 ECP 時的 MCA 平均血流速度分別記錄 3 分鐘。計算 CAI 并根據梗死灶的同側及對側進行分析。

支架臨床實驗的亞組分析表明支架治療組和非支架治療組在基本臨床資料方面沒有區別。所有病人在隨機分組后都沒有卒中復發。兩組的 MCA 平均血流速度在 ECP 作用下明顯增加（$p<0.05$）。支架治療組的 CAI 明顯低於非支架治療組。（同側 3.89±2.90 versus 7.47±3.42，p=0.051；對側 3.00±4.03 versus 7.04±2.82，p=0.013）。兩組病人梗死灶側與對側的 CAI 沒有區別。

我們記錄了 ECP 治療的卒中患者的臨床資料，并在發病后 3 個月用 modified Rankin Scale（mRS）評價卒中後果。將患者分為後果較好（mRS 0~2）和後果不良（mRS 3~6）兩組。然後分析腦血流增加與 ECP 治療后卒中後果的關係。

在進行了 TCD 監測并完成 3 個月隨訪的 36 例患者中，二十一例（58.3%）有著較好的卒中結果（mRS 0~2）而 41.7%患者的後果較差（mRS 3~6）。統計分析表明入院 NIHSS 在後果良好組較低。在 ECP 過程中，梗死灶同側基線平均血流速

度和增大的平均流速在後果良好組相對較高，但沒有顯著差別。基於現有數據，我們沒有發現特定的血流動力學指數可以預測 ECP 治療後果。

我們分析威爾斯親王醫院的 ECP 數據庫，其收集了接受 ECP 治療的帶有顱內大血管阻塞的急性缺血性腦卒中患者資料。ECP 標準治療包括 35 次每天 1 小時的治療。我們收集了 155 例成功完成了至少 10 小時 ECP 治療及 3 個月隨訪的患者資料。我們將患者根據 mRS 分成後果不同的兩組，比較兩組間臨床資料及 ECP 治療時間的不同。

ECP 數據庫中至少完成了 10 次 ECP 治療及 3 個月隨訪的 155 例患者中，99 例（63.87%）在 3 個月隨訪時有較好的臨床結果。相對結果不良組，結果良好組的患者較年輕，入院時 NIHSS 評分較低，總膽固醇水平較低，入院收縮壓值較高及 ECP 的治療持續時間較長。這些患者更可能有 TIA 病史，他們由卒中發作到 ECP 治療開始的間隔也較長。多因素回歸分析表明 ECP 持續時間（OR 1.073，95%CI 1.012~1.137，p=0.018），入院 NIHSS （OR 0.744，95%CI 0.658~0.841，p<0.001）和入院收縮壓值（OR 1.024，95%CI 1.008~1.041，p=0.003）是較好結果的獨立影響因素。

我們進行了一項試驗性的研究，利用新型的頸支架進行頸動脈多普勒監測，研究頸內動脈在 ECP 作用下的血流增加作用。比較頸內動脈 CAI 和 MCA CAI，研究二者是否存在聯繫，從而檢測頸內動脈監測的可行性。

我們收集了 14 例患者進行 ICA 監測的初步試驗。他們的平均年齡是 60.93，入院 NIHSS 中位數是 3。在這個試驗中，MCA 和 ICA 的平均血流速度在 ECP 治

療時都下降。MCA 的 CAI 中位數是-5.08，ICA 的 CAI 中位數是-4.79。ECP 引起的 MCA 和 ICA 的血流增加效應沒有顯著關聯。因而， 此研究結果并不支持在顯窗不良的病人用 ICA 取代 MCA。

綜上所述，急性缺血性卒中患者在 ECP 作用下腦血流速度，在梗死灶側和對側的增加程度相似。這表明 ECP 可以同時增加梗死灶同側及對側腦血流以促進缺血區域的側枝循環。由 CAI 評價的動態增加效應與普遍認可的血管運動反應性不同。CAI 是一個獨立于血管運動反應性，衡量大腦血流增加儲備力的指標。運用 CAI 進行評價，顱內動脈粥樣硬化的支架治療長遠來說可以促進大腦自主調節功能及血流增加的儲備力。150mmHg 是增加腦血流的最佳反博壓力，更高的壓力并不會增加腦血流速度。除了廣泛認可的預後因子，如入院 NIHSS 和血壓值，ECP 治療時間是經 ECP 治療的卒中患者康復的重要影響因子。這一系列研究闡明了 ECP 對卒中病人可能有效的重要機制。

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| | |
|---|---|
| ASU | acute stroke unit |
| ACA | anterior cerebral circulation |
| BP | blood pressure |
| BHI | breathholding index |
| BMI | body mass index |
| CABG | coronary artery bypass grafting |
| CAD | coronary artery disease |
| CAI | cerebral augmentation index |
| CBF | cerebral blood flow |
| CCA | common carotid artery |
| CCS | Canadian Cardiovascular Society |
| CEA | carotid endarterectomy |
| cGMP | cyclic guanosine monophosphate |
| CI | confidence interval |
| $CO_2$ | Carbon dioxide |
| CTA | computed tomography angiography |
| DM | diabetes mellitus |
| DVT | deep vein thrombosis |
| ECG | electrocardiogram |
| ECP | external counterpulsation |
| EDV | end diastolic velocity |
| EECP | enhanced external counterpulsation |

| | |
|---|---|
| EPCs | endothelial progenitor cells |
| HPCs | haematopoietic progenitor cells |
| HT | hypertension |
| ICA | internal carotid artery |
| ICP | intracranial pressure |
| IEPR | the international EECP patient Registry |
| IHD | ischemic heart disease |
| LDL | low-density lipoprotein |
| LV | left ventricular |
| MACE | major adverse cardiovascular events |
| MCA | middle cerebral artery |
| MES | microembolic signals |
| MPa | mega Pascal |
| MRA | magnetic resonance angiography |
| MRI | magnetic resonance imaging |
| mRS | modified Rankin scale |
| MUST-EECP | the multicenter study of enhanced external counterpulsation |
| MV | mean velocity |
| NIHSS | national institute of health stroke scale |
| NO | nitric oxide |
| NYHA | New York Heart Assoication |
| OCSP | Oxfordshire Community Stroke Project |
| OR | odd ratios |

| | |
|---|---|
| $PaCO_2$ | arterial carbon dioxide tension |
| PAD | peripheral arterial disease |
| PCA | posterior cerebral circulation |
| PCI | percutaneous coronary intervention |
| PDAV | peak diastolic augmented velocity |
| PFO | patent foramen ovale |
| PSV | peak systolic velocity |
| PTCA | percutaneous transcoronary angioplasty |
| SAH | subarachoid hemorrhage |
| TCD | transcranial Doppler ultrasonography |
| TIA | transient ischemic attack |
| TOAST | the Trial of Org 10172 in Acute Stroke Treatment |
| tPA | tissue plasminogen activator |
| VEGF | vascular endothelial growth factor |

# SECTION I

# CHAPTER 1 GENERAL INTRODUCTION

Ischemic stroke is a worldwide disease with upcoming morbility as the aging of population happens. It has become the second cause of death worldwide and an important leading cause of burden of disease. [1-2]

The cerebral circulation plays an important role in the onset, development and prognosis of ischemic stroke. Cerebral hemodynamics is different from systemic circulation with unique features. Complex regulatory mechanisms in cerebral circulation allow the brain to finely regulate its blood flow to fulfill the requirement of metabolism and function.

External counterpulsation is a novel, noninvasive, highly beneficial and safe treatment for chronic coronary artery disease. As many randomized controlled clinical trials reported, ECP could reduce the angina symptoms of patients, improve their function class and exercise tolerance. [3-4] Our previous study showed ECP may help the recovery of acute ischemic stroke patients. [5]

In ECP system, there are three pairs of pneumatic cuffs wrapped around the lower extremities, including the calves, lower thighs and upper thighs (buttocks). It is triggered by ECG to inflate at the early phase of diastole and deflate at the end of diastole. The cuffs are inflated sequentially from distal to proximal during diastole. The rapid compression on the vascular bed of the lower body increases venous return and diastolic pressure. The aortic flow is retrograded and coronary circulation is also improved. The

deflation before the start of systole increases systolic uploading, which reduces heart workload and systemic resistance. Therefore it could augment cardiac output and perfusion of vital organs, such as brain, liver and kidneys. [6]

ECP has been demonstrated as an effective treatment for angina pectoris, especially for those patients with refractory stable angina or stable angina but not suitable for invasive therapy or medical management. After ECP treatment, angina pectoris patients with left ventricular dysfunction improve angina status, LV function and quality of life. ECP also exerts beneficial effects on patients with heart failure. The benefits of ECP treatment may due to its effects on augmentation of coronary flow, promotion of angiogenesis, enhancement of left ventricular function, increase of oxygen consumption, improvement of endothelial function and regression of atherosclerosis. [7]

Hemodynamic researches on effects of ECP showed coronary perfusion significantly increased during ECP as well as coronary collateral flows. [8-9] Carotid artery flow was also demonstrated to be improved by ECP, particularly with diastolic flow velocity increase. [10] ECP has augmentation effects on blood flow of renal artery, and induces flow velocity dramatically elevated from baseline. [11] Investigations of ECP on ophthalmic artery flow reported that blood flow velocity significantly increased by ECP in elderly patients with atherosclerosis. [12]

However, the hemodynamic effects of ECP on cerebral circulation are mainly unknown. ECP is a potential treatment for ischemic stroke patients. But there is limited

data of cerebral augmentation of ECP on ischemic stroke patients. We aim to explore the cerebral hemodynamic effects of ECP for patients with recent ischemic stroke. Does ECP induce the increase of cerebral blood flow in the infarct cerebral hemisphere? How about the non-affected side? Is the augmentation effect of blood flow produced by ECP similar in the mechanism of well-known cerebrovascluar reactivity or it work through cerebral autoregulation or other pathways? Can we use ECP as a new method to assess the cerebral autoregulation of patients? Since the cuff pressures on lower extremities lead to diastolic augmentation during ECP, how does the TCD waveform change responding to diastolic augmentation and what is the optimal pressure to reach the maximal augmentation effect? ECP may benefits ischemic stroke patients with large artery occlusive disease. For better outcome after ECP treatment, what could give us a clue to choose patient to perform treatment or to identify good responders? How about the effects of ECP on distal internal carotid artery rather than middle cerebral artery? Are they correlated with each other?

Therefore, the research focuses of this study were as follows:

1. To explore the changes of cerebral blood flow velocities in the infarct side as well as the contralateral side during ECP.
2. To investigate the association between hemodynamic effects of ECP and vasomotor reactivity.
3. To explore the use of ECP as a measure of cerebral autoregulation in long term follow up of patients received stenting.

4. To determine the optimal counterpulsation pressure of ECP treatment for acute ischemic stroke patients in order to achieve the maximal hemodynamic effect on the cerebral blood flow.

5. To identify promising hemodynamic parameters to predict better functional outcome for ECP-treated ischemic stroke patients.

6. To find out clinical independent predictive factors of good outcome of ischemic stroke patients under ECP treatment.

7. To explore a new method of ICA monitoring from cervical internal carotid artery to investigate cerebral hemodynamic effects of ECP

Accordingly this thesis is divided into six sections. Section I generally introduces the background information and bring forward research interests (Chapter 1); reviews the cerebral circulation, regulations of cerebral hemodynamics, TCD as a method to evaluate cerebral blood flow, brief overview of ischemic stroke, cerebral autoregulation in ischemic stroke, clinical applications and mechanisms of ECP treatment, as well as relative hemodynamic studies on ECP effects (Chapter 2); and clearly presents the main methods used in this study (Chapter 3). Section II demonstrates the cerebral blood flow changes of both sides under ECP on ischemic stroke patients (Chapter 4); investigates the cerebral hemodynamic effects induced by ECP compared with vasomotor reactivity (Chapter 5); evaluates the cerebral autoregulation of patients with large artery high-grade stenosis in stenting group as well as medical group using ECP (Chapter 6). Section III studies the optimal pressure of ECP treatment for ischemic stroke based on cerebral blood flow (Chapter 7); Section IV identifies if the hemodynamic parameters were

predictors for better functional outcome after ECP treatment (Chapter 8), and also points out some predictive factors for good response of ECP-treated ischemic stroke patients based on data of our ECP registry (Chapter 9). Section V explores a new method of cervical internal carotid artery monitoring and tests its feasibility (Chapter 10). Section VI generally discusses our findings (Chapter 11), then concludes the whole study and puts forward the directions of future works (Chapter 12).

# CHAPTER 2 LITERATURE REVIEW

## 2.1 Cerebral circulation

### 2.1.1 Brief overview of anatomy of cerebral circulation

The cerebral circulation is very important for intact brain function. Human brain receives nearly 15% of the cardiac output although it just takes around 2% of total body weight. Because of lacking substrate and oxygen stores, the brain is highly dependent on the current cerebral blood flow. Interruption of cerebral blood supply impairs neural function, and produces irreversible damage if sufficient reperfusion does not happen within a limited time period. [13]

The vasculature of brain is a complex and unique network, which specifically irrigates blood flow to various brain regions. The arterial system transports oxygenated and nutrient-rich blood to brain tissue via arteries, arterioles and capillaries. The venous drainage system removes deoxygenated blood with waste products back to heart.

**Arterial supply**

In man, the brain is supplied majorly by four arteries, two carotid and two vertebral arteries. The vertebral arteries unite intracranially to form the basilar artery, which then coalesce with both internal carotid arteries to form a complete anastomotic ring, the circle of Willis. The ring consists of three pairs of arteries, the anterior, middle and posterior arteries (ACA, MCA and PCA). Each internal carotid artery becomes ACA and MCA,

where ACA supplies blood to the frontal lobe, parietal lobe and a small part of the occipital lobe. The MCA is the major branch of the internal carotid artery, and supplies most of the lateral aspects of the cerebral hemisphere. Two PCAs come from the basilar artery and join with the posterior communicating arteries to complete the circle. Branches of the vertebral artery, basilar artery and PCA supply the cerebellum, brain stem and occipital lobe. The circle of Willis has the indispensable physiologic significance to collaterals, which arranges adequate perfusion to all parts of the brain despite the block of one part of the circle. There are considerable anatomical variations of the circle of Willis, and only 52% of healthy people to be considered with a normal circle from a dissection examine of 350 healthy brains. [14]

The measurements of intraluminal pressure in cerebral arteries have been demonstrated variable with vessel size. [15] Thirty-nine percent of aortic pressure was lost distal to the major cerebral arterial, and twenty-one percent of the pressure was lost between the major arterial to the cortical surface arterioles. Meanwhile, the cerebral circulation is devoid of precapillary sphincter, which is different with the peripheral circulation. The arterial and arteriolar segments of brain mainly regulate vascular resistance. Arterioles were observed constricted to remain blood flow constant when arterial pressure increased. [16-17] Studies by Faraci and Heistad et al. showed large arteries contributed substantially to overall cerebrovascular resistance in normal non-pathologic conditions. [18] Large arteries were the major determinants of local microvascular pressure, and humoral stimuli such as vasopressin produced selective responses of large arteries to regulate microvascular pressure.

**Venous drainage**

The cerebral venous system is a complex network, which provides the opportunities to mix the blood from various brain regions. Three groups of valveless vessels are comprised of this venous system, including the superficial cortical veins located in the pia mater, the deep or central veins draining the interior of the brain, and the venous sinuses within the dura mater.

The cerebral veins all terminate in the dural sinuses, which are endothelium-lined spaces between two layers of the dura mater. The posterosuperior group of dural sinuses contains the superior sagittal sinus, inferior sagittal sinus, straight sinus, two transverse sinuses, sigmoid sinus, and occipital sinus. The sinuses in anteroinferior group include the cavernous sinuses, superior petrosal sinus and basilar plexus. [19]

The superior sagittal sinus starts at the foramen cecum and its posterior part is attached at the border of the falx cerebri. The superior sagittal sinus mainly receives blood from superior cerebral veins, which drain the superior, lateral and medial surface of the hemisphere. The inferior sagittal sinus is located at the posterior half of the free margin of the falx cerebri, and it joins with the great cerebral vein of Galen to form the straight sinus. The inferior sagittal sinus drains the medial surface of the cerebrum through medial cerebral veins. The occipital sinus, formed by several small veins surrounding the foramen magnum, ascends in the margin of the falx cerebelli. It connects with the superior sagittal sinus and straight sinus in the confluence of sinuses (torcular herophili).

Transverse sinuses, one on each side of the cortex, begin at the occipital protuberance and travel laterally along the attached margin of the tentorium. They drain the blood from the confluence of sinuses and also receive the blood from inferior cerebral and cerebellar veins. Sigmoid sinus is the ultimate part of the transverse sinus, and ends in the internal jugular vein.

The cavernous sinuses lie on the base of the skull, and they connect with each other. They receive the ophthalmic and the middle cerebral veins, then drain blood via intracavernous and the inferior or superior petrosal sinuses into transverse sinuses.

Most of the cerebral venous return drains through the sigmoid sinuses into the internal jugular veins. A small proportion of cerebral blood empties into the cavernous sinuses, the internal jugular veins, the ophthalmic veins, and the vertebral venous plexus.

### 2.1.2 Cerebral autoregulation

Cerebral blood flow (CBF) is proportional to the cerebral perfusion pressure divided by the cerebrovascular resistance. According to Poiseuille's law, the major determinants of CBF are cerebral perfusion pressure, blood viscosity and vessel radius. Cerebral perfusion pressure is the difference between systemic blood pressure and the intracranial pressure (ICP). Blood viscosity is the internal frictional resistance of blood flow, which is mainly affected by the hematocrit and aggregation state of blood celluar components. The vessel radius is significantly related to CBF, and fine tuning of vascular diameter

responds to fluctuant perfusion pressure to maintain CBF constant via the cerebral autoregulation, as discussed below.

Cerebral autoregulation is an important regulatory mechanism that allows CBF keep relative constant within a wide range of perfusion pressure. [20] The autoregulation protects the brain against the risks of hypoxia at low perfusion pressure and brain edema at high perfusion pressure. Cerebral arteries and arterioles dilate with decreases of cerebral perfusion pressure to maintain CBF, in contrast, arteries constrict with increases of perfusion pressure. In normotensive individuals, the lower limit of CBF autoregulation is approximately at the mean arterial pressure of 50 to 60mmHg and the upper limit is around 150 to 160mmHg. [21] Between lower and upper limits, CBF is relatively constant but not absolutely stable. However, many factors may modify both limits, such as chronic arterial hypertension, arterial $CO_2$ tension, pharmacologic agents and sympathetic nerve activity. When the cerebral pressure exceeds the limits of autoregulation, the regulation mechanism is exhausted and CBF changes passively with fluctuation of perfusion pressure.

There are several hypotheses proposed to contribute to the mechanisms of cerebral autoregulation, including myogenic hypothesis, metabolic hypothesis and neurogenic hypothesis.

**Myogenic hypothesis**

Myogenic hypothesis tells that the vascular smooth muscle cells constrict or dilate to change calibers of vessels in response to the change of transmural pressure. Rapidity of

autoregulatory responses tends to support this hypothesis. [22] Responses of cerebral arteries occur within a few seconds after a change in transmural pressure. According to myogenic theory, cerebral vessels would constrict following the increase of cerebral arterial pressure. But the responses of cerebral arterioles to the increased jugular venous pressure, which leads to the increase of cerebral arterial pressure and decrease of cerebral perfusion, conversely observed vasodilation of pial arterioles but not constriction. [23] Evidences from in vitro and in vivo experiments show that endothelium plays a significant role in cerebral autoregulation, and it possibly contributes to myogenic hypothesis. The pressure induced activation of isolated cerebral arteries is suggested depend on the release of endothelium-derived contracting and relaxing factors. [24-26] The endothelium may act as a pressure transducer and also a flow transducer to modulate vascular smooth muscle tone by detection of the changes in shear stress and release of vasoactive factors. [27] However, some studies obtained in vivo oppositely suggested endothelial injury by the light-dye technique did not impair the autoregulatory responses [28] and cerebral vasodilatation preserved after inhibition of nitric oxide (NO) synthesis when arterial blood pressure reduced [29].

**Metabolic hypothesis**

Metabolic regulation suggests that CBF is controlled by the cerebral metabolism demand and the reduction of blood flow results in the release of vasoactive substances from nonvascular central nervous system cells to stimulate the dilatation of cerebral resistance arteries. Several vasoactive molecules have been proposed to relate with cerebral autoreglation, such as $H^+$, $K^+$, $Ca^{2+}$, carbon dioxide, oxygen and adenosine [30],

but their definite roles remain unclearly. Adenosine has been found to be a vasodilator with the increase of its concentration level when mean arterial blood pressure decreased. [31] Investigations by Kontos et al. showed that hypoxia was the dominant factor involved in the vasodilation associated with hypotension, possibly via the release of a vasodilator or a local reflex mediated through intrinsic nerves. [32] The observation of vasodilatation induced by increased venous pressure and reversed by local hyperoxia [33], also favors the predominant effects of metabolic regulation via an oxygen-sensitive mechanism.

Carbon dioxide ($CO_2$) is the most pronounced and consistent cerebral vasodilator. An increase of arterial carbon dioxide tension ($PaCO_2$) raises cerebral blood flow. The studies on relationship of CBF and $PaCO_2$ demonstrated it was an S-shaped curve, with minimal and maximal CBF reached at $PaCO_2$ of around 10~15mmHg and 150mmHg respectively when $PaCO_2$ varied from 5 to 418mmHg. [34] Although all cerebral vessels respond to $PaCO_2$ changes, hypercapnia dilates small cerebral arterioles more than large cerebral artery, but the vasoconstriction caused by hypocapnia is size independent. Prolonged hypercapnia and hypocapnia both reduce CBF responsiveness to acute change of $PaCO_2$. The effect of $CO_2$ on the cerebrovasculature is local, which is proposed to be mediated by extracelluar $H^+$, prostaglandins, NO and neural pathway. [35-37]

**Neurogenic hypothesis**

Neurogenic hypothesis states that the sympathetic or parasympathetic nervous system participates in the regulation of CBF. But autonomic nervous system is not the major

factor of autoregulation, because the sympathetically and parasympathetically denervated animals were shown preserved autoregulation. [38] The nervous modulation effects on autoregulation are reflected by activation of sympathetic system shifting both lower and upper limits of autoregulation to higher cerebral perfusion pressures and inhibition downward shifting both limits. [39] The intrinsic nerve fibers may exert direct influence on cerebral vascular tone thus regulate cerebral blood flow. [40]

These hypotheses may collaboratively contribute to the cerebral autoregulation rather than one mechanism independently controls everything. It is possible that one hypothesis may take the dominant role under a given particular condition.

### 2.1.3 Transcranial Doppler ultrasonography to evaluate cerebral blood flow

Transcranial Doppler ultrasound was firstly introduced into applications of cerebral hemodynamics by Aaslid R et al. in 1982 [41], and it provided a new noninvasive method to assess the cerebral blood flow. Ultrasound technology is based on its Doppler effects, which is known that a sound wave is reflected with a different frequency when it strikes a moving object. The received frequency is higher than the emitted one if the object approaches towards the sound source, whereas the received frequency is lower when the object recedes from the sound source. TCD uses this principle to measure cerebral blood flow velocity and displays as a flow velocity-time waveform. The flow velocities of intracranial artery in the circle of Willis as well as the vertebrobasilar artery could be evaluated by TCD through three acoustic windows, including transtemproal, transorbital

and transforaminal windows. With better understanding of TCD technique and development of TCD system, nowadays TCD is a helpful tool applied in the clinical practice, especially in cerebrovascular disease.

**Ischemic stroke**

In 1986, Lindegaard et al. introduced the pulsed wave 2MHz Doppler as a useful means to evaluate the patients with intracranial artery occlusive disorders. [42] Velocity criteria applied to TCD signal has been established for diagnosis of intracranial stenosis. [43] The sensitivity and specificity of TCD to detect arterial lesion are generally higher in the anterior circulation than in the posterior circulation. TCD is an important noninvasive, cheap and convenient screening tool to localize the presence of intracranial large artery occlusive disease, and it is also practical to predict clinical outcome of patients with occlusive arteries. [44]

The combination of TCD with carotid duplex could be used to identify the available candidate for thrombolysis intervention, and the ultrasound exams were shown to predict the large artery occlusive lesion with very high sensitivity and specificity. [45] In addition TCD provides real-time information to evaluate the speed and degree of artery recanalization during thrombolysis as well as valuable prognostic information. Alexandrov AV and his collaborators developed a TIBI system (Thrombolysis in Brain Ischemia) to classify the intracranial vessel residual flow after intravenous administration of tissue plasminogen activator (tPA). [46] TIBI waveforms were graded from 0 to 5 respectively as absent, minimal, blunt, dampened, stenotic and normal. The flow grades

predicted stroke severity, clinical recovery and mortality. CLOTBUST trial (combined lysis of thrombus in brain ischemia using transcranial ultrasound and systemic TPA), a phase II multicenter randomized trial, demonstrated 2-hour continuous TCD monitoring safely enhanced the tPA-induced arterial recanalization in acute ischemic stroke. [47]

**Microembolic singal detection**

Middle cerebral artery emboli, which was composed of thrombus and platelet aggregates, was detected by TCD during carotid endarterectomy (CEA) in 1990. [48] The audible, visible transient and high-pitched signals caused by microembolic within TCD frequency spectrum are called microemoblic signals (MES), or high-intensity transient signals. In patients with large artery atherosclerotic disease, carotid artery stenosis or MCA stenosis, MES independently predicts the risk of stroke, transient attack (TIA) and recurrent ischemic events. [49-50] MES becomes a surrogate marker for cerebral infarction, hence the measurement of MES is used to assess the therapeutic efficiency of antiplatelet agents. [51] The combination of clopidogrel and aspirin was recommended for prevention of stroke from recent randomized trials since the combined therapy was more effective than aspirin alone to reduce the presence and number of MES. [52-53]

TCD has been applied in intraoperative and perioperative management of surgical procedure, such as CEA, cardiopulmonary bypass and surgical repair of aortic dissection. TCD monitoring during surgery immediately detects intraoperative cerebral emboli as well as problems with shunt function. [54-55] MES has been shown associated with postoperative neurological deficits and complications. [56-57]

**Cerebral vasospasm**

Vasospasm significantly associates with mortality following subarachnoid hemorrhage. Although cerebral angiography is the gold standard in diagnosis of vasospasm, TCD offers a portable and easy method to detect vasospasm and guide clinical treatment. In 1984, Aaslid et al. evaluated cerebrovascular spasm with TCD and found there was an inverse relationship between vessel diameter and flow velocities. [58] The Lindegaard ratio is calculated as the ratio of the MCA flow velocity to the ipsilateral extracranial ICA flow velocity [59], which could be to distinguish true vasospasm from hyperdynamic state and classify the severity of vasospasm. The ratio will be high in vasospasm due to increase of flow velocities only in the intracranial vessels, whereas all vessels increase velocity in a hyperdynamic state leading to little change of the ratio. A systemic review found TCD diagnosis with flow velocity of 120cm/s for MCA vasospasm had 67% sensitivity and 99% specificity thus TCD may be used to identify patients with MCA spasm. [60] For basilar artery spasm, the ratio of BA velocity divided by extracranial VA velocity was associated with 92% sensitivity and 97% specificity for 50% or greater narrowing of basilar artery when the ratio was higher than 3.0. [61]

**Sickle cell disease**

Adams R et al. explored the use of TCD in the children with sickle cell disease, and they reported abnormal high flow velocity detected by TCD predicted the risk of ischemic stroke. [62] When the time averaged mean maximum blood flow velocity of ICA and MCA is 200cm/s or greater evaluated by TCD, it was strongly associated with the long-term risk of stroke. [63] STOP (Stroke Prevention in Sickle Cell Disease) trial

used TCD to screen children with abnormal flow velocity results, and found transfusion greatly prevented the risk of a first stroke. [64]

**Patent foramen ovale**

Patent foramen ovale (PFO) is a remnant of the fetal formen ovale and it provides a right-to-left shunt, which is a risk for stroke or TIA. [65] TCD could discover the right-to-left shunt when the injection of agitated saline induces microbubble signals on TCD spectrum. A Valsalva maneuver could increase the sensitivity of PFO detection during TCD. TCD has a high rate of concordance with transesophageal echocardiography in diagnosis of PFO and right-to-left quantification. [66]

**ICP and Brain death**

In 1987, Klingerhofer firstly described the use of TCD to detect acute change changes of intracranial pressure, which could be recorded quantitatively by means of pulsatility index and mean flow velocity. [67] TCD provides the instantaneous information of cerebral hemodynamics and has been applied in traumatic brain injury. TCD identifies brain hypoperfusion in severely brain-injured patients and instructs therapy with cerebral invasive monitoring. [68] The quantitative variables of TCD findings, such as low mean flow velocity of MCA (<40cm/s) and high pulsatility indices (>1.5), predict poor outcome of severe traumatic injury at 6 months. [69] As ICP increases, pulsatility of cerebral blood flow increases. In brain death, there is no antegrade cerebral blood flow. The typical abnormal patterns of TCD waveform in at least two intracranial arteries show absent or reversed diastolic flow and brief early systolic spikes, indicating brain death.

[70] The use of TCD to confirm brain death is shown with 100% specificity and 96.5% sensitivity. [71]

**Autoregulation**

TCD has been extensively used in studies of cerebral autoregulation due to its noninvasiveness and reproducibility of measurement on cerebral blood flow velocities. The study of cerebral autoregulation classically evaluates the changes of cerebral perfusion pressure secondary to fluctuation in systemic blood pressure. TCD studies allow estimate of the static and dynamic components of cerebral autoregulation. [72] The method to assess static autoregulation is quantification of steady-state CBF at baseline, followed by another steady-state measurement after BP manipulation. The changes of BP in the static autoregulation occur gradually, and the measured CBF is outcome of cerebral autoregulation rather than the process. TCD is used to measure the changes of cerebral flow velocity before and after BP manipulation. The single steady-state evaluation is easy to be confounded by other variabilites, such as pharmacological interventions, $CO_2$ changes, haematocrit, and so on. Developments of TCD and servo-controlled finger photoplethysmography provide technical supports to assess dynamic autoregulation, with real-time beat-to-beat information of CBF. The dynamic method induces rapid BP changes as the autoregulatory stimuli and observes the CBF alteration respond to BP. The classical thigh cuff method was introduced by Aaslid et al. to cause sudden drop of blood pressure by rapid deflation of thigh pressure cuff. [73] Other dynamic autoregulation models contain Valsalva maneuver and transient hyperaemic response test by brief compression of the ipsilateral ICA. There are other evaluation methods, like frequency

domain analysis, time domain analysis and spontaneous autoregulation, to evaluate cerebral autoregulation.

Assessment of autoregulation responding to changes of $PaCO_2$ is defined as vasomotor reactivity. The vasodilation effects of CO2 are primarily confined to the arterioles and precapillary sphincters, lightly affecting basal cerebral arterials. The CBF velocity measured by TCD is approximately proportional to CBF. Therefore, TCD is a convenient tool to evaluate cerebral vasoreactivity. The common methods to induce PaCO2 changes contain acetazolamide injection and simple breathholding test.

The estimation of cerebral autoregulation by TCD is widely applied in clinical practice, such as brain injury, subarachnoid hemorrhage, ischemic stroke, and so on. The use of TCD in cerebral autoregulation impairment after ischemic stroke will be discussed later.

## 2.2 Ischemic stroke

### 2.2.1 Epidemiology, etiology and management of ischemic stroke

Stroke is the second most common cause of death worldwide, and it causes around 9% of all death. [1] Over the past four decades, there is an increasing trend of stroke incidence in low to middle income countries with more than 100% increase, whereas in high-income countries the stroke incidence shows decrease trend with a 42% decrease. [2] In 2005, cerebrovascular disease caused an estimated 5.7 million deaths with 87% of these deaths occurring in low to middle income countries. Without intervention, the

number of global deaths is expected to rise to 6.5 million by 2015 and 7.8 million by 2030. [74]

From a report in 2001, cerebrovascular disease was the fifth leading cause of burden of disease in low- and middle-income countries and took the second place in high-income countries, evaluated by the disability-adjusted life years. [1] Stroke consumes 2~4% of total health care costs worldwide, and it accounts for more than 4% of health-care costs in industrialized countries. [75]

Strokes are divided into ischemic and haemorrhagic stroke. Ischemic stroke takes around 80% of all strokes. [76] There are many different subtypes of ischemic stroke. Based on etiology, the subtypes of ischemic stroke were categorized by the classification of TOAST (the Trial of Org 10172 in Acute Stroke Treatment) as followings: 1) large-artery atherosclerosis, 2) cardioembolism, 3) small-vessel occlusion, 4) stroke of other determined etiology, and 5) stroke of undertermined etiology. [77] Based on a study of first ischemic stroke in European population, the age-standardized incidence rates were reported following divided by ischemic stroke subtypes: cardioembolism 30.2%; small artery occlusion 25.8%; large artery atherosclerosis 15.3%. [78] In that study, the subtype of ischemic stroke according to the TOAST criteria was demonstrated as a significant predictor for long term survival.

Another classification of ischemic stroke subtypes called OCSP (Oxfordshire Community Stroke Project) is a simple clinical classification based on neurologic signs

and syndrome, which reveals important prognostic information. OCSP classification divided ischemic stroke into four subtypes, including lacunar infarct, total anterior circulation infarcts, partial anterior circulation infarcts and posterior circulation infarcts. [79-80] Subtype of total anterior circulation infarcts was found to be associated with highest mortality and frequency of complication and risk factors. The one-year mortality of patients with partial anterior or posterior circulation infarcts was much lower than that for those with total anterior circulation infarcts (about 15~20% versus 60%). The prognosis was best in the subtype of lacunar infarct. [81-82]

Primary prevention of ischemic stroke is largely attributable to the control of risk factors, which markedly reduces mortality from stroke. A recent publication of the INTERSTROKE study, a case-control study of risk factors for ischemic and intracerebral hemorrhagic stroke in 22 countries, identified ten significant risk factors for ischemic stroke, including history of hypertension, current smoking, waist-to-hip ratio, diet risk score, regular physical activity, diabetes mellitus, alcohol intake, psychosocial stress and depression, cardiac causes, and ratio of apolipoproteins B to A1. [83] All these factors account for 90% of the combined population-attributable risk of all strokes. It suggested targeted interventions of these factors, especially modification of life style, could reduce the burden of stroke.

For those who have an acute ischemic stroke, the critical treatment is early reperfusion of ischemic brain without adverse effects, such as intracranial hemorrhage. In acute phase of stroke onset within 3~4.5 hours, intravenous thrombolysis with recombinant tPA is a

safe and effective option to reduce disability caused by stroke events. [84-85] If the occlusion of major cerebral vessel is proven but intravenous thrombolysis is contraindicated, intra-arterial thrombolysis with tPA can be performed within 6 hours after stroke onset.[86] The combination of intravenous and intra-arterial thrombolysis is demonstrated to be safe and the combination improves recanalization rate. [87]

For survivors of ischemic stroke or TIA, the control of risk factors is still a major part for secondary prevention for stroke from evidence-based recommendation according to the recent guideline of American Stroke Association. [88] It recommends BP reduction, glycemic control, statin therapy, elimination of smoking and alcohol consumption, physical exercise, and so on.

Among the antiplatelet agents to treat ischemic stroke, aspirin is an effective drug in the secondary prevention of ischemic events for noncardioemoblic stroke or TIA. which was introduced in 1978. [89] The early administration of oral aspirin within 48 hours of stroke onset, significantly reduced mortality and recurrence rate in the first 4 weeks. [90] The CAPRIE study (trial of clopidogrel versus aspirin in patients at risk of ischaemic events) pointed out clopidogrel was superior to aspirin in patient with atherosclerotic vascular disease to reduce the combined risk of ischemic stroke, myocardial infarction and vascular death. [91] The use of clopidogrel plus aspirin, proved by CLAIR study (The CLopidogrel plus Aspirin for Infarction Reduction in acute stroke or transient ischaemic attack patients with large artery stenosis and microembolic signals), was more

effective than aspirin alone to reduce microembolic signals in patients with large artery stenosis. [53]

In patients with non-rheumatic atrial fibrillation, anticoagulation therapy with warfarin has been shown to effectively prevent thromboembolic complications, better than aspirin. [92] Warfarin, the oral vitamin K antagonist, is preferable to aspirin in the secondary prevention of recurrent vascular events with overall relative risk reduction of about 70%. [93] The optimal intensity of prophylactic anticoagulation is suggested as target INR of 2.0 to 3.0, since the lower level of anticoagulation significantly declines its efficacy. [94]

Prospective, randomized clinical trials demonstrated carotid endarterectomy plus medical therapy was superior to medical therapy alone in terms of reduction of stroke risk, in symptomatic patients with a at least 70% high-grade atherosclerotic carotid stenosis. [95-96] Carotid angioplasty and stenting is a therapeutic alternative to CEA to treat the extracranial carotid artery occlusive disease with features of less invasiveness, less patient discomfort and shorter recuperation time. Initial trials indicated stenting was comparable to CEA in perioperative risks (30-day stroke, death or myocardial infarction) for symptomatic surgery candidate. [97-98] The long-term effectiveness and relative procedural risks of stenting remain unclear.

### 2.2.2 Cerebral autoregulation in ischemic stroke

Markus HS pointed out in a review of "cerebral perfusion and stroke" that cerebral autoregulation may become impaired after ischemic stroke. [99] The limits of autoregulation plateau could be shifted upwards under sympathetic activation and chronic hypertension, but this protective response may turn to harmful if blood pressure excessively reduces and ischemia may happen at a relatively higher blood pressure. Impairment of cerebral autoregulation in moderate to severe ischemia may exacerbate the damage of penumbral tissue under fluctuant blood pressure. Autoregulation impairment may be crucial to the survival of ischemic penumbra.

The autoregulation impairment in ischemia stroke is hypothesized resulted by the damage to cerebral arteriole and capillaries. The obstruction of cerebral blood supply initiates a series of processes involving endothelial dysfunction and smooth muscle activation, which all may correlate with impaired autoregulation. [100]

TCD combined with continuous BP monitoring allows noninvasive beside investigation of cerebral autoregulation in stroke patients with high temporal resolution. There are many TCD studies of cerebral autoregulation in ischemic stroke with various analytic methods. Schwarz S and his collaborators found cerebral static autoregulation was impaired in acute stroke, especially in patients with large ischemic stroke, using different stimuli like induced hypertension, body position and $CO_2$ changes. [101-103] Dynamic autoregulation is demonstrated impaired after acute ischemic stroke [104-106] and even at follow-up [107], through the means of thigh cuff method, transient pressor and depressor BP stimuli and transfer function analysis. Autoregulation impairment is

also observed in minor stroke or lacunar stroke. [108-109] Autoregulation is globally impaired after ischemic stroke both in the infarct affected side and contralateral side. [106-108] The impairment appears more pronounced on the affected side of large hemispheric stroke, but seems bilaterally parallel in lacunar infarction. [102, 108]

Cerebral vasomotor reactivity was shown impaired in patients with lacunar infarction or cortical infarction as well as patients with carotid obstruction. [110] Impaired cerebral vasoreactivity was suggested as a risk marker for first-even lacunar infarction. [111] In patients with asymptomatic high-grade carotid artery stenosis (at least 70%), impaired cerebrovascular reactivity was associated with risks of ipsilateral ischemic events. [112]

Autoregulation impairment after stroke may progress and recovery as time goes by. Reinhard et al. showed autoregulation was increasingly impaired over the first five days of major ischemic stroke after unsuccessful recombinant tPA thrombolysis, mainly on the affected side. [105] They also found dynamic autoregulation did not present within 22 ± 11 hours after minor MCA stroke but slight autoregulatory disturbance may occur at the subacute stage. [113] Kwan et al. investigated dynamic cerebral autoregualtion in patients with MCA territory ischemic stroke at <7 days, 6 weeks, and 3 months after stroke, and they found the improvement of dynamic autoregulation over the first 3 months. [114]

Some clinical conditions may confound the assessment of cerebral autoregulation after stroke. In patients with chronic hypertension, dynamic autoregulation was observed impaired as well as static autoregulation. [115] Autonomic neuropathy due to diabetes

mellitus may lead to dysfunction of cerebral autoergulation. [116] In patients with large artery occlusive disease, such as stenosis or occlusion of MCA or carotid artery, cerebral autoregulation was shown impaired, and these patients had higher risk of stroke events. [117-119] Meanwhile physiological parameters may affects investigation results, such as sympathetic activation, $CO_2$ levels, cerebral venous pressure, and so on. Autoregulation impairment after stroke may be attributable to these underlying correlative factors.

The TCD technique is noninvasive, convenient and repeatable with excellent temporal resolution for studies of autoregulation. But there are some issues calling for cautions when TCD results of autoregulation assessments are interpreted: 1. Flow velocity measured by TCD is proportional to the CBF based on the assumption that the diameter of insonated artery is kept constant during exam. 2. Flow velocity of the insonated vessel by TCD represents the capacity of regional autoregulation, nevertheless the perfusion territory supplied by insonated vessel may alter under some pathological or nonpathological conditions. 3. With limited spatial resolution of ultrasound images, TCD could not allocate the area of impaired autoregulation. 4. Lack of sufficient acoustic temporal window is a restriction of TCD, whereas a considerable number of elder stroke patients have insufficient bone windows. 5. Although various methods used in autoregulation assessment are mentioned above, there is not a gold standard method established for evaluation. Therefore no available data is obtained on its accuracy and specificity when TCD is used to estimate cerebral autoregulation. [120]

## 2.3 External counterpulsation

### 2.3.1 Introduction of external counterpulsation therapy

External counterpulsation is a noninvasive, highly beneficial and effective treatment for angina pectoris. This technique contains three pairs of pneumatic cuffs wrapped around the lower extremities of patients, including the calves, lower thighs, upper thighs and buttocks (**Figure 2.1**). The system is synchronized with patients' electrocardiogram to trigger inflation of pressure on cuffs at the earlier diastole and deflation at the end of diastole. (**Figure 2.2** and **Figure 2.3**) Rapid compression of lower limbs proceeded sequentially from distal to proximal in the early diastolic phase of the cardiac cycle. The vascular bed of lower extremity is compressed and it increases the volume of venous return. The retrograde counterpulse effect of this technique increases diastolic blood pressure and aortic pressure. Since the myocardium is at rest during diastole and resistance of blood flow to coronary circulation is lowest at the same time, the augmented aortic pressure enhances coronary artery flow. Simultaneous release of pressure at the end of diastole improves systolic unloading because the vascular bed of lower extremity is relatively empty and the peripheral vascular resistance is decreased when pressure deflated. Eventually cardiac output is augmented, on the basis of increased blood flow in ventricle from venous return and reduced heart workload.

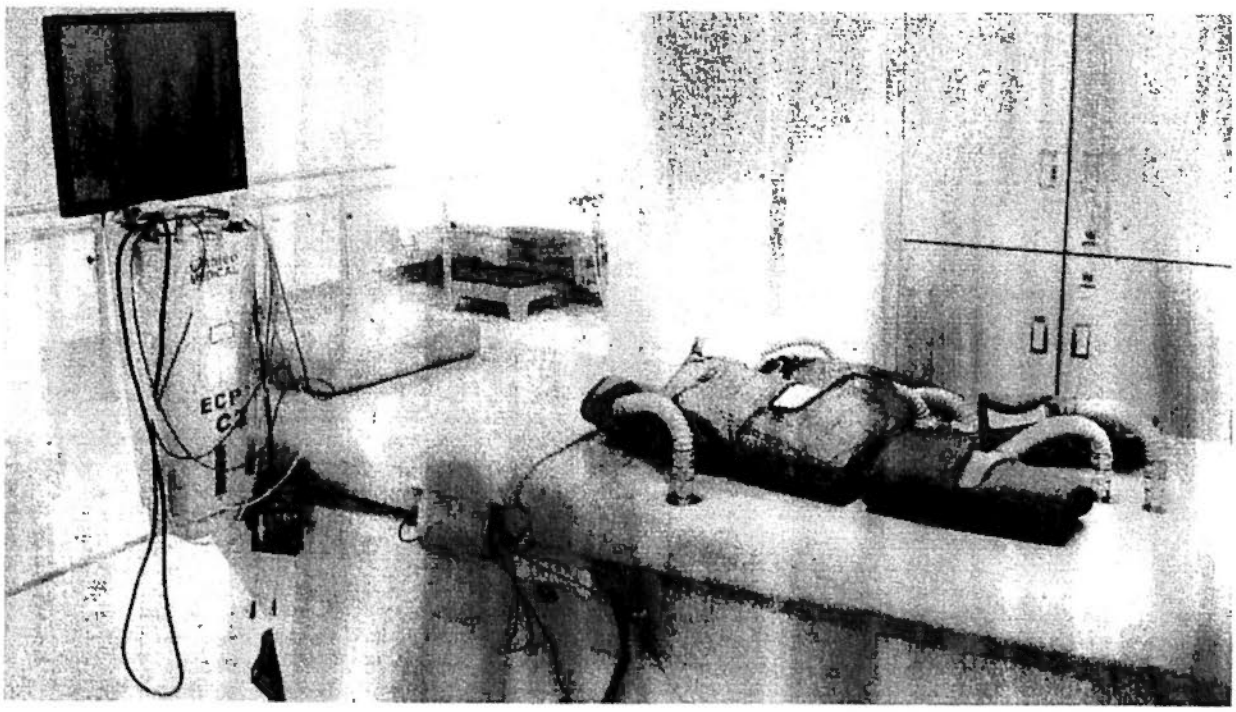


**Figure 2.1** External counterpulsation

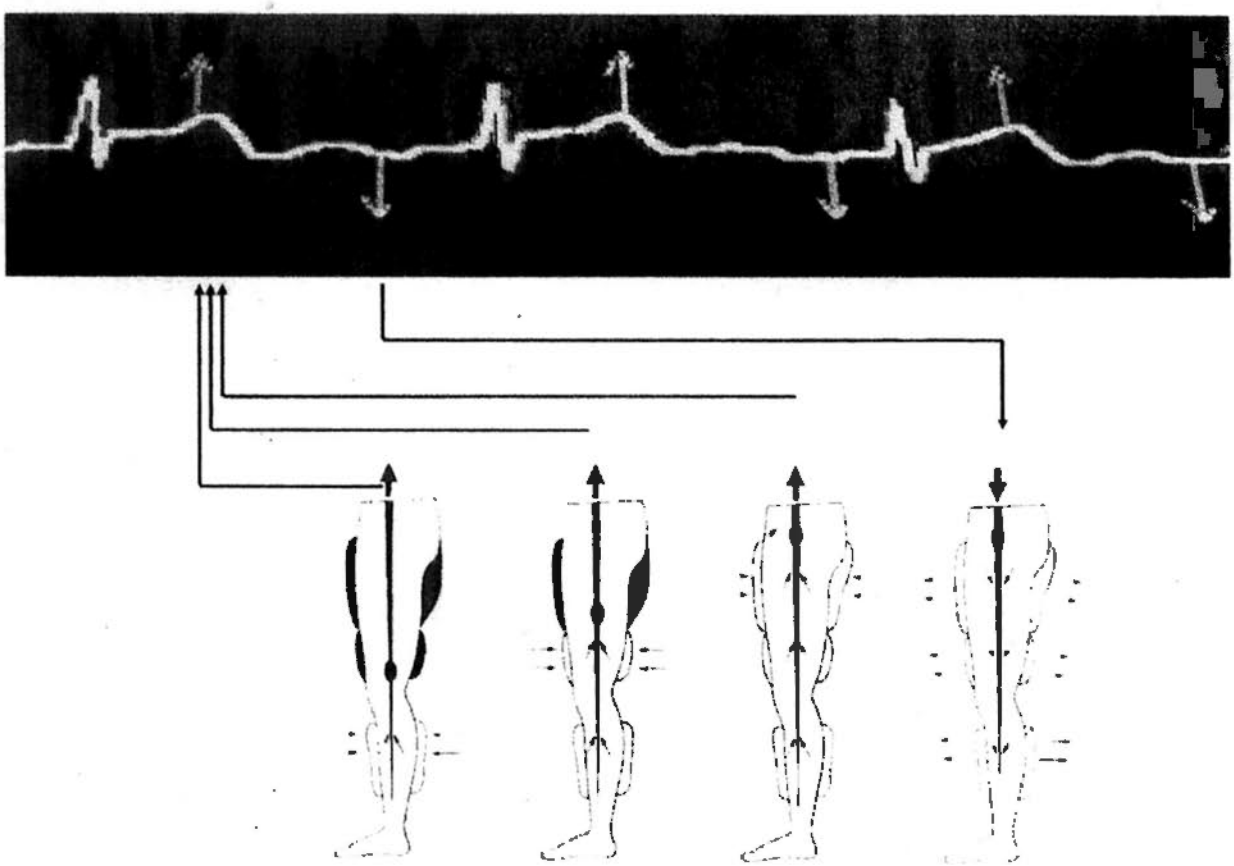


**Figure 2.2** Technique of enhanced external counterpulsation

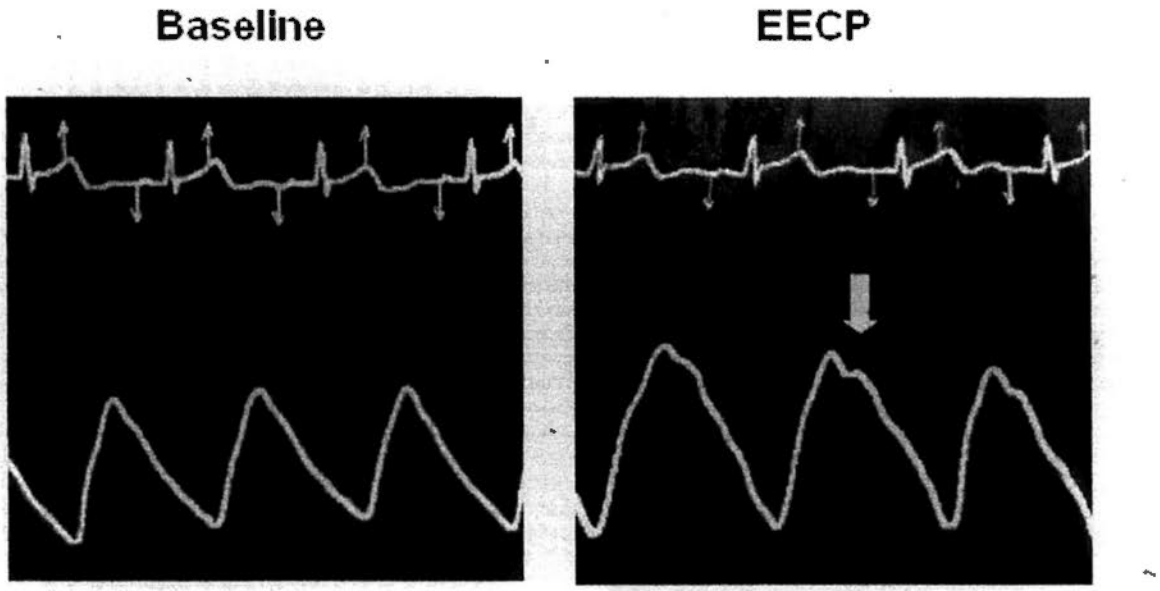


**Figure 2.3** Recording of electrocardiogram and finger plethymography before and during EECP. Wide arrow indicates the corresponding diastolic changes in finger plethymography during EECP.

**Historical perspectives and development of external counterpulsation**

In 1953, the principle of diastolic augmentation was firstly described by Kantrowitz bothers that the elevations of diastolic pressure in the arteries could improve coronary blood flow. [121] In 1958, Sarnoff et al. proposed a theory that tension-time index is an important determinant of myocardial oxygen consumption, which means heart workload is proportional to the pressure generated by the left ventricular (LV) as well as its contraction time. [122] Birtwell and his coworkers combined two concepts together to design a synchronized clinical device for external left ventricular assist. [123] In 1960s, early counterpulsation technique was developed at Harvard University by the remove of a certain blood volume from femoral artery during systole and return of them to the arterial system during diastole. Also in 1960s, three groups (Birtwell and Soroff, Dennis, and Osborne) independently developed and evolved the counterpulsation system to a non-invasive hydraulically activated external device. The use of external pulsatile pressure to the lower extremities was found to increase aortic diastolic pressure by 50 mmHg and cardiac output by 20%, based on data of 5 normal subjects. [124] This early model of external counterpulsation was effective to improve survival of patients suffering from cardiogenic shock after myocardial infarction. [125] The initial use of external counterpulsation in stable angina pectoris was evaluated by Banas et al., and it provided the evidence of symptom relief accompanied by increased myocardial vascularity. [126]

In 1968, the principle of counter pulsator with sequenced pressurization on the lower extremities was applied to advance the development of new sequential external counterpulsation. The leg sections of sequential pulsator were divided into multiple zones.

Each zone had a larger water filled bladder and a smaller air-filled bladder. All air pressure was evacuated from the entire system at the start of the QRS complex. Then at the onset of diastole air was pumped sequentially to bladders from the distal ankle to the upper thigh. Comparison between the effects of sequential and non-sequential external counterpulsation demonstrated that cardiac output was increased by 17% with sequential system while it didn't significantly change under non-sequential one, although both systems resulted in equivalent diastolic augmentation.[127]

From 1960s to 1970s, there were many experiments done in US using ECP to treat acute myocardial infarction ad cardiogenic shock. The definitive hemodynamic effects of ECP were found but the clinical benefits were uncertain. Therefore, the ECP technique failed to gain wide acceptance and application during that time.

In early of 1980s, a Chinese group lead by Zheng ZS developed a new sequential pneumatic system of external counterpulsation. In addition to balloons around the calves, lower thighs and upper thighs, a pair of counter pulsatile trousers with buttock balloons was developed. The new device was named enhanced external counterpulsation (EECP), which was proved to increase diastolic augmentation more effectively. [128]

In 1990s, there were numerous open-label studies on ischemic heart disease performed with the enhanced system in China as well as US [129-130]. Although many of these studies were not randomized or lack of control group, they actually provided the preliminary evidences of significant clinical improvement of patients with reduced

symptoms and increased exercise tolerance. From then on, the enhanced external counterpulsation emerged as an effective and noninvasive treatment for coronary artery disease.

### 2.3.2 Clinical application

**Angina pectoris**

Since 1960s, a variety of clinical trials have investigated the use of ECP in patients with angina pectoris. The first multicenter, prospective, randomized, controlled trial (The multicenter study of enhanced external counterpulsation, MUST-EECP) was reported by Arora et al in 1999. [131] This study was purposed to evaluate the safety and efficacy of EECP treatment on 139 outpatient subjects with angina, documented CAD or a positive exercise treadmill test. Subjects were randomized to receive either active (300mmHg applied pressure) or inactive (75 mmHg) counterpulsation for 35 hours of EECP sessions over 4 to 7 weeks. ST-segment depression in active group significantly improved from baseline after treatment compared with inactive group. EECP also reduced the frequency of angina and extended time to exercise-induced angina episode in patients with symptomatic CAD. The use of nitroglycerin was lower in active group but did not change in inactive group. According to the subgroup study of the MUST-EECP [132], active EECP group reported significantly greater improvement in health-related quality of life at the end of treatment and at 12-month follow up compared with controls.

From the organization in 1998, the international EECP patient Registry (IEPR) invited all centers that used EECP for treatment of patients with angina pectoris to join in. The

first report from this trial recruited 978 patients from 43 clinical centers and collected their data before the first EECP treatment and upon completion of final treatment. The result showed EECP was a safe and effective treatment for angina pectoris with 81% patients in full treatment improved at least one angina class after the last treatment. [133] Study of two-year outcomes in patients with mild refractory angina (Canadian Cardiovascular Society [CCS] class II) in IEPR [134], demonstrated that EECP significantly reduced the angina frequency, reduced nitroglycerin use and improved quality of life both in mild angina group and severe angina group (CCS III~IV). Seventy-four percent of patients with mild angina achieved a durable improvement of at least one CCS class at 2 years after treatment, as seventy percent of patients with severe angina did. The first phase of the IEPR enrolled more than 5, 000 consecutive patients and followed up them at least 3 years. The 3-year follow-up results of IEPR further confirmed EECP is a long-term effective treatment for chronic refractory angina. [4] Of 1,061 patients completed their follow-up, 78% patients had at least one CCS class improvement and 38% improved by at least two classes. The treatment benefits were sustained in 74% of the patients during follow up. This study also identified the independent predictors of unfavorable outcome, including more severe baseline angina, a history of heart failure and a history of diabetes.

If combined with heparin pretreatment on patients with stable angina, EECP significantly extended longer treadmill exercise time compared with EECP treatment alone. [135] With 5000 IU heparin pretreatment followed by EECP therapy, the index of regional myocardial oxygen metabolism markedly elevated in the ischemic region

whereas the index left unchanged in non-ischemic region, assessed by ammonia positron emission tomography.

In severe chronic angina pectoris with dobutamine-induced left ventricular wall motion abnormalities, EECP reduced the CCS class and prolonged exercise tolerance according to a study of Bagger et al. in 2004. [136] In that study, stress-induced wall motion score improved by more than two grades in 43% of the patients after EECP. Patients with improved wall motion score had higher diastolic/systolic augmentation ratio increase than the remaining patients at the end of full course of EECP.

Compared with Percutaneous Coronary Intervention (PCI) [137], EECP helped PCI candidates with stable angina to reach the comparable survival rate after one year and rates of coronary artery bypass grafting during one year, although patients in EECP group had a higher prevalence of many risk factors and lower LV ejection fraction. EECP was suggested as an alternative option for selective patients with obstructive coronary disease. In patients received percutaneous transcoronary angioplasty (PTCA), following EECP therapy has been demonstrated to improve angioplasty restenosis. [138] At 6 months after PTCA, the recurrence rate of ischemia in the PTCA related regions by scintigraphy was significantly lower in EECP group compared with that of controls (13% vs. 44%).

Another study compared effects of EECP and Spinal Cord Stimulation on patients with refractory angina pectoris [139], EECP showed more effective than Spinal Cord

Stimulation on reduction of angina CCS class. It became an alternative treatment for refractory angina patients not responding to electrical stimulation.

A recent meta-analysis of EECP in patients with chronic stable angina investigated 13 prospective studies with a total of 949 patients. The CCS angina class was reduced at least one score in 86% of the patients. The definite evidence of clinical improvement encouraged EECP to be used for refractory stable angina or stable angina but not suitable for invasive therapy or medical management.

**Angina pectoris with left ventricular dysfunction**

Soran et al. investigated 363 patients with refractory angina and LV dysfunction (LV ejection fraction < or =35%) from IEPR and followed up patients for two years. After treatment 72% of the patients improved from severe angina to mild or no angina, there was a significant decline in severity of angina class. At 2 years 55% of the patients maintained the decrease of angina class. The total survival rate after 2 years was 83% and survival rate of major adverse cardiovascular event-free (MACE-free) was 70%. Forty-three percents of patients reported no cardiac hospitalization and eighty-one percents reported no congestive heart failure. The authors concluded that for patients of refractory angina with high risk LV dysfunction, EECP was an effective and durable treatment to improve angina symptom and quality of life. These authors also found EECP substantially reduced rates of all-cause Emergency Department visits and hospitalization on this group of patients at 6-month follow up [140].

Indian experience in EECP therapy pointed out that the improvement of LV ejection fraction mainly because of reduction in end systolic volume, which suggested LV contractility improved after EECP. [141]

**Heart failure**

A large randomized controlled clinical trial titled as PEECH (the Prospective Evaluation of EECP in Congestive Heart Failure) assessed the effects of EECP in 187 patients with symptomatic but stable heart failure. [142] Subjects were randomized into EECP group (applied pressure of 300mmHg for 35 hourly EECP seesions over 7 weeks) or usual medical care group. As shown in this randomized, single-blinded study [143], more patients in EECP group increased exercise tolerance at 6 months. New York Heart Assoication (NYHA) functional class of patients in EECP group significantly improved at 1 week, 3 months and 6 months. Their quality of life determined by The Minnesota Living with Heart Failure score also improved after treatment. There was no difference found on the Peak volume of oxygen uptake between two groups. A subgroup analysis of PEECH trial confirmed the benefits of EECP in elderly patients (65 years or older) with chronic stable heart failure [144]. Additionally, the elderly EECP group had significantly higher responder rate for peak oxygen consumption, different from the findings of PEECH trial.

Lawson et al. analyzed 746 patients with heart failure from IEPR and divided them into systolic dysfunction (355 patients, mean LV ejection fraction = 26.3±6.9%) and diastolic dysfunction (391 patients, mean LV ejection fraction = 51.0±10.2%). This study compared the immediate and one-year benefits of EECP between two groups. It showed

angina classes of both group were similarly reduced after 32 hours EECP session as well as angina episodes and nitroglycerin use. The improvement was sustained at one year with comparable rate of MACE. For heart failure patients, EECP brought similar benefits either systolic or diastolic dysfunction. [145]

**Coronary artery disease-associated erectile dysfunction**

EECP has been demonstrated to improve erectile dysfunction in patients with angina pectoris. A cohort of 120 male refractory severe angina patients with erectile dysfunction from IEPR, the investigators assessed erectile dysfunction by the International Index of Erectile function scores. [146] The scores were significantly increased after 35 hours EECP treatment at the same time of improvement of angina status. There were significant elevation of intercourse satisfaction and overall satisfaction at the end of treatment. From another study of erectile dysfunction on patients with severe angina refractory to aggressive surgical and medical treatment [147], risk factors such as diabetes, hypertension, dyslipidemia, myocardial infarction and obesity, did not affect the efficacy and satisfaction of EECP treatment. However, smoking and the presence of more than two risk factors negatively influenced overall satisfaction and global efficacy of EECP. Meanwhile, patients who only received one EECP course improved better than patients with repeat therapy and patients who had less than five-year duration of IHD got higher efficacy and satisfaction rate than patients with more than five-year IHD duration. [148]

**Peripheral artery disease (PAD)**

PAD is common in patients with coronary artery disease but it used to be listed in the contraindication to external counterpulsation. A report from IEPR investigated the safety and effectiveness of ECP for PAD patients with two-year clinical outcome follow up. [149] After initial therapy, the reduction in angina CCS class and improvement of quality of life were similar in patients with and without PAD. The effects were sustained at 2-year follow-up. PAD patients stopped EECP more frequently but their lower extremity ulceration did not happen more frequently. The adverse rates (death and myocardial infarction) were higher in patient with PAD. EECP still was an effective and safe treatment for angina pectoris in patients with PAD in short-term and long-term.

**Diabetes**

According to a report of external counterpulsation on angina patients with diabetes in 2003 from IEPR [150], EECP was a safe and effective treatment option to relieve angina symptoms. Diabetic patients bore high risk of cardiovascular events and received less benefit from revascularization than patients without diabetes. In that study, 86% of patients with diabetes had been revascularized with prior PCI or coronary bypass grafting surgery and 87% of them were unsuitable for additional treatment. After EECP, 69% of those patients significantly reduced at least one CCS class as well as improved quality of life. Seventy-two percents of those patients maintained the angina class reduction after one year. The one-year mortality rate of those diabetic patients was similar with other coronary intervention patients.

**Other disease**

EECP was found to ameliorate rotational vertebrobasilar insufficiency caused by cervical spondylosis, and its effects were better if combined with traction therapy in a recent paper. [151] Three days of EECP plus traction therapy relieved 84% patients' symptoms of rotational vertebrobasilar insufficiency, much higher than EECP alone (61%) and traction alone (15%). Moreover the successful outcome maintained at 3-month follow-up. The percentage of rotational reduction of blood flow in vertebrobasilar artery in patients with EECP and traction therapy, reached highest compared with that in EECP group and traction group.

EECP accelerated the reperfusion of ischemic retinal area in patients with acute central or branch retinal artery occlusion with only 2 hours of adjunctive EECP treatment. [152] A significant increase of perfusion in the ischemic retinal areas occurred immediately after EECP but there was no reperfusion in control group with hemodilution therapy. Nevertheless, both groups observed significant perfusion increase after 48 hours without group differences.

EECP was proved to improve renal excretory function in patients with liver cirrhosis. [153] In cirrhosis patients, the mean blood pressure and concentration of arterial natriuretic peptide increased after EECP, while plasma rennin concentration decreased. EECP was associated with the improvement of urinary flow rate and the sodium and chloride excretion rates. Glomeruler filtration rate and renal plasma flow in patients did not change but vascular resistance increased during EECP.

For Restless Legs Syndrome, EECP was suggested as a novel treatment in an open-label preliminary study. [154] EECP clinically improved symptoms of Restless Legs Syndrome. The improvement lasted months even one year after completion of full course treatment.

As reported, EECP improved skin oxygenation and perfusion in healthy subjects as well as patients with coronary artery disease. [155] Transdermal oxygen pressure and concentration of moving blood cells increased during EECP, whereas transdermal carbon dioxide pressure and concentration of moving blood cells decreased. After EECP, only transdermal carbon dioxide remained reduced and other aspects returned to baseline.

**Contraindication**

There are many contraindications for EECP treatment as mentioned in the selection of patient for EECP. [156] The contraindications are followings: arrhythmias that interfere with machine triggering; decompensated heart failure (i.e. central venous pressure >7mmHg, and pulmonary edema); severe pulmonary hypertension (pulmonary artery mean pressure >50mmHg); uncontrolled systemic hypertension (>118/110mmHg); severe aortic insufficiency; severe lower extremity peripheral vascular disease with rest claudication or non-healing ischemic ulcers; aortic aneurysm requiring surgical repair; current or recent (within 2 months) lower extremity thrombophlebitis; lower extremity deep venous thrombosis; bleeding diathesis or warfarin therapy with INR ≥3.0; pregnancy.

**Adverse effects**

ECP is a non-invasive and safety therapy and well tolerated by patients, but there are some adverse effects reported. The commonest adverse effects are musculoskeletal and skin trauma, such as joint and muscle pain of legs or back, edema or swelling, skin abrasion and bruise. Wearing a tight fitting treatment pant made of stretchy, elastic material help to lessen this kind of adverse effects.

**Predictive factors related with clinical outcome**

Many papers were published on predictors of outcomes in angina patients with enhanced external counterpulsation. Immediately after EECP treatment, angina class improvement was associated with male gender, baseline severe disabling angina and no smoking history. [157] And patients with diabetes, prior bypass surgery and heart failure were more likely to be non-responders to EECP treatment. For one year outcome after EECP, Lawson et al. investigated IEPR data for factors affecting angina class reduction. [158] They found 82.7% of 2,007 patients initially responded to EECP treatment with at least one CCS class reduction. One year later, 70.6% of responders and 35.4% of initial non-responders still remained angina improvement and free of MACE. Predictors for one-year improvement were initial response, baseline angina class and no history of congestive heart failure. For two year outcome, baseline angina class was still an important factor to predict benefits according to a report from Scandinavian medical center of 86 refractory angina patients with EECP. [159] CCS class III–IV angina pectoris was more likely to benefit after treatment and sustained effectiveness at two years follow-up. Patients with CCS class II initially improved but failed to maintain

improvement after one year. Diabetes was more common in the non-responders, as well as the use of calcium channel antagonists.

Multiple vessel coronary artery disease also impacts the clinical outcome of angina pectoris after EECP. The extent of coronary artery disease was suggested negatively associated with the reversible radionuclide stress perfusion defects. [160] In patients with single vessel CAD, 95% of them improved perfusion defects after 35 hours EECP sessions. Ninety percents of double vessel CAD patients benefited reversible perfusion defects after treatment but only forty-two percents of patients with triple vessel CAD benefited. For patients with 3-vessel CAD, prior coronary artery bypass grafting (CABG) significantly increased the benefits from EECP. [161] Compared with 20% of unrevascularized triple vessel disease patients, 80% of CABG patients with triple vessel disease responded to EECP with improvement of radionuclide stress testing. However, the effectiveness of EECP was comparable in unrevascularized group and CABG group for patient with single or double vessel CAD.

Lawson et al. also analyzed 2,899 angina patients [162] for predictors of adverse outcomes and suggested that diabetes and multivessel CAD were predictors of MACE (death, myocardial infarction, CABG and PCI) during the course of EECP treatment. For high risk group (even diabetic and multivessel CAD group), the overall risk of MACE was low in mostly unrevascularizable patients with EECP. Investigation of patients with residual angina CCS class III or IV after EECP treatment in IEPR-2 [163], has revealed that those patients had more severe angina class and multivessel disease at baseline.

Furthermore, the residual high-grade angina after treatment was associated with cardiac events at three-year follow up.

McCullough et al. investigated 2,730 patients in IEPR-2 to identify the association of body mass index (BMI) and the outcome of EECP therapy. [164] Among patients with severe CAD for EECP treatment, 40.6% of them were obesity with BMI > 30kg/m$^2$. The reduction of weekly angina episodes from baseline was greater across the ascending levels of BMI. The clinical events (myocardial infarction, heart failure and death) tender to be higher across ascending levels of BMI, however, multivariate analysis showed the predictors of clinical events were older age, diabetes, history of stroke and history of heart failure but not BMI level.

For the impact of passive smoke exposure, Efstratiadis et al. reported that non-smokers with second-hand smoke benefited less angina relief after treatment compared with non-smokers without second-hand smoke. [165] The current smokers achieved the least angina relief compared with non-smokers with or without second-hand smoke. The passive smoke exposure was an independent predictor for failure of clinical improvement among non-smokers in multilogistic regression.

**Treatment duration**

The standard treatment of external counterpulsation consists of a total of 35 hourly ECP sessions, based on empiric data from studies in China. [156] Usually the treatment is performed one session a day, five times a week, for seven weeks. Treatment session

could be given twice per day to reduce total treatment time to 4 weeks as described in MUST-EECP study. [131] Patients respond well and completely tolerate to 1 or 2 hours daily treatment. However, currently there is no data showing which treatment regimen is better for a favorable outcome.

Data from the International EECP Patient Registry showed that additional extended therapy (more than 35 hours) or even repeat treatment was proved to help patients gain further symptom improvement. In IEPR-2, 75 patients (7%) extended EECP treatment with a mean of 10.3 ± 9.8 hours after completion of initial therapy. Angina class decreased further after completion of the extended course than after standard therapy. The improvement of angina severity and functional class were maintained at 6-month follow up. [156] Among 1,192 patients with stable angina pectoris, 18% of the patients repeated EECP therapy at a mean interval of 378 days after initial EECP within 2 years. [166] Seventy percent of these repeat EECP patients improved at least one CCS class and reduced nitroglycerin use at the end of repeat EECP. For those who failed to complete their initial EECP course, repeat EECP therapy showed comparable results with similar reduction of CCS class compared with those who completed initial treatment. [167] Predictors of failure to complete the initial course included female gender, heart failure, use of nitroglycerin, and the use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. And the independent predictors for those who successfully completed repeat therapy were patients stopped initial course due to clinical events and candidates for coronary artery bypass grafting at the initial course.

### 2.3.3 Mechanisms of ECP benefits

EECP has been demonstrated as a beneficial treatment for chronic angina pectoris with long term effects, but the mechanisms of its benefits are largely unknown. There are many diverse hypotheses of its mechanisms, including coronary collateral promotion, left ventricular function enhancement, endothelial function improvement, arterial stiffness reduction, atherosclerosis regression, and so on.

**Coronary flow augmentation**

Coronary collateral growth is suggested as one of the mechanisms for therapeutic effects of external counterpulsation for coronary artery disease. Myocardial perfusion of ischemic regions at rest and with dipyridamole, evaluated by 13N-ammonia positron emission tomography, has significantly increased after therapy, as well as the collateral flow reserve. [168] Michaels et al. used coronary angiography and intracoronary Doppler to assess coronary flow, and they found EECP significantly increased coronary perfusion. [8] The diastolic and mean pressure of coronary artery and aortic artery increased during therapy whereas systolic pressure decreased. It provided the evidence of coronary flow improvement combined with increased left ventricular systolic unloading. More sound evidences came from invasive collateral flow measurement in cardiac catheterization. [9, 169] The pressure-derived collateral flow index in ECP treatment group improved significantly from baseline as well as reduction of clinical CCS and NYHA class, but no change in the sham control group was found. The corresponding changes of collateral flow index were associated with flow mediated dilatation, which suggested that collateral growth was related with systemic endothelial function.

**Angiogenic factors inducement**

In a dog model of acute coronary occlusion, EECP significantly increased newly developed microvessels in the infarct regions, which were determined by alpha-actin and von Willebrand factor. The expression of systemic and local vascular endothelial growth factors (VEGF) in the dog model was markedly increased after EECP. Changes of these angiogenic factors corresponded to the improvement of myocardial perfusion. [170]

EECP in a porcine model of hypercholesterolemia [171], demonstrated its angiogenesis effects by the increase of endogenous granulocyte colony-stimulating factor. The counts of growth cytokines-mediated progenitor cells and the expression of VEGF and stromal cell derived factor 1α in myocardium were significantly increased after EECP treatment.

Research of angiogenic factors on patients with CAD revealed that the serum level of VEGF progressively increased during the course of EECP. [172] The VEGF level was higher than baseline at the 24$^{th}$ hours of EECP and reached the maximum at 3 months after therapy. In the meantime, the concentration of nitrite was significantly observed increased at 1 month and 3 months after therapy compared with baseline, although only an insignificant trend detected initially after completion of treatment.

**Left ventricular function enhancement**

Arora et al. examined 14 patients with CAD using echocardiography and performed 35 hours of EECP treatment on these subjects. [173] The systolic function, including LV ejection fraction at rest and peak dobutamine stress, significantly improved after ECP but

diastolic function didn't change. Mean resting LV ejection fraction was elevated to 52.1% after EECP compared with 47.2% at baseline, and mean peak stress LV ejection fraction increased from baseline 65.3% to 70.3% after therapy. The LV function evaluated by lung/heart ratio at stress in thallium-201 single-photon emission computed tomography, showed that EECP significantly decreased lung/heart ratio at stress of patients with CAD both at 1-month and 6-month follow up. [174] Another echocardiographic study suggested EECP could improve the left ventricular systolic and diastolic function of patients with CAD both regionally and globally. [175]

EECP may improve left ventricular systolic and diastolic function in selective patients. Estahbanaty et al. reported that patients with baseline LV ejection fraction ≤ 50%, baseline peak early diastolic transmitral flow velocity / early diastolic wave >14, baseline grade II or III diastolic dysfunction, baseline early diastolic wave < 7 cm/s and baseline systolic wave < 7cm/s were shown to reduce end-systolic volume and end-diastolic volume after EECP. The LV ejection fraction was significantly increased by EECP in these patients. On the contrary, patients with baseline LV ejection fraction > 50%, baseline peak early diastolic transmitral flow velocity / early diastolic wave <14, baseline normal diastolic function or grade I diastolic dysfunction, baseline early diastolic wave ≥ 7 cm/s and baseline systolic wave ≥ 7cm/s, didn't benefit LV function improvement from EECP.

**Oxygen consumption increase**

The consumption of oxygen at rest during EECP was reported as significantly increased compared with baseline in both CAD patients with previous coronary revascularization and healthy subjects. [176] The increase degrees were similar in patients and healthy controls. The oxygen uptake enhanced by EECP may contribute to the therapeutic effects of EECP on improvement of exercise tolerance. The elderly subgroup study of PEECH trial on patients with congestive heart failure, pointed out that more patients with EECP had peak oxygen consumption increase than controls. [144] Moreover, the percentage of patients with exercise duration extension more than 1 minute was higher with EECP treatment.

**Endothelial function improvement**

EECP exerts its clinical benefits possibly via endothelial function improvement. Bonetti et al. applied reactive hyperemia-peripheral arterial tonometry to assess peripheral endothelial function on patient with refractory angina pectoris undergoing EECP. [177] After each EECP treatment, the average reactive hyperemia-peripheral arterial tonometry index significantly increased and the increase of index was associated with EECP. At 1 month after 35 hours of EECP, the index increase was only found in patients with reduction of clinical CCS class. Hashemi et al. investigated the effects of EECP on endothelial function by flow-mediated dilation and nitroglycerine-mediated dilation measurement. [178] The flow-mediated dilation index significantly increased immediately after EECP therapy, but returned to baseline at one month follow-up. EECP was associated with the improvement of flow-mediated dilation index after treatment. Nitroglycerine-mediated dilation didn't change by EECP. A recent randomized sham-

controlled study also measured the flow-mediated dilation of the brachial and femoral arteries. [179] The peripheral artery flow-mediated dilation was significantly increased in EECP group compared with sham group. Meanwhile EECP exerted beneficial effects on endothelial-derive vasoactive agents, such as nitric oxide, endothelial-1, 6-ketoprostaglandin F1α and asymmetrical dimethylarginine.

The improvement of endothelia function induced by ECP also has been investigated via the pathway of angiogenic factors. The nitrite level only showed an increased trend without significance immediately after full course of EECP. However, the plasma level of nitrite was significantly elevated compared with baseline at one month and three months after treatment. [172] During the course of therapy, VEGF progressively increased [172, 180] and endotheline-1 levels decreased [180], moreover the releasing effects still remained at 3 months after therapy completion. Recent studies on circulating HPCs (haematopoietic progenitor cells) and EPCs (endothelial progenitor cells) provide an explanation for the lasting effects of ECP and further suggest ECP may be considered as a regenerative therapy [181]. The numbers of HPCs and EPCs were significantly increased over the ECP treatment course and these effects were maintained at follow up. meanwhile the changes of progenitor cell correlated with prolonged clinical benefits on patients with angina pectoris. [182-183]

The level of cyclic guanosine monophosphate (cGMP) was proven increased by immediately after single session of EECP. [184] cGMP is a regulator of vascular smooth muscle to relax vessels and lead to vasodilation. One hour of EECP treatment increased

plasma cGMP by 52% and platelet cGMP by 19%. CAD patients with low level of low density lipoprotein cholesterol showed particular marked increase of plasma cGMP. The authors suggested that EECP increased platelet cGMP level via activation of nitric oxide synthase after analysis of the change modulation of platelet cGMP content.

**Atherosclerosis regression**

In hypercholesterolemia porcine model, the peak diastolic arterial wall shear stress during EECP was increased markedly from baseline. [185] The intimal hyperplasia assessed by intima-to-media area ratio was significantly decreased with EECP treatment. EECP modified the expression of endothelial NO synthase and extracellular signal-regulated kinases ½ and inhibited the development of atherosclerosis. The same research team also found a mark reduction of atherosclerotic lesion size in the coronary and aortic artery of pigs receiving EECP. [186] The atherosclerosis regression effect of EECP was associated with the decrease of macrophage accumulation. The expression of proinflammatory gene were suppressed after EECP, including C-reactive protein, complement 3a, vascular cell adhesion molecule-1, inducible nitric oxide synthase, mitogen-activated protein kinase-p38 phosphyorylation and nuclear factor- κB. The authors suggested that the increased arterial wall stress by EECP reduced hypercholesterolemia-induced endothelial damage and overactivation of proinflammatory signal pathway.

In patients with symptomatic coronary artery disease, EECP decreased the circulating level of proinflammatory biomarkers. Patients with EECP treatment showed a mark

reduction of circulating levels of tumor necrosis factor-alpha and monocyte chemoattractant protein-1 after therapy. However, the relative changes were not detected in the sham group. Attenuation of chronic low-level inflammation in coronary artery disease via increased shear stress, was considered as one of the mechanisms to contribute to clinical benefits of EECP on CAD.

**Arterial stiffness reduction**

Nichols et al. investigated the properties of arterial walls and characteristics of wave reflection during EECP. [187] EECP reduced the augmentation index and increased the travel time of reflected wave. The reduced arterial stiffness decreased the left ventricular afterload and myocardial oxygen demand. The frequency of angina episode and CCS class both declined after EECP. The improvement of arterial wall properties and wave reflection provided a possible mechanism for long term effects of EECP.

### 2.3.4 Hemodynamic studies on external counterpulsation

In 1960s, the technique of external counterpulsation started to be developed as a noninvasive alternative therapy to intra-aortic balloon counterpulsation. The well known current EECP system with pneumatic cuffs was firstly described in 1983. From then on, many studies reported its safety, clinical benefits, long term efficiency and potential mechanisms. Let's have a look at the studies on hemodynamics of external counterpulsation.

ECP is a novel method used to improve the perfusion of vital organs. Werner D et al. published in 1999 that ECP lead to a significant increase in perfusion of brain, liver, kidneys and myocardium on healthy volunteer. The flow augmentation in the carotid, renal and hepatic arteries varied from 20% to 25%, and coronary arteries flow increased from 20% to 40%. Meanwhile the augmentation of flow volume is accompanied by an increase in mean arterial pressure and a downregulation of vasoconstrictive hormones (endothelin and rennin). [6]

ECP is a noninvasive, highly beneficial and well established treatment for coronary artery disease. There are many hemodynamic studies of ECP on coronary perfusion. It is believed that the ability of ECP to be an effective treatment for angina pectoris resulting from the recruitment or development of coronary collaterals. In patients with stable coronary artery disease, direct evidences of invasive coronary catheterization pointed out that pressure-derived collateral flow index significantly improved after sessions of ECP treatment accompanied by reduction of CCS and NYHA classes of patients, suggesting that ECP helps to stimulate coronary arteriogenesis and promote collateral growth. [9, 169] Coronary perfusion determined by angiographic TIMI frame count, was also shown dramatic augmentation with a 28% increase of coronary flow during ECP, while the intracoronary Doppler measurement of average peak velocity increased 109% from baseline [8]. Nitrogen-13 (13N) ammonia positron emission tomography study demonstrated myocardial perfusion significantly improved at rest and with dipyridamole as well as coronary flow reserve after therapy. [168] Interestingly, myocardial perfusion in regions with CAD significantly increased after EECP both at rest and with

dipyridamole. However, there was no perfusion difference after EECP found in regions without CAD. The increased exercise tolerance and nitric oxide levels were associated with EECP in this study.

Data on cerebral circulation under ECP was rare and inconclusive. Two studies recorded MCA blood flow under ECP, and suggested that ECP did not increase mean CBF velocity in healthy subjects even though blood waveform and diastolic velocity markedly changed. [188-189] However, ECP was reported to significantly augment mean MCA flow velocity on both sides in 5 healthy subjects at 5 and 20 minutes (Right MCA velocity median: baseline 48 cm/s vs. 5 minutes 58 cm/s and 20 minutes 61 cm/s; Left MCA velocity median: baseline 55 cm/s vs. 5 minutes 67 cm/s and 20 minutes 68 cm/s) using a different methodology.[190] The peak diastolic velocities of both MCAs were also dramatically higher than baseline end diastolic velocities. Our previous randomized controlled study of ischemic stroke patient with large artery occlusive disease, evaluated cerebral blood flow by color velocity imaging quantification. CBF changes tended to increase more with ECP therapy (48.6±146.7 vs. 13.9±110.1) although no significance was detected. [5]

There is an important mechanism regulating hemodynamics of cerebral circulation, cerebral autoregulation, which also strongly influences ECP effects on cerebral blood flow. Cerebral autoregulation is a protective mechanism of cerebral circulation regulation, and it ensures the constancy of cerebral blood flow supply under fluctuant blood pressure. Research of ECP effects on dynamic cerebral autoregulation showed ECP does not

compromise cerebral autoregulation either in elder patients with atherosclerosis or in young healthy subjects. [191] The transfer function gain and phase shift between mean blood pressure and mean cerebral blood flow velocity remained stable during EECP in both groups although ECP induces marked systemic changes. Impaired cerebral autoregulation may play a critical role in the pathways to mediate increased cerebral blood flow induced by ECP, but its specific role needs further investigations and more evidences.

Research on patients with atherosclerotic heart disease provided the evidence of external counterpulsation on carotid flow, suggesting mean carotid flow velocity integral increased by 22% during ECP with peak carotid diastolic flow velocity 75% as high as the systolic wave. [11] Levenson J et al. also investigated carotid circulation during ECP therapy in patients with coronary artery disease. The blood flow of carotid artery increased from baseline during three timepoints of EECP treatment, including 1 hour, 17 hours and 35 hours. The vascular resistance of carotid artery reduced from baseline at all three timepoints in active EECP group. They found that the reduced arterial stiffness and resistance of carotid circulation was probably due to increased regional blood flow. [10]

ECP also exerts impacts on ocular perfusion in elderly patients with atherosclerosis, which significantly increased ophthalmic artery blood flow velocity by 11.4% but no change in young healthy subjects. [12] Patients with central retinal artery occlusion or branch retinal artery occlusion increased reperfusion in ischemic retinal areas from baseline 57±19 arbitrary units to after ECP treatment 99±14 arbitrary units, proved by

laser Doppler flowmetry scan. [152] Ophthalmic artery is a key collateral pathway between intracranial and extracranial circulation especially when internal carotid artery occludes or high grade stenosis exists, therefore studies on ophthalmic artery help to have an insight into ECP effects to cerebral circulation.

Kidney is the other organ with autoregulation mechanism in human body, where renal blood flow and glomerular filtration rate remain relative constant within a range of blood pressure. [192] ECP also has augmentation effects on renal artery blood flow. Applebaum et al. reported in 1997 that the mean renal artery flow velocity integral increased 19% during sequential ECP. [11] The diastolic wave of renal artery blood flow was increased 68% as high as systolic wave under ECP, and the systolic wave increased 8% from baseline. Werner D. and his group investigated renal function and renal plasma flow in patients with liver cirrhosis as well as healthy controls. [153] As their results shown, glomerular filtration rate and renal plasma flow significantly increased on healthy subjects during EECP but didn't change on patients. Renal vascular resistance increased by 20% in cirrhosis group but kept unchanged in controls. However, both two groups demonstrated the improvement of renal excretory function after EECP. They found that EECP did not influence the vasoconstrictive dysfunction of the kidneys in patients with liver cirrhosis.

There were two reports on ECP and peripheral circulation, both on patients with coronary artery disease. As reported, averaged flow volume of the posterior tibial artery decreased to 69% during ECP and increased to 133% of baseline 1 hour after ECP, but

average flow volume of the brachial artery increased 9% during ECP and returned to baseline values after ECP. [193] ECP increased flow-mediated dilation of brachial and femoral arteries respectively by 51% and 30% from ultrasound exam, and the endothelial-derived vasoactive agents improved as well. [179] Both studies support that one of extracardiac effects of ECP is the improvement of endothelial function by reactive hyperemia-peripheral arterial tonometry.

Investigations on hemodynamics under EECP in patients with acute myocardial infarction demonstrated that right atrial pressure and pulmonary capillary wedge pressure significantly increased during EECP and after one hour EECP treatment. [194] Cardiac index was also significantly elevated during EECP. Heart rate changes were not observed following EECP treatment. The concentration of blood atrial natriuretic peptide markedly increased but brain natriuretic peptide did not. The time- or frequency-domain heart rate variability didn't change after EECP. [195] The increase of low frequency heart rate variability in diabetic patients among the angina cohort was associated with the reduced mortality.

For effects of EECP on blood pressure, the systolic blood pressures of patients with EECP were improved after treatment. [196] The decrease of blood pressure was sustained at 6 weeks after the last session of EECP. No significant changes were observed in diastolic blood pressure as well as heart rate. Interestingly, after stratified by baseline systolic blood pressure, it showed EECP increased systolic blood pressure for patients

with low baseline value (<110mmHg). The stratified differences were independent of cardiovascular medication changes.

Investigations on hemodynamic benefits of external counterpulsation are interesting but mainly unknown. Previous findings were mostly focused on ECP hemodynamic effects on angina patients. Suresh K et al. studied EECP effective ratio in order to reach maximal therapeutic effect in 1998. [197] The EECP effective ratio was calculated by the relative magnitude of diastolic augmentation (DA) and systolic unloading (SU) assessed by finger plethysmography. They suggested that hemodynamic effects were optimal when cuff pr sures caused DA/SU in the range of 1.5-2.0 since systolic flow maximized at ratio of 1.5 and diastolic flow at 2.0. Do the patients have better response to ECP treatment with special hemodynamic pattern or patients achieved higher DA ratio derive better clinical benefits? In the IEPR, patients with higher DA ratio ( 1.5) trended to have a greater reduction in angina class at 6-month follow up (p 0.069), although there were no significant difference between high and low DA ratio groups at the end of therapy in terms of myocardial infarction, revascularization rates and nitroglycerin use. [198] The lower DA ratio group had a higher rate of unstable angina and congestive heart failure. The predictors for achieving higher DA ratio were young age, male, nonsmoking and without multivessel coronary or noncardiac vascular disease. Another study of IEPR demonstrated patients received ECP increased DA ratio from 0.7 to 1.0 from the beginning to the end of treatment, and those had the greatest increase in the DA ratio had the greatest reduction in angina class. [199]

In Summary, external counterpulsation exerts strong effects on systemic and orangic hemodynamics. ECP is a new treatment for ischemic stroke, and there are many veiled mysteries of hemodynamic effects of ECP on cerebral blood flow, such as optimal treatment pressure, maximal hemodynamic effect, hemodynamic parameter predictors on clinical outcome after treatment, and so on. It is important to research hemodynamic effects of ECP on cerebral circulation and instruct its clinical application on ischemic stroke in the future.

# CHAPTER 3 MATERIALS AND METHODS

The chapter introduced the materials and methods commonly used in the experiments of the study, including subjects, EECP, TCD monitoring and systemic parameters.

**Subjects**

In most experiments, we recruited patients with recent ischemic stroke as subjects. These patients were hospitalized into Acute Stroke Unit in Prince of Wales Hospital, The Chinese University of Hong Kong. Patients presented with neurological deficits as a result of stroke when examined. They were verified with cerebral large artery occlusive disease using TCD, MRA, CTA or DSA. The clinical characteristics of subjects were documented for analysis, including demographics, medical history, medication, and so on. The study was approved by the local medical ethics committee (Joint CUHK-NTEC Clinical Research Ethics Committee). All subjects gave informed consents and agreed to join the study.

The exclusion criteria of patient enrollment contained followings: brain CT showed evidence of intracranial hemorrhage; history of intracranial hemorrhage; cardioembolic stroke such as atrial fibrillation and rheumatic heart disease; stroke onset relevant pontine infarct or medullary infarct; evidence of arteriovenous malformation, arterial fistula or aneurysm; sustained hypertension (systolic>180mmHg or diastolic>100mmHg); co-existing systemic disease, such as renal failure, cirrhosis, severe dementia or psychosis;

brain tumor or other significant non-ischemia brain lesion on CT; thrombocytopenia (platelet count <100,000/mm$^3$); pregnancy.

**EECP**

EECP treatment was performed using the Enhanced External Counterpulsation system, model number MC2 or MC3 (Vamed Medical Instrument Company device, Foshan, China). Usually the treatment was given one hour daily. Subjects were instructed to lie on the EECP treatment bed then leg cuffs were wrapped around lower extremities (the calves, lower thighs, upper thighs and buttocks). The ECG was connected to synchronize and trigger EECP system. The cuff inflation pressure for treatment varied from 150 to 262.5mmHg.

**TCD monitoring**

TCD monitoring was applied to evaluate the hemodynamic effects of EECP on cerebral circulation. We used the ST3 Transcranial Doppler system (Spencer Technologies, Seattle, USA) or SONARA TCD system (BioBeat Medical Limited, California, USA) to perform examination. The subject was asked to lie on the EECP treatment bed and wear a head frame with two 2 MHz probes mounted. Bilateral M1 segments of MCAs were insonated at the depth of highest mean flow velocity between 50 to 60 mm. The blood flow velocity changes during the whole examination were recorded. Cerebral augmentation index CAI was used to evaluate the augmentation effect of ECP on cerebral circulation, calculated by the increase percentage of mean flow velocity during ECP compared with baseline.

**Systemic parameters**

At the same time of TCD monitoring, we recorded the systemic parameters during exam, such as heart rate, brachial blood pressure, continuous blood pressure, and so on. We used Task Force Monitor system (CNSystems Medizintechnik AG, Graz, Austria) to assist recording and analysis of systemic data. TCD was also connected to this system, and the TCD data was automatically recorded by the system.

# SECTION II

# CHAPTER 4 FLOW VELOCITIES INCREASE BY THE SAME EXTENT ON BOTH SIDES OF ISCHEMIC STROKE PATIENTS DURING EXTERNAL COUNTERPULSATION

## 4.1 Background

Enhanced external counterpulsation is a noninvasive, highly beneficial and long term effective treatment for ischemic heart disease. The technique of ECP has been demonstrated to improve the perfusion of vital organs through diastolic augmentation. For patients with coronary artery disease, ECP treatment helped to reduce the angina symptom, extend the exercise tolerance and improve quality of life with sustained effects in the long term. [131, 134] The augmentation of coronary perfusion or promotion of coronary collaterals may contribute to clinical benefits of ECP. The myocardial perfusion in the ischemic regions, assessed by Nitrogen-13 ammonia positron emission tomography, markedly increased after ECP. [168] Studies of invasive cardiac catheterization suggested that coronary collateral flow improved after ECP treatment. [9, 169]

Our previous randomized controlled study of ischemic stroke patient with large artery occlusive disease showed ECP treatment was significantly associated with favorable clinical improvement of neurological deficits. The changes of cerebral blood flow evaluated by color velocity imaging quantification tended to increase more with ECP therapy. [5] The hemodynamic effects of external counterpulsation on cerebral circulation are largely unknown. The conclusions of published data studied the effects of ECP on cerebral blood flow were controversial. The mean blood flow velocity of MCA

during ECP did not differ from baseline in healthy subjects as well as patients with atherosclerosis. [188-189] However, ECP was shown to significantly augment mean flow velocity and peak diastolic velocities of bilateral MCAs in 5 healthy subjects using a different methodology. [190] Hemodynamics of ECP on ischemic stroke patients remains unclear. We aim to explore cerebral hemodynamic changes under ECP on patients with recent ischemic stroke.

## 4.2 Methods

### 4.2.1 Subjects

We recruited ischemic stroke patients with large artery occlusive disease into this study. These patients received ECP as adjunctive treatment of conventional medical therapy and had good temporal window for TCD monitoring. The exclusion criteria were mentioned above in Chapter 3. Since the previous findings were inconsistent with two different analysis methods of cerebral blood flow velocity data, we recruited two groups of patients to conduct investigations with two data interpretation methods. There were 32 patients with recent ischemic stroke due to large artery disease in group 1 and another 30 ischemic stroke patients in group 2. We also recruited 20 elderly healthy without cerebrovascular events and risk factors as controls.

### 4.2.2 ECP and TCD monitoring

All subjects were performed TCD monitoring at their first ECP session, and ECP was performed using the Enhanced External Counterpulsation system, model number MC2 (Vamed Medical Instrument Company device, Foshan, China). The treatment pressure of

ECP was 150mmHg. ST3 Transcranial Doppler system (Spencer Technologies, Seattle, USA) was used to monitor blood flow velocities of bilateral MCAs. Two 2 MHz probes mounted on a head frame were fixed on bilateral temporal windows. M1 segments of MCA were insonated at the depth of highest mean velocity (MV) between 50 to 60mm. We recorded blood flow velocity of MCAs before and during ECP respectively for 3 minutes. (**Figure 4.1**) The physiological correlates associated with ECP-TCD waveform morphology were identified.

4.2.3 Data interpretation

We interpreted TCD data of stroke patients with two different methods. For subjects in group 1, mean flow velocity was automatically recorded by TCD system, which was the mean value of area under the envelope curve in a cardiac cycle beat. In group 2, TCD parameters were manually read at first 4 beats of each minute. We recorded the value of peak systolic velocity PSV, peak diastolic augmentation velocity PDAV and end diastolic velocity EDV. The mean flow velocity of group 2 was calculated as (PSV+2*EDV)/3 at baseline and (PSV+PDAV+EDV)/3 during ECP as previously described. [200] All data were analyzed based on whether it was ipsilateral to the infarct side or the contralateral side. In the control group, we recorded the mean flow velocity automatically from TCD system. Cerebral augmentation index CAI was calculated by the increase percentage of mean flow velocity during ECP compared with baseline. Significance level was inferred at $p<0.05$.

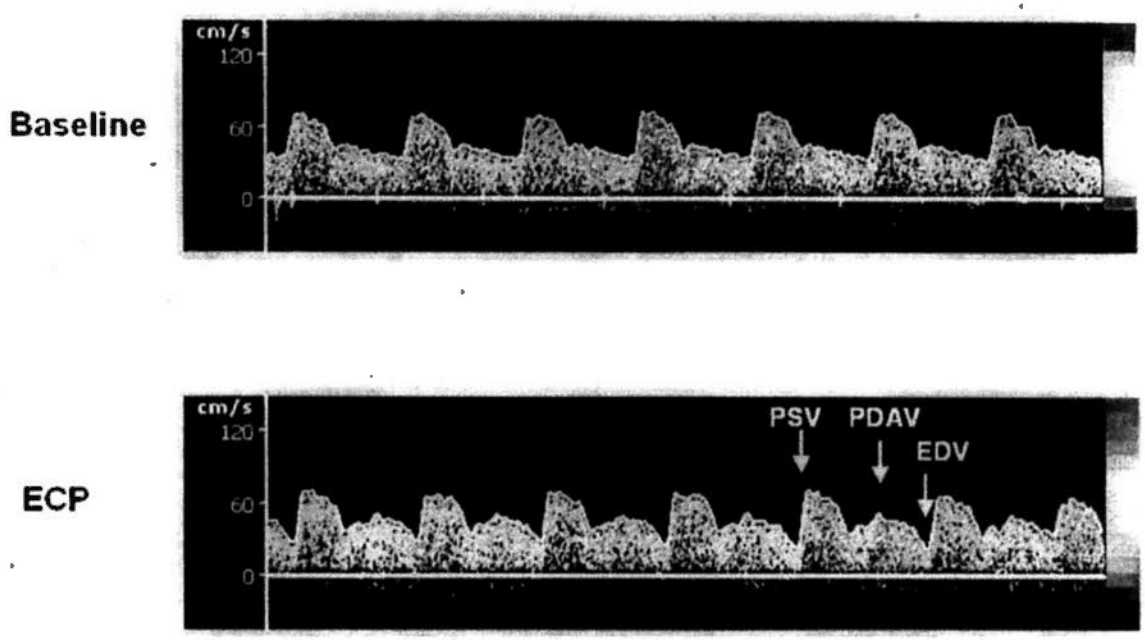


**Figure 4.1** TCD waveform of MCA blood flow at baseline and during ECP. EDV, end diastolic velocity; PDAV, peak diastolic augmentation velocity; PSV, peak systolic velocity.

### 4.3 Results

The healthy controls were younger than stroke patients and had more female subjects. Stroke patients in group 1 and group 2 were similar in age, interval of stroke onset to start of ECP and distribution of cerebrovascular risk factors (**Table 4.1**). The age of stroke patients was around 68 years old, and mean interval of stroke onset to exam was around 6 days. Stroke patients both had moderate neurological deficits according to admission NIHSS, but admission NIHSS scores in group 2 stroke patients was higher. Comparison between two groups of stroke patients, also found that group 2 had less patients with dyslipidemia and baseline systolic BP of patients in group 2 was relatively higher.

For stroke patients group 1, mean flow velocity significantly increased after ECP on both sides compared with baseline (ipsilateral side 9.62% and contralateral side 9.57%, both $p<0.001$, **Table 4.2**). CAIs between infarct ipsilateral side and contralateral side were similar. Similarly in group 2, mean flow velocities during ECP increased significantly on ipsilateral side (18.49%) and contralateral side (18.93%) when compared with baseline, but there was no increase difference between two sides when compared with each other as well. Peak diastolic augmentation velocities significantly increased on ipsilateral side (78.70%) and contralateral side (93.10%) compared with baseline end diastolic velocity. No peak diastolic increase difference was found between two sides. Peak systolic velocity and end diastolic velocity didn't show significant change during ECP. (**Table 4.3**) However, MCA mean flow velocity of elderly controls did not change during ECP on left side or right side.

**Table 4.1** Characteristics of subjects

| | Group1 Patients (32) | Group 2 Patients (30) | Controls (20) | P value # |
|---|---|---|---|---|
| Age (year) | 68.84 ± 10.82 | 68.6 ± 9.45 | 60.30 ± 6.57 | 0.919 |
| Gender, Male (n, %) | 31, 96.9% | 26, 86.7% | 10, 50% | 0.189 |
| Hypertension (n, %) | 25, 78.1% | 25, 83.3% | | 0.604 |
| Diabetes Mellitus (n, %) | 15, 46.9% | 14, 46.7% | | 0.987 |
| Dyslipidaemia (n, %) | 19, 59.4% | 9, 30% | | 0.020* |
| History of IHD (n, %) | 4, 12.5% | 4, 13.3% | | 1.000 |
| Previous CVA history (n, %) | 10, 31.2% | 15, 50% | | 0.133 |
| Smoking (n, %) | 12, 37.5% | 9, 30% | | 0.533 |
| Alcoholism (n, %) | 6, 18.8% | 1, 3.3% | | 0.105 |
| Interval of Stroke onset to exam (day) | 6.18 ± 5.41 | 6.41 ± 4.30 | | 0.876 |
| NIHSS on admission | 4 (0~17) | 6 (2~12) | | 0.012* |
| Left side infarct (n, %) | 16, 50% | 11, 36.7% | | 0.290 |
| Baseline systolic BP (mmHg) | 128.55 ± 17.80 | 143.19 ± 19.85 | | 0.010* |
| Baseline diastolic BP (mmHg) | 86.16 ± 15.58 | 87.04 ± 11.82 | | 0.824 |

Notes: # stroke patients in group 1 vs. group 2. * $p<0.05$. Ages of three groups were analyzed using ANOVA, $p=0.004$.

**Table 4.2** Blood flow velocity changes of group 1 and control group on both sides

| Velocity | Baseline (cm/s) | ECP (cm/s) | CAI (%) | P value |
|---|---|---|---|---|
| Stroke ipsilateral | 49.93±18.94 | 54.37±20.61 | 9.62±8.81 | <0.001* |
| Stroke contralateral | 52.87±20.25 | 58.07±23.07 | 9.57±7.71 | <0.001* |
| Stroke ipsilateral CAI vs. contralateral CAI | | | | 0.688 |
| Control | | | -0.47±2.89 | |
| Control L side | 60.88±13.61 | 60.50±12.86 | -0.59±3.35 | 0.435 |
| Control R side | 54.16±11.84 | 53.72±11.24 | -0.35±3.30 | 0.328 |

Notes: Using Paired-T test to compare baseline and ECP. *, baseline vs. ECP, $p<0.05$.

**Table 4.3** Blood flow velocity changes of group 2 on both sides

| Velocity (cm/s) | Baseline | ECP | Increase percentage | P value |
|---|---|---|---|---|
| Ipsilateral | | | | |
| Mean velocity | 58.32 ± 20.77 | 68.43 ± 23.69 | 18.49 ± 15.56 | <0.001* |
| Peak systolic velocity | 96.35 ± 33.19 | 99.44 ± 34.50 | 3.60 ± 10.98 | 0.313 |
| End diastolic velocity | 39.31 ± 16.06 | 38.41 ± 15.65 | -1.35 ± 14.24 | 0.294 |
| Peak diastolic augmentation velocity | | 67.46 ± 24.14 | 78.70 ± 45.03 | <0.001* |
| Contralateral | | | | |
| Mean velocity | 50.69± 18.05 | 59.87 ± 20.29 | 18.93 ± 12.44 | <0.001* |
| Peak systolic velocity | 86.60 ± 29.93 | 89.28 ± 30.24 | 3.48 ± 9.02 | 0.056 |
| End diastolic velocity | 32.74 ± 13.39 | 32.79 ± 13.60 | 0.94 ± 12.95 | 0.926 |
| Peak diastolic augmentation velocity | | 57.54± 19.74 | 93.10 ± 42.00 | <0.001* |
| Ipsilateral CAI vs. Contralateral CAI | | | | 0.288 |
| Peak diastolic augmentation velocity increase percentage on ipsilateral side vs. that of contralateral side | | | | 0.061 |

Notes: Wilcoxon Signed Ranks Test was used to compare the velocity change at baseline and ECP. *, baseline vs. ECP, $p<0.05$.

**4.4 Discussion**

The results of two TCD data interpretation methods coincidentally point out ECP induces the increases of mean MCA blood flow velocities on cerebral both sides of ischemic stroke patients. However, it does not change cerebral blood flow of healthy controls even tends to decrease mean blood flow velocity based on our findings. It is consistent with previous finding [188-189], that ECP does not augment cerebral blood flow in the healthy brain. The distinct responses to ECP between stroke patients and healthy controls may attribute to cerebral autoregulation. The autoregulation of cerebral circulation ensures the constancy of cerebral blood flow under fluctuant cerebral perfusion pressure. Whereas the autoregulation is impaired after stroke [99], and it is hypothesized to caused by damage of cerebral arterioles and capillaries during ischemia or other chronic illness, such as malignant hypertension. [115] Cerebral blood flow of ischemic stroke patient increases during ECP possibly via impaired cerebral autoregulation. Peak diastolic augmentation velocity significantly increases during ECP but not peak systolic velocity and end diastolic velocity. It suggests cerebral blood flow is mainly improved by the diastolic augmentation of ECP.

The CAIs of stroke patients are similar on infract ipsilateral side and contralateral side either in group 1 or in group 2. There is no increase difference of mean MCA flow velocity between the two sides when compared with each other, as well as the increase extent of peak diastolic augmentation velocity. It indicates the level of cerebral blood flow augmentation during ECP in recent stroke patients seems the same on both sides. It is consisted with the finding that cerebral autoregulation is globally impaired on the

ischemia affected side and non-affected side after ischemic stroke [106-107] The presence and extent of collateral circulation affects the reperfusion of ischemia and is associated with clinical prognosis after acute ischemic stroke. [201-202] Our results suggest that potentially circulation may be enhanced by ECP to improve the collateral blood supply of ischemic territories not only from the infarct ipsilateral side but also from contralateral side.

From **Figure 4.2**, the mean CAI of group 2 is higher than that of stroke patients in group 1. Although there are some differences on clinical characteristics of two stroke groups, the increase percentage difference between two groups is partially related to the methods used to interpret TCD data. The Manually reading method may enlarge the augmentation effect of ECP on cerebral blood flow. It may provide an explanation why manually reading detects cerebral augmentation of ECP on healthy subjects but other studies do not. [200]

There are several limitations in this study. The sample sizes are all relative small in all three groups. The age and gender differences of controls compared with stroke patients may partially contribute to their distinct hemodynamic responses to ECP, although we believe they are not the major reasons. It needs further study with age and gender matched control group to confirm this finding.

In summary, we investigate the cerebral blood flow changes during ECP in stroke patients as well as elderly controls. ECP equally induces cerebral blood flow increase on

both sides even using different data interpretation methods. These findings suggest that potentially circulation may be enhanced by ECP to improve the collateral blood supply of ischemic territories both from the infarct ipsilateral side and contralateral side.

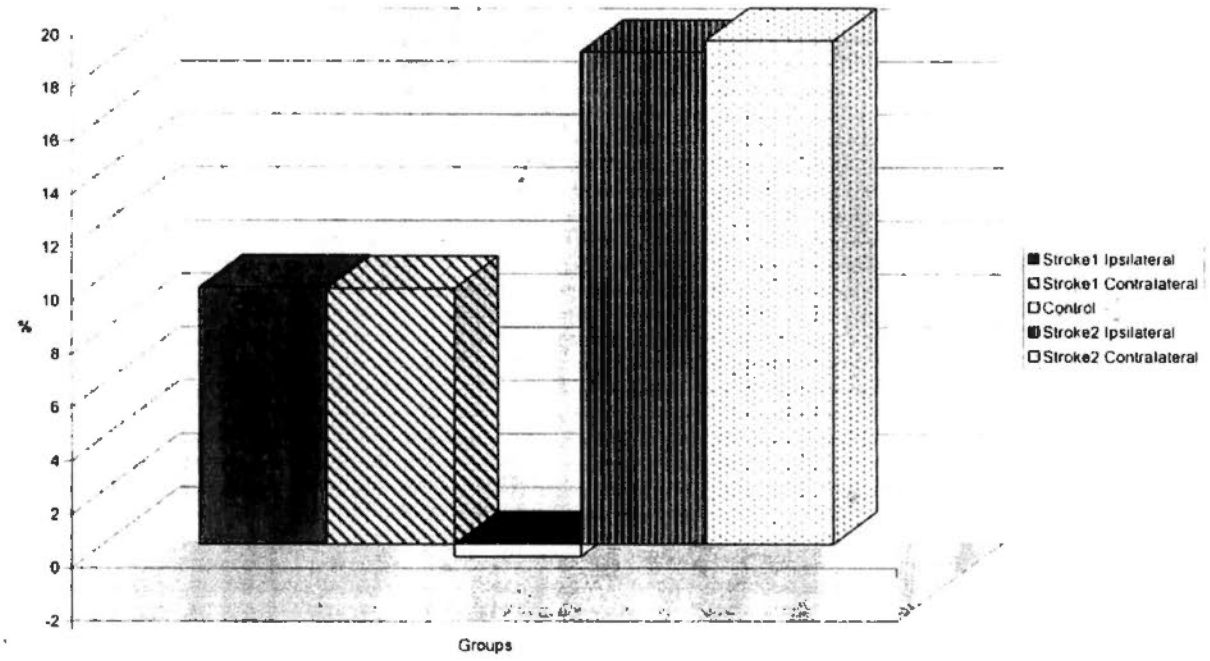


**Figure 4.2** Mean flow velocity increase percentages during ECP

# SECTION III

# CHAPTER 5 HEMODYNAMIC EFFECTS OF EXTERNAL COUNTERPULSATION ON CEREBRAL CIRCULATION IS A DIFFERENT MEASURE OF IMPAIRED CEREBRAL AUTOREGULATION FROM VASOMOTOR REACTIVITY

## 5.1 Background

External counterpulsation is a noninvasive, highly beneficial and long term effective treatment for ischemic heart disease. There are three pairs of pneumonic cuffs applied to the calves, lower thighs, and upper thighs (buttocks) in the enhanced external counterpulsation system. ECG triggers cuff inflation sequentially from distal to proximal during diastole and releases cuff pressure before the start of systole. ECP has been demonstrated to improve the perfusion of vital organs through diastolic augmentation [6], and our previous finding showed cerebral blood flow velocities of ischemic stroke patients significantly increased during ECP as described in Chapter 4. The cerebral hemodynamic augmentation effects induced by ECP in ischemic stroke patients possibly work via impaired cerebral autoregulation. The hemodynamic effect during ECP on cerebral blood has not been quantified. We proposed a measurement of cerebral augmentation index (CAI) to evaluate the augmentation effect induced by ECP.

Cerebral autoregulation is one of the important regulatory mechanisms of brain flow, which keeps the cerebral blood flow relatively constant within a wide range of cerebral perfusion pressure. There are many different hypotheses about its regulation mechanisms, including metabolic, myogenic and neurogenic regulation. [203] Breathholding is a

simple and effective method to assess cerebral autoregulation through vasomotor reactivity. Breathholding Index (BHI) is used to evaluate cerebral vasoreactivity, calculated by increase percentage of cerebral mean flow velocity after breathholding divided by the time of breathholding. Cerebral vasoreactivity is considered as impaired when BHI is less than 0.69. And impaired cerebrovascular reactivity is associated with risk of ischemic stroke events for patients with asymptomatic carotid artery stenosis. [112] Thigh cuff method is one of methods to assess dynamic cerebral autoregulation firstly described in 1989, which usually maintains thigh cuff pressure for 2 minutes then rapidly deflates pressure leading to transient blood pressure drop. [73, 204] ECP is similar as thigh cuff method using external pressure applied on low limbs, but different with cyclic inflation and deflation resulting in increased diastolic blood pressure and aortic pressure.

We aim to explore the cerebral hemodynamic response to ECP and breathholding test on ischemic stroke patients respectively assessed by CAI and BHI. Then further to investigate the correlation between the augmented hemodynamic effect of ECP and cerebral vasomotor reactivity.

## 5.2 Methods

### 5.2.1 Subjects

We recruited 37 recent ischemic stroke patients with large artery occlusive disease and 20 healthy elderly controls into this study. Patients were hospitalized into Acute Stroke Unit, Prince Wales of Hospital, The Chinese University of Hong Kong. They were verified with ischemic stroke due to large artery occlusive disease. Exclusion criteria

included intracranial hemorrhage, cardioembolic stroke, stroke onset relevant pontine infarct, and so on, as described in Chapter 3. Controls were elderly healthy subjects without any cerebrovascular risk factors and cerebrovascular events. The clinical characteristics of all subjects were documented for analysis.

5.2.2 Procedure

All subjects underwent ECP treatment and breathholding test combined with transcranial Doppler monitoring on bilateral MCAs. Two 2MHz probes were mounted at bilateral temporal windows on a fixed headframe. Bilateral M1 segments of MCA were insonated at depth of 50~60mm. Firstly, we asked subjects to hold their breath for 30 seconds, and recorded mean flow velocities at baseline and after breathholding. Then after a break of 2 minutes, we went ahead to ECP-TCD monitoring using ECP treatment pressure of 150mmHg. We documented the MCA mean flow velocity before and during ECP respectively for 3 minutes. We also monitored the beat-to-beat blood pressure using Task Force Monitor system during the whole procedure.

5.2.3 Data analysis

We designated the ipsilateral or contralateral MCA based on the side of the recent infarct. CAI was calculated as change percentage of mean flow velocity during ECP compared with baseline. And BHI was measured by increase percentage of cerebral mean flow velocity after breathholding divided by the time of breathholding. Data of stroke patients were analyzed according to the infarct ipsilateral side or contralateral side. We performed the Correlation analysis between BHI and CAI in all groups as well as

correlation between CAI and BP changes in stroke groups. Significance level was inferred at $p<0.05$.

**5.3 Results**

There were 37 cases in stroke patients group. They were older and had more male compared with controls. Median of admission NIHSS score in patients group was 3, and the mean interval from stroke onset to exam was around 6 days. (**Table 5.1**)

Mean flow velocity of stroke patients both significantly increased at breathholding and ECP as **Table 5.2** shown. In control group, flow velocity increased after breathholding but decreased during ECP. BHI was smaller in the stroke group, (ipsilateral $0.79 \pm 0.51$, contralateral $0.93 \pm 0.53$), than that of the controls ($1.40 \pm 0.45$). Stroke patients had much higher CAI compared with controls (stroke CAI ipsilateral $8.11 \pm 9.79$ and contralateral $7.74 \pm 8.99$, control CAI $-0.47 \pm 2.89$). From **Table 5.3**, CAI did not correlate with BHI in the ipsilateral or contralateral side of stroke group as well as in controls. BHI was significantly lower on the ipsilateral side than the contralateral side, $p=0.011$, but CAI showed no difference on both sides. CAI of stroke patients was strongly related to the systolic and diastolic blood pressure change on ipsilateral side as illustrated in **Table 5.4**.

**Table 5.1** Subject characteristics

| | Patients (37) | Controls (20) |
|---|---|---|
| Age (year) | 67.46 ± 11.23 | 60.30 ± 6.57 * |
| Gender, Male (n, %) | 36, 97.3% | 10, 50% |
| Hypertension (n, %) | 29, 78.4% | |
| Diabetes Mellitus (n, %) | 17, 45.9% | |
| Dyslipidaemia (n, %) | 22, 59.5% | |
| History of IHD (n, %) | 4, 10.8% | |
| Previous CVA history (n, %) | 12, 32.4% | |
| Smoking (n, %) | 14, 37.8% | |
| Alcoholism (n, %) | 6, 16.2% | |
| Stroke onset to exam (day) | 6.14 ± 4.80 | |
| NIHSS on admission | 3 (0~17) | |
| Left side infarct (n, %) | 21, 56.8% | |
| Baseline systolic BP (mmHg) | 128.78 ± 17.99 | |
| Baseline diastolic BP (mmHg) | 86.33 ± 15.16 | |

Notes: * p= 0.004

**Table 5.2** Velocity changes after breathholding and ECP

| Velocity (cm/s) | Stroke ipsilateral | Stroke contralateral | Control L side | Control R side |
|---|---|---|---|---|
| BH Baseline | 47.96±14.41 | 53.19±18.30 | 55.59±11.31 | 50.25±12.05 |
| Breathholding | 58.58±17.87 | 67.00±24.50 | 78.46±18.29 | 72.00±18.55 |
| ECP baseline | 48.56±17.51 | 51.32±17.07 | 60.88±13.61 | 54.16±11.84 |
| ECP | 52.29±19.51 | 55.35±19.09 | 60.50±12.86 | 53.72±11.24 |

**Table 5.3** BHI and CAI changes

| | Stroke ipsilateral | Stroke contralateral | Control | P value (Ipsilateral vs. contralateral ) |
|---|---|---|---|---|
| BHI | 0.79±0.51 | 0.93±0.53 | 1.40±0.45 | 0.011* |
| CAI | 8.11±9.79 | 7.74±8.99 | -0.47±2.89 | 0.449 |
| Pearson Correlation | 0.333 | 0.307 | 0.873 | |
| P value | | | | |

Notes: Paired T test was used to compare the difference between ipsilateral and contralateral side. Pearson correlation was used to analyze the relationship between BHI and CAI. * $p<0.05$.

**Table 5.4** CAI and BP changes in stroke patient group

| | BP change (%) | | Pearson Correlation Coefficient | Pearson Correlation P value |
|---|---|---|---|---|
| | | Ipsilateral CAI | | |
| Systolic BP change | 7.41±7.38 | 8.11±9.79 | 0.594 | 0.001* |
| Diastolic BP change | 8.72±9.91 | 8.11±9.79 | 0.566 | 0.002* |
| | | Contralateral CAI | | |
| Systolic BP change | 7.41±7.38 | 7.74±8.99 | 0.200 | 0.339 |
| Diastolic BP change | 8.72±9.91 | 7.74±8.99 | 0.098 | 0.639 |

Notes: Pearson correlation was used to analyze the relationship between CAI and BP change. * $p<0.05$.

When divided stroke patients into subgroups according to intact or impaired vasoreactivity (BHI ≥ or <0.69), we found patients with impaired vasoreactivity were relatively older. (**Table 5.5**) CAI of impaired vasoreactivity patients was higher than intact stroke patients on both sides. (**Table 5.6**) BHI in impaired vasoreactivity group was lower on ipsilateral side when compared with contralateral side, whereas CAIs were similar on both sides in two subgroups.

### 5.4 Discussion

Stroke patients show distinct hemodynamic responses at breathholding or ECP compared with controls. MCA mean flow velocity of control increases after breathholding, but decreases during ECP. However, flow velocities of stroke patients both significantly increase when exposed to either breathholding or ECP. BHI and CAI of stroke patient group are different from those of control group. It points out that cerebral hemodynamic reserve is impaired on stroke patients. This finding is consisted with other studies that cerebral vasomotor reactivity is observed impaired in patients with acute ischemic stroke [205-206] and regulation of cerebral perfusion is impaired after stroke [99].

**Table 5.5** Stroke subgroup characteristics

| | **BHI≥0.69** | **BHI<0.69** | **P value** |
|---|---|---|---|
| | **19 patients** | **18 patients** | |
| Age (year) | 64.11±10.27 | 71.00±11.39 | 0.004* |
| Male (n, %) | 18, 94.7% | 18, 100% | 1.000 |
| HT (n, %) | 13, 68.4% | 16, 88.9% | 0.232 |
| DM (n, %) | 7, 36.8% | 10, 55.6% | 0.254 |
| Dyslipidemia (n, %) | 14, 73.7% | 8, 44.4% | 0.070 |
| CVA history (n, %) | 7, 36.8% | 5, 27.8% | 0.556 |
| IHD histroy (n, %) | 3, 15.8% | 1, 5.6% | 0.604 |
| Smoker (n, %) | 8, 42.1% | 6, 33.3% | 0.582 |
| Drinker (n, %) | 3, 15.8% | 3, 16.7% | 1.000 |
| Left side infarct (n, %) | 9, 47.4% | 12, 66.7% | 0.236 |
| NIHSS on admission | 3.89±3.14 | 4.83±3.76 | 0.518 |
| Stroke onset to exam (days) | 6.62±4.48 | 5.44±5.43 | 0.393 |
| Systolic BP (mmHg) | 125.04±17.97 | 134.11±17.37 | 0.303 |
| Diastolic BP (mmHg) | 84.40 ±15.65 | 89.08 ±14.65 | 0.471 |

Notes: Mann-Whitney test was used to compare two subgroups. * $p<0.05$.

**Table 5.6** Stroke subgroup analysis according to breathholding index

| Stroke subgroup | BHI≥0.69<br>19 patients | BHI<0.69<br>18 patients | P value |
|---|---|---|---|
| BHI ipsilateral | 1.15 ± 0.42 | 0.39 ± 0.23 | <0.001* |
| BHI contralateral | 1.27 ± 0.47 | 0.56 ± 0.30 | <0.001* |
| BHI ispilateral vs. contralateral | p=0.856 | p=0.049* | |
| CAI ipsilateral | 6.24 ± 9.27 | 10.20 ± 1020 | 0.009* |
| CAI contralateral | 4.01 ± 7.29 | 11.47 ± 9.15 | <0.001* |
| CAI ispilateral vs. contralateral | p=0.540 | p=0.569 | |

Notes: Wilcoxon Signed Ranks test was used to compare the difference between ispsilateral and contralateral side. Mann-Whitney test was used to compare the BHI≥0.69 group and BHI<0.69 group. * p<0.05.

Hemodynamics of both sides in stroke group show remarkable changes after breathholding and ECP. BHI was a little lower on the infarct symptomatic side and contralateral side. It is consistent with vasomotor reactivity studies that infarct symptomatic side suffers more severe dysfunction. [110] The ipsilateral side gains higher CAI from ECP although it does not reach the significance level. Cerebral autoregulation is globally impaired on the ischemia affected side and non-affected side after ischemic stroke. [106-107] Stroke patients with impaired vasomotor reactivity also have relatively higher CAI. This patient group may have severe cerebral autoregulation impairment, involving multiple pathways of cerebral autoregulation.

CAI does not associate with BHI either on stroke patients or elderly controls. It indicates velocity change of breathholding and ECP augmentation possibly operated under different mechanisms or pathways. Cerebral augmentation effects of ECP are uncorrelated with the well-known vasoreactivity. CAI reveals another aspect of autoregulation impairment different from vasomotor reactivity. CAI of stroke patients is strongly related to the systolic and diastolic blood pressure change on ipsilateral side. It illustrates autoregulation impairment reflected by CAI probably majorly work via the myogenic but not metabolic mechanism [203]. The reason why contralateral CAI does not associated with the changes of blood pressure, may relate to complex network of collateral circulation improved by ECP. [9, 169]

ECP may become a new tool to assess the dynamic cerebral autoregulation. It induces the changes of blood flow in patients with impaired autoregulation, with corresponding

blood pressure changes. Compared with thigh cuff method, ECP method is superior in the safety and subject tolerance. The safety and well patient tolerance of ECP treatment are proved by many clinical trials. [131, 207] However, thigh cuff method bears some risks with rapid drop of blood pressure, especially when subjects have symptoms of systemic or cerebral hypoperfusion. The application of CAI will be discussed later in Chapter 6.

There are several limitations in this study. Firstly, healthy control group is younger and has more female subjects. Current results may not fully demonstrate hemodynamic response difference between stroke patients and elderly healthy. Secondly, our sample size is relative small. It needs further study with more cases of age and gender matched controls.

In summary, hemodynamic effects of external counterpulsation on cerebral circulation may be considered as a new approach to assess cerebral autoregulation. Dynamic augmentation effects as measured by CAI were different from the well established vasomotor reactivity. CAI is a measure of how well the brain accommodates blood flow augmentation, independent of vasomotor reactivity.

# CHAPTER 6 STENTING IMPROVES CEREBRAL AUTOREGULATION IN STROKE PATIENTS WITH INTRACRANIAL LARGE ARTERY HIGH-GRADE STENOSIS

## 6.1 Background

Intracranial stenting is an adjunctive treatment option in symptomatic intracranial large artery stenosis. Intracranial atherosclerosis is an important etiology for cerebrovascular disease, and it is demonstrated with higher risk for populations of Asian, African and Hispanics. [208-209] Despite receiving maximal medical treatment, patients with intracranial atherosclerosis are at high risks of recurrent stroke or transient ischemic attack. [210-211] Stenting provides a treatment option for medical refractory intracranial stenosis to remodel vasculature and achieve favorable outcome. [212-213]

External counterpulation is a noninvasive technique to improve the perfusion of vital organs by inflation of pneumonic cuffs applied on the lower extremities. [6] Our previous study demonstrated ECP could help the recovery of ischemic stroke patients with large artery occlusive disease. [5] ECP augmented cerebral blood flow of ischemic stroke patients described in Chapter 4, and it could be used to assess cerebral autoregulation as Chapter 5 mentioned.

The benefits of intracranial stenting for symptomatic stensosis compared with conventional medical treatment remains investigational and controversial. We aimed to

explore the intermediate-term effects of stenting on cerebral autoregulation in patients with intracranial large artery occlusive disease receiving external counterpulsation.

## 6.2 Methods

### 6.2.1 Subjects

This is a subgroup study of a randomized controlled study of intracranial stenting, and we assessed 18 ischemic stroke patients around 2 years after randomization. They all had symptomatic high-grade (≥ 70%) intracranial internal carotid artery or middle cerebral artery (MCA) stenosis. There were 10 patients randomized to receive stenting angioplasty (Wingspan, Boston Scientific, CA, USA), and 8 patients in intensive medical treatment group. Pre-specified therapeutic targets of intensive medical treatment were low-density lipoprotein (LDL) ≤ 70 mg/dL, HbA1c ≤ 6.5%, systolic blood pressure ≤ 140 mmHg, and abstinence from smoking. We used NIHSS to evaluate neurological deficits of all subjects at randomization and examination. Clinical characteristics of all subjects were documented for analysis.

### 6.2.2 ECP-TCD monitoring

We performed ECP treatment on all subjects and monitored their mean flow velocity changes of MCA with transcranial Doppler at the same time. ECP treatment was given with cuff pressure of 150mmHg. Two 2MHz probes were mounted at bilateral temporal windows on a fixed headframe. Bilateral M1 segments of MCA were insonated at the depth of highest mean flow velocity between 50 to 60mm. We recorded TCD data before

and during ECP respectively for 3 minutes. Beat-to-beat blood pressure was monitored by Task Force Monitor system during the whole procedure.

6.2.3 Data analysis

Cerebral augmentation index CAI was calculated by the increase percentage of mean blood flow velocity induced by ECP compared with baseline. Velocity data were analyzed based on the presence of infarction (symptomatic versus contralateral side). Due to small number of group patients, non-parametric tests were used for statistical analysis. Significance level was inferred at $p < 0.05$.

**6.3 Results**

Demographics data were totally comparable between stenting and medical groups. Both NIHSS scores at randomization and examination were balanced in two groups. They were investigated around 2 years after randomization. (**Table 6.1**) All patients had no stroke recurrence after randomization.

MCA mean flow velocities significantly increased during ECP in both stenting and medical groups as shown in **Table 6.2**, all $p < 0.05$. CAI of stenting group on contralateral sides was significantly lower than that of non-stenting group, $p=0.013$. And the symptomatic side CAI had lower tendency with stenting treatment with p value 0.051. (**Table 6.3**) CAI was not different between symptomatic and contralateral sides in both groups. (**Figure 6.1**)

**Table 6.1** Patient characteristics

| | Stented group (10) | Medical (8) | P value |
|---|---|---|---|
| Age (year) | 60.80±11.21 | 66.00±6.14 | 0.173 |
| Male (n, %) | 9, 90% | 7, 87.5% | 1.000 |
| NIHSS when recruited | 2.5 (1~5) | 2 (1~6) | 0.696 |
| NIHSS when examed | 1 (0~3) | 2 (1~3) | 0.321 |
| HT (n, %) | 5, 50% | 3, 37.5% | 0.664 |
| DM (n, %) | 2, 20% | 2, 25% | 1.000 |
| Dyslipidemia (n, %) | 9, 90% | 6, 75% | 0.559 |
| Smoker (n, %) | 7, 70% | 6, 75% | 1.000 |
| Drinker (n, %) | 0 | 2, 25% | 0.183 |
| Left side infarct (n, %) | 5, 50% | 7, 87.5% | 0.152 |
| Months from recruitment to exam (month) | 24.10±14.33 | 29.88±14.07 | 0.315 |
| Systolic BP (mmHg) | 108.19±14.54 | 117.46±11.39 | 0.122 |
| Diastolic BP (mmHg) | 72.28±7.56 | 79.76±10.58 | 0.146 |

Notes: Mann-Whitney test was used to compare differences between two groups.

**Table 6.2** Velocity change after ECP in groups

| Velocity (cm/s) | Stented symptomatic | Stented contralateral | Medical symptomatic | Medical contralateral |
|---|---|---|---|---|
| Baseline | 57.65 | 51.95 | 49.10 | 46.15 |
| | (33.10 ~ 108.70) | (41.00 ~ 82.10) | (32.70 ~ 101.70) | (39.30 ~ 144.50) |
| ECP | 59.85 | 52.75 | 53.05 | 49.40 |
| | (33.50 ~ 111.60) | (41.40 ~ 85.10) | (33.60 ~ 107.00) | (41.70 ~ 152.80) |
| P value | 0.009* | 0.025* | 0.012* | 0.012* |

Notes: Wilcoxon Signed Rank test was used to compare velocities at baseline and ECP.

* $p < 0.05$.

**Table 6.3** CAI on both sides of two groups

| | Stented | Medical | P value |
|---|---|---|---|
| CAI symptomatic | 3.89 ± 2.90 | 7.47 ± 3.42 | 0.051 |
| CAI contralateral | 3.00 ± 4.03 | 7.04 ± 2.82 | 0.013* |
| P value (symptomatic vs contralateral) | 0.575 | 0.386 | |

Notes: Mann-Whitney test was used to compare differences between stented group and medical group. Comparison of symptomatic and contralateral side was done with Wilcoxon Signed Rank test. * $p < 0.05$.

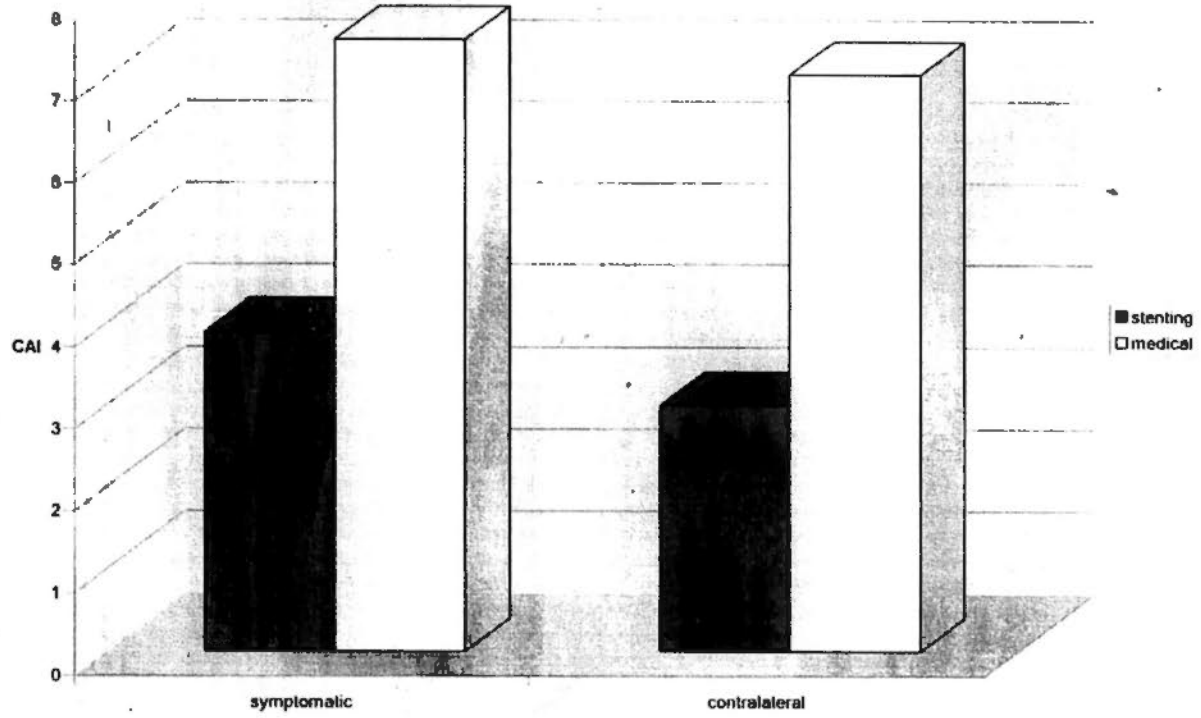


**Figure 6.1** CAI on both sides of two groups

### 6.4 Discussion

ECP induces increase of cerebral blood flow velocity in both stenting and non-stenting group, possibly via impaired cerebral autoregulation. All subjects are ischemic stroke patients with symptomatic intracranial large artery high-grade stenosis. Cerebral autoregulation is impaired in patients with ischemic stroke as well as large artery occlusive disease. [120] CAI of stenting group is much lower than that of medical group. Patients received stenting have less flow velocity change during ECP compared with those without stenting. The cerebral augmentation effect of ECP is more significant in non-stenting group than in stenting group. In the long term of stenting treatment, the cerebral autoregulation may be improved after remodel of cerebral vasculature. Stenting also improves the ability of ischemic brain to accommodate flow augmentation. A study of patients with severe carotid obstruction showed that cerebral dysautoregulation could be remedied by recanalization through carotid endarterectomy or stenting. [214]

Intracranial angioplasty with stenting is a promising treatment option for symptomatic patients with large artery stenosis. After stenting, the target vessel is recanalized and cerebral blood flow is enhanced. There are several clinical evidences suggesting stenting as a safe, less invasive and effective alternative to carotid endarterectomy in patients with symptomatic carotid high-grade stenosis to prevent first stroke or recurrent stroke. [215] Although there are some risks of inter-procedure complication and restenosis, stenting is beneficial to improve the prognostic outcome after atherosclerotic cerebrovascular disease. However, the long term effect of stenting is still under investigation, particularly the newly emerging treatment of intracranial stenting. Our findings of improved cerebral

autoregulation after intracranial stenting compared with medical treatment, maybe one of mechanisms contributed to clinical benefits of stenting.

CAI is similar on the symptomatic and contralateral side in both groups. It may due to the global impairment of cerebral autoregulation as mentioned above. The parallel pattern of both sides suggests the impaired autoregulation may bilaterally simultaneously recovery in the long term. The significant difference of CAI in stenting and non-stenting group is found on the contralateral side but not the ipsilateral side. It may be caused by the limited sample size with relative weak power. A further study of large number of stenting patients is warranted.

In this study, CAI is firstly applied to assess cerebral autoregulation of stroke patients with stenting treatment through diastolic augmentation of ECP. ECP may introduce a new approach to measure autoregulation evaluated by CAI. In future clinical practice, CAI could help physicians to make treatment decision based on the identification of cerebral autoregulation level. Such as cerebral vascular intervention, for some high risk candidates, pre-operative CAI may provide the information of cerebral vascular reserve and predictive value of the outcome after intervention. For ECP treatment, CAI may help to choose suitable ischemic stroke patients responding well with appropriate cerebral blood flow increase.

There are several limitations. First of all, two patients groups are just comparable based on current data. The evaluation of baseline information such as neurological

deficits at stroke onset and infarct size may strengthen the match of two groups. Second, the sample size is currently quite limited, which needs further study to confirm these results.

In summary, assessed by CAI of ECP, stenting of intracranial atherosclerosis may improve the cerebral autoregulation in the long term. The ability for the ischemic brain to accommodate flow augmentation is significantly enhanced in the patient receiving stenting treatment. Effects of stenting on cerebral autoregulation may contribute to its clinical benefits.

# SECTION IV

# CHAPTER 7 OPTIMAL PRESSURE OF EXTERNAL COUNTERPULSATION FOR CEREBRAL BLOOD FLOW AUGMENTATION IN ISCHEMIC STROKE PATIENTS

## 7.1 Background

External counterpulsation (ECP) is a noninvasive, highly beneficial and long term effective treatment for ischemic heart disease. [3, 207] There are three pairs of pneumonic cuffs applied to the calves, lower thighs, and upper thighs (buttocks) in the enhanced external counterpulsation system. ECG triggers cuff inflation sequentially from distal to proximal during diastole and releases cuff pressure before the start of systole. Diastolic pressure on the lower extremities improves venous return and cardiac output, while deflation before systole leads to increased systolic unloading. ECP has been demonstrated to improve the perfusion of vital organs through diastolic augmentation. [6] The improvement of myocardial perfusion and promotion of coronary collaterals were suggested to contribute to clinical benefits of ECP on ischemic heart disease. [8-9] In the application of ECP on coronary artery disease, effectiveness ratio was used to investigate optimal hemodynamic benefits. Effectiveness ratio was calculated by the relative magnitude of diastolic augmentation and systolic unloading, assessed by finger plethysmography. When cuff pressures caused the effectiveness ratio in the range of 1.5~2.0, the aortic flow was maximized. [197] Patients with higher ratio (>1.5) during ECP trended to have a greater reduction of angina class at 6-month follow up. [198] Angina patients with ECP increased the effectiveness ratio from 0.7 to 1.0 from the

beginning to the end of treatment, and those had the greatest increase in the ratio had the greatest reduction in angina class. [199]

The hemodynamic effects of external counterpulsation on cerebral circulation are largely unknown. Our previous study of ischemic stroke patient with ECP treatment demonstrated ECP was significantly associated with favorable clinical improvement and blood flow velocity of MCA increased after ECP compared with baseline. [5] The optimal pressure of ECP on cerebral augmentation has not been well documented. In this study, we aimed to find the optimal pressure of ECP treatment in relation to the cerebral blood flow on patients with recent ischemic stroke. We hypothesized that cerebral blood flow velocity of stroke patients may increase as ECP treatment pressure raises.

## 7.2 Methods

### 7.2.1 Subjects

We recruited subjects from ischemic stroke patients, who were hospitalized in Acute Stroke Unit, Prince of Wales Hospital from Sept 2006 to July 2009. Those with cardioembolic stroke, history of intracranial haemorrhage and stroke onset relevant pontine infarct were excluded. Contraindication for ECP (such as sustained hypertension, aortic aneurysm, carotid dissection, severe peripheral artery disease and so on), severe systemic diseases and malignancy were also exclusion criteria. For ischemic stroke patients who were verified with cerebral large artery occlusive disease by magnetic resonance angiography or computed tomography angiography, 101 patients agreed external counterpulsation as an adjunctive treatment based on medical treatment. ECP

was performed using the Enhanced External Counterpulsation system, model number MC2 (Vamed Medical Instrument Company device, Foshan, China). Among 101 patients, 53 patients received the standard 35 daily ECP sessions (each session 1 hour) and 48 patients failed to finish the whole course of treatment. Some of elder stroke patients had no adequate acoustic temporal windows for TCD monitoring, particularly female elder patients. Thirty cases with good temporal window from those fifty-three patients were enrolled into this study. The study was approved by the local medical ethics committee (Joint CUHK-NTEC Clinical Research Ethics Committee). All subjects gave informed consent and their clinical data were documented.

7.2.2 TCD monitoring and data interpretation

We monitored their cerebral blood flow velocities of bilateral MCAs using SONARA TCD system (BioBeat Medical Limited, California, USA) during their first ECP session. Patients were asked to lie on the EECP treatment bed with two 2MHz probes mounted on a headframe. Bilateral M1 segments of MCA were insonated at the depth of highest mean velocity between 50 to 56mm. Treatment pressure started from 150mmHg (0.02MPa), then gradually increased to 187.5mmHg (0.025MPa), 225mmHg (0.03MPa), 262.5mmHg (0.035MPa). (**Figure 7.1**) Blood flow velocities at baseline and under different ECP pressures were respectively recorded for 3 minutes, and the physiological correlates associated with ECP-TCD waveform morphology were identified.

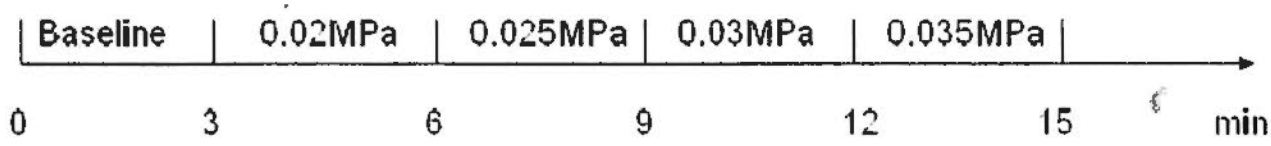


**Figure 7.1** ECP-TCD monitoring procedure

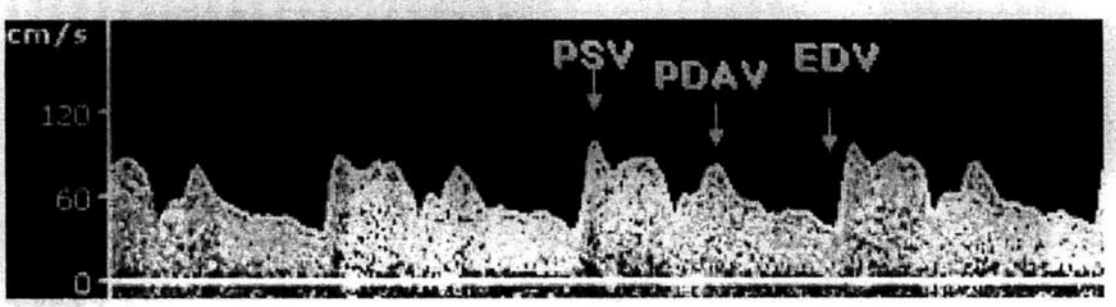


**Figure 7.2** ECP-TCD waveform during ECP. EDV, end diastolic velocity; PDAV, peak diastolic augmentation velocity; PSV, peak systolic velocity.

Flow velocities were manually read at first 4 beats of each minute by the same experienced technician, including peak systolic velocity PSV, end diastolic velocity EDV and peak diastolic augmentation velocity PDAV. PDAV was induced by ECP diastolic augmentation as shown in **Figure 7.2**. The mean flow velocity MV was calculated as (PSV+2*EDV)/3 at baseline and (PSV+PDAV+EDV)/3 during ECP as previously reported. [200] PDAV/PSV ratio was used to investigate the pressure effect, which was determined by the value of PDAV divided by PSV. PDAV/PSV ratio reduced the influence of individual cerebral blood flow velocity difference.

### 7.2.3 Data Analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Continuous data were expressed as means ± standard deviations, while category data were expressed as frequency and percentage. Data of MCA velocity was compared based on whether it was infarct side or the contralateral side. TCD waveforms were analyzed in relation to cardiac cycle. The percentage changes of augmented velocities from baseline were calculated to manifest augmentation effects of ECP. Repeat measurements were used to detect changes of blood flow velocities under different pressures. Statistical significance level was inferred at $p<0.05$.

## 7.3 Results

Mean age of 30 subjects was 68.6 ± 9.45 and 86.7% of patients was male. (**Table 7.1**) Mean interval of stroke onset to ECP-TCD monitoring were 6.41 days. The median of admission National Institutes of Health Stroke Scale (NIHSS) of patients was 6. Of 30

patients, 19 patients (63.3%) had right side infarct and 26 patients (86.7%) had infarcts located in MCA and ACA territories as **Table 7.2** shown. Anterior circulation artery occlusive disease was found in all patients, meanwhile posterior circulation artery occlusive disease was also involved in 43.3% of patients. Twenty-one patients (70%) had multiple large artery stenosis.

Mean blood pressure during different ECP pressures significantly increased from baseline, but the increases of blood pressures under 4 ECP pressures were different ($p<0.001$, **Table 7.3**). The mean flow velocities on the symptomatic side under different ECP pressures increased 18.49% (0.02MPa), 19.33% (0.025MPa), 19.16% (0.03MPa) and 18.46% (0.035MPa), as illustrated in **Table 7.4** and **Figure 7.3**. All were significantly higher than baseline but did not differ among different pressures. Under increasing pressures, PDAV gradually increased (78.70%, 88.24%, 96.66%, 103.02% respectively compared with baseline EDV, $p<0.001$) while PSV decreased (3.60%, 2.64%, 1.02%, -1.88% compared with baseline PSV, $p=0.001$) and EDV decreased (-1.35%, -4.18%, -9.28%, -11.87% compared with baseline EDV, $p=0.002$). The contralateral side showed similar picture (**Table 7.5** and **Figure 7.4**). No significant change of contralateral MV was detected, however, PDAV increased ($p<0.001$) and PSV decreased ($p<0.001$) as well as EDV did ($p=0.01$). PDAV/PSV ratio significantly increased following the elevated pressure on both sides ($p<0.001$, **Table 7.6** and **Figure 7.5**).

**Table 7.1** Subjects clinical characteristics

| | Patient (30) |
|---|---|
| Age (year) | 68.6 ± 9.45 |
| Gender, Male (n, %) | 26, 86.7% |
| Hypertension (n, %) | 25. 83.3% |
| Diabetes Mellitus (n, %) | 14. 46.7% |
| Dyslipidaemia (n, %) | 9, 30% |
| Chronic heart disease (n, %) | 4. 13.3% |
| Peripheral vascular disease (n, %) | 1, 3.3% |
| Previous CVA history (n, %) | 15, 50% |
| Smoking (n, %) | 9, 30% |
| Alcoholism (n, %) | 1, 3.3% |
| Stroke onset to exam day (day) | 6.41 ± 4.30 |
| NIHSS on admission | 6 (2~12) |
| Baseline heart rate (times/minute) | 69.76 ± 11.81 |
| Baseline systolic BP (mmHg) | 143.19 ± 19.85 |
| Baseline diastolic BP (mmHg) | 87.04 ± 11.82 |

**Table 7.2** Infarct location and stenotic artery

| | Patient (30) |
|---|---|
| Left side infarct (n, %) | 11, 36.7% |
| Right side infarct (n, %) | 19, 63.3% |
| MCA+ACA territory infarct (n, %) | 26, 86.7% |
| PCA territory infarct (n, %) | 4, 13.3% |
| Anterior circulation artery stenoses (n, %) | 17, 56.7% |
| Both anterior and posterior circulation artery stenoses (n, %) | 13, 43.3% |
| Single artery stenoses (n, %) | 9, 30% |
| Multiple artery stenoses (n, %) | 21, 70% |

**Table 7.3** Mean blood pressure changes under different pressures

| Baseline | 150mmHg | 187.5mmHg | 225mmHg | 262.5mmHg | P value |
|---|---|---|---|---|---|
| Mean blood pressure (mmHg) | | | | | |
| 103.54 ± 13.36 | 110.40 ± 14.30 | 116.16 ± 16.75 | 116.08 ± 17.74 | 117.78 ± 17.30 | *<0.001* |
| Blood pressure increase percentage (%) | | | | | |
| | 6.76 ± 5.77 | 12.17 ± 7.56 | 12.17 ± 10.46 | 13.90 ± 10.71 | *<0.001* |

Notes: * Using Repeat Measurement to analysis differences among 4 different pressures.

**Table 7.4** MCA velocity changes on symptomatic side under different pressures

| Ipsilateral (cm/s) | Baseline | 0.02 MPa | 0.025 MPa | 0.03 MPa | 0.035 MPa | P value |
|---|---|---|---|---|---|---|
| MV | 58.32 ± 20.77 | 68.43 ± 23.69 | 68.55 ± 22.81 | 68.51 ± 22.76 | 68.40 ± 23.41 | 0.985 |
| MV change % | | 18.49±15.56 | 19.33±14.49 | 19.16±14.68 | 18.46±12.68 | 0.853 |
| PSV | 96.35 ± 33.19 | 99.44 ± 34.50 | 97.97 ± 33.37 | 96.64 ± 33.63 | 94.20 ± 34.09 | 0.001* |
| PSV change % | | 3.60±10.98 | 2.64±11.99 | 1.02±12.41 | -1.88±11.34 | 0.001* |
| PDAV | | 67.46± 24.14 | 70.54 ± 23.63 | 73.61 ± 24.78 | 76.52 ± 26.33 | <0.001* |
| PDAV change % ** | | 78.70±45.03 | 88.24±44.32 | 96.66±48.99 | 103.02±45.73 | <0.001* |
| EDV | 39.31 ± 16.06 | 38.41 ± 15.65 | 37.15 ± 15.23 | 35.28 ± 15.21 | 34.47 ± 15.96 | 0.002* |
| EDV change % | | -1.35±14.24 | -4.18±15.13 | -9.28±16.47 | -11.87±19.21 | 0.002* |

Notes: Using Repeat Measurement to analysis change under different pressures.

* p<0.05. ** PDAV under ECP compared with baseline EDV. MV= mean flow velocity; PSV= peak systolic velocity; PDAV= peak diastolic augmentation velocity; EDV= end diastolic velocity.

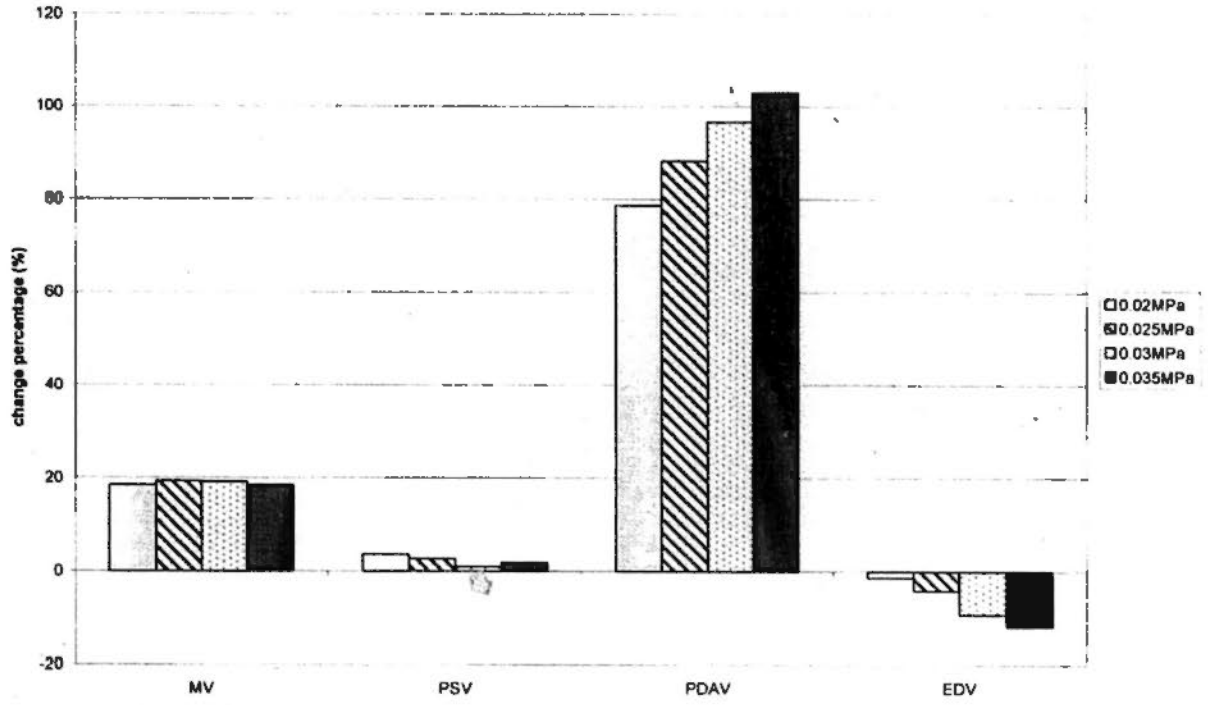


**Figure 7.3** Flow velocity change percentages from baseline on the symptomatic side under different pressures.

**Table 7.5** MCA velocity changes on contralateral side under different pressures

| Contralateral (cm/s) | Baseline | 0.02 MPa | 0.025 MPa | 0.03 MPa | 0.035 MPa | P value |
|---|---|---|---|---|---|---|
| MV | 50.69 ± 18.05 | 59.87 ± 20.29 | 60.01 ± 20.48 | 59.74 ± 20.41 | 60.45 ± 20.90 | 0.666 |
| MV change % | | 18.93±12.44 | 19.29±12.91 | 18.64±12.94 | 19.91±12.95 | 0.719 |
| PSV | 86.60 ± 29.93 | 89.28 ± 30.24 | 87.70 ± 30.15 | 86.01 ± 30.24 | 84.46 ± 29.95 | <0.001* |
| PSV change % | | 3.48±9.02 | 1.67±9.95 | -0.49±10.79 | -2.48±10.62 | <0.001* |
| PDAV | | 57.54 ± 19.74 | 60.65 ± 20.36 | 63.31 ± 20.61 | 66.99 ± 22.50 | <0.001* |
| PDAV change % ** | | 83.10±42.00 | 93.97±44.52 | 102.68±46.27 | 113.55±46.71 | <0.001* |
| EDV | 32.74 ± 13.39 | 32.79 ± 13.60 | 31.69 ± 13.86 | 29.92 ± 14.38 | 29.89 ± 14.71 | 0.010* |
| EDV change % | | 0.94±12.95 | -2.61±16.70 | -8.93±19.13 | -8.89±22.31 | 0.002* |

Notes: Using Repeat Measurement to analysis change under different pressures.

* $p<0.05$. ** PDAV under ECP compared with baseline EDV. MV= mean flow velocity; PSV= peak systolic velocity; PDAV= peak diastolic augmentation velocity; EDV= end diastolic velocity.

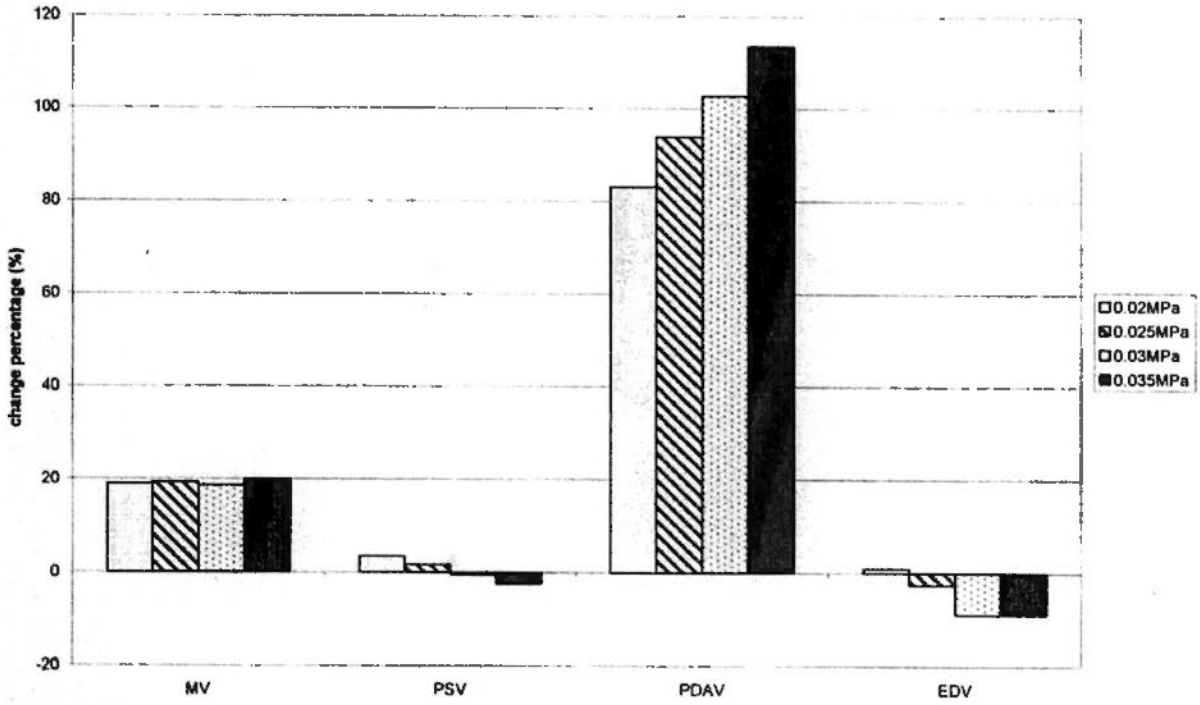


**Figure 7.4** Flow velocity change percentages from baseline on the contralateral side under different pressures.

**Table 7.6** PDAV/PSV ratio changes under different pressures

| **PDAV/PSV** | 0.02 MPa | 0.025 MPa | 0.03 MPa | 0.035 MPa | P value |
|---|---|---|---|---|---|
| Symptomatic | 0.68 ± 0.10 | 0.73 ± 0.10 | 0.77 ± 0.12 | 0.83 ± 0.15 | <0.001* |
| Contralateral | 0.65 ± 0.10 | 0.70 ± 0.10 | 0.75 ± 0.12 | 0.81 ± 0.15 | <0.001* |

Notes: Using Repeat Measurement to analysis change under different pressures.

* $p<0.05$. PDAV/PSV = peak diastolic velocity / peak systolic velocity

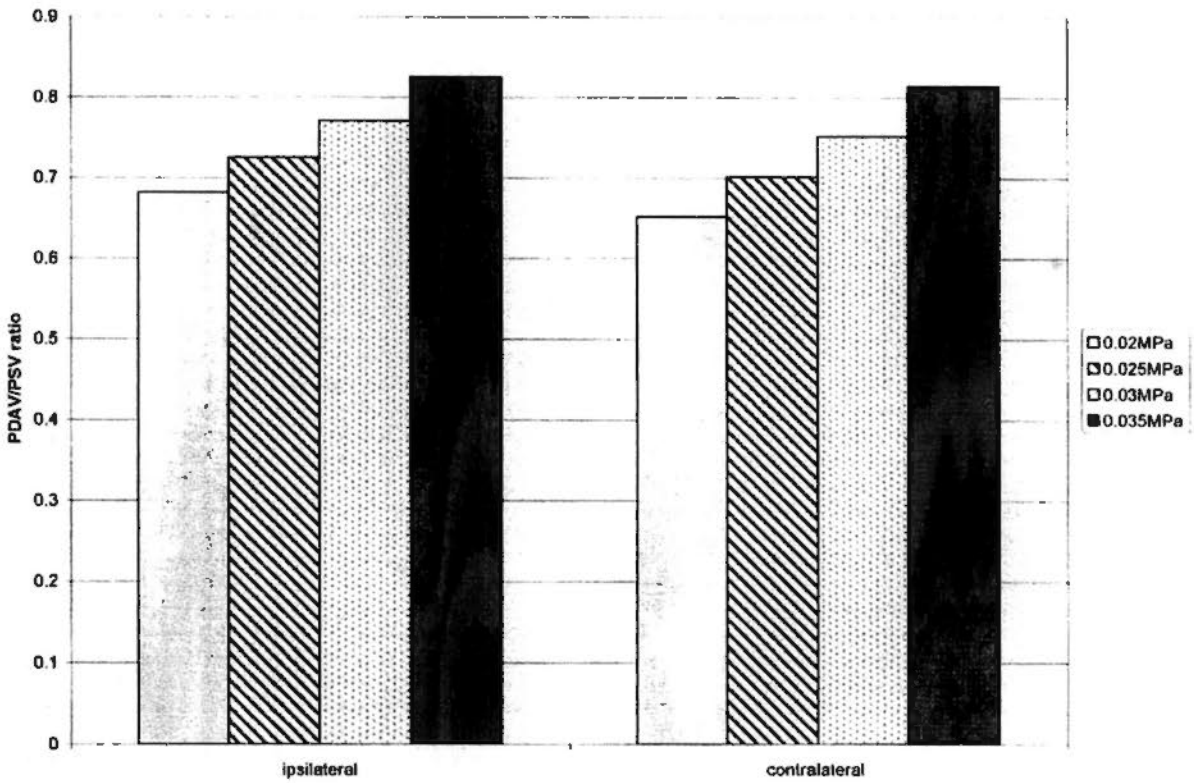


**Figure 7.5** PDAV/PSV ratio changes under different pressures.

### 7.4 Discussion

In this pilot study, we investigate hemodynamic effects of ECP on cerebral circulation in patients with recent ischemic stroke, in order to find the optimal treatment pressure of ECP used for ischemic stroke. Blood pressure and MCA mean flow velocity under different ECP pressures significantly increase from baseline. However, MCA mean flow velocity does not increase following the elevated cuff pressure and mean blood pressure. At the treatment pressure of 150mmHg, mean flow velocities of bilateral MCAs are remarkably augmented by ECP. But the increase of MV seems reach a plateau after 150mmHg. Cerebral blood flow velocity is detected at a steady angle when monitored. Presumed artery territory perfusion and artery diameter unchanged, our results indicate that cerebral blood flow volume is kept constant under different cuff pressure stimuli from 150 to 262.5mmHg.

This study reveals a critical issue when the novel treatment concept ECP is applied to ischemic stroke, which is to identify the optimal treatment pressure. Ideally the optimal cuff pressure produces maximal effective hemodynamic augmentation with minimal risk of adverse events. The commonest ECP device-related adverse effects are musculoskeletal and skin barotraumas, such as joint and muscle pain of legs or back, edema or swelling, skin abrasion and bruise. Higher cuff pressure predisposes patients to relative higher risk of barotraumas, although the incidence rate of adverse effects is very low. Of 30 ischemic stroke patients in this study, only 1 case reported haematuria during other sessions of the whole treatment course but not the first session when TCD monitoring was performed. Since mean flow velocity does not further increase as

treatment pressure is more than 150mmHg, this finding suggests 150mmHg as the effective and safe optimal treatment pressure.

PDAV increases following the applied pressure while PSV and EDV relatively decrease on the both sides. This result is consistent with a recently report [189], which reveal peak systolic velocity and end diastolic velocity of MCA blood flow in healthy subjects reduce during ECP. Cerebral autoregulation mechanism may play an important role in the change of cerebral blood flow. Intact cerebral autoregulation enables the brain maintain constant cerebral blood flow despite dynamic fluctuation of cerebral perfusion pressure. To remain the brain stable circulation, autoregulation mechanism may extenuate the sudden and upward effect of diastolic augmentation induced by ECP with the compensation of systolic and end diastolic flow decline. ECP does not change mean flow velocity of MCA in healthy subjects [188-189]. However, in ischemic stroke patients the cerebral autoregulation is partially impaired [216]. From a different viewpoint it does provide a chance to improve blood flow supply for ECP treatment to increase ischemia reperfusion. According to one study of ECP and dynamic cerebral autoregulation [191], ECP did not compromise cerebral autoregulation and remain transfer function gain and phase shift between mean arterial BP and mean cerebral blood flow velocity. Therefore, cerebral autoregulation may explain the change trend of PDAV, PSV and EDV under increasing cuff pressures.

PDAV/PSV ratio gradually increases following the elevated cuff pressure. This ratio is different from the effectiveness ratio used in coronary augmentation, because

PDAV/PSV ratio is calculated by velocity change rather than magnitude change of finger plethysmography. As the applied cuff pressure rises, venous return and cardiac output simultaneously increase. The effect of diastolic augmentation is more powerful although mean flow velocity does not further increase.

Patients with higher diastolic augmentation tended to have a greater reduction in angina class at 6-month follow up from international EECP Patient Registry [198]. Nevertheless, for ECP treatment on ischemic stroke patients, relative data of hemodynamic effect and clinical outcome is rare. Further research of short term and long term clinical outcome under different pressure treatment is warranted to confirm the efficiency and safety of low treatment pressure.

There are some limitations in this study need to be discussed. First, we read the first 4 beats TCD data of each minute (total 12 timepoints for 3 minutes) to present the whole during treatment of each pressure. We may miss some velocity variation due to ECP duration time adaption [188] at those skipped timepoints, but our data did catch the change trends of flow velocities. The sample size is relatively small due to limited numbers of ECP-treated stroke patients with good temporal window. Although the large artery occlusive disease, like MCA or internal carotid artery stenosis, may influence MCA flow velocity from normal range, we compare flow velocities at baseline and under different cuff pressures to investigate the ECP-induced velocity changes.

In summary, 150mmHg appears to be the optimal pressure to be used to increase cerebral blood flow in terms of mean flow velocity from this pilot study of ECP and ischemic stroke. Further increase in pressure does not increase cerebral blood flow velocity. Augmented mean flow velocities mostly remain stable on both sides under different pressures. Blood flow velocities show distinct responses in the cardiac cycle. Peak diastolic augmentation velocity increases but peak systolic and end diastolic velocity decrease.

# SECTION V

# CHAPTER 8 CEREBRAL BLOOD FLOW VELOCITY AND GOOD OUTCOME FOR COUNTERPULSATION-TREATED STROKE PATIENTS

## 8.1 Background

External counterpulsation is a novel and noninvasive method used to improve the perfusion of vital organs [6], which also induces hemodynamic changes of cerebral circulation. [188, 200] ECP inflates pressure on three pairs of pneumonic cuffs applied to lower extremities during diastole and releases cuff pressure before the start of systole, resulting in diastolic augmentation. ECP could help the recovery of ischemic stroke patients with large artery occlusive disease accompanied by cerebral blood flow increase. [5] We observed ischemic patients had different hemodynamic responses to external counterpulsation treatment in our previous studies. The data on hemodynamic effects of ECP on ischemic stroke patients are rare, especially on cerebral hemodynamics and clinical outcome of patients.

We hypothesize that hemodynamic response pattern of ECP may correlate with the clinical outcome of ischemic stroke patients after ECP treatment, which indicates some certain response pattern may bring more benefits on suitable ischemic stroke patients. We aim to further explore ECP hemodynamic effects on acute ischemic stroke patients and investigate its relationship with short term clinical outcome.

## 8.2 Methods

### 8.2.1 Patients

We performed external counterpulsation on ischemic stroke patients in Acute Stroke Unit, Prince of Wales Hospital, as an adjunctive treatment of conventional medical treatment. Inclusion criteria and exclusion criteria for ECP treatment were described above in Chapter 3. We treated patients using the Enhanced External Counterpulsation system, model number MC2 (Vamed Medical Instrument Company device, Foshan, China). Among ischemic stroke patients with cerebral large artery stenosis received ECP treatment, we recruited those with good temporal window for TCD monitoring and patients completed ECP sessions for more than 10 times. The clinical characteristics of patients were documented for analysis, including demorgraphics, medical history and ischemic stroke events. All cases enrolled were followed up with modified Rankin Scale (mRS) at 3 months after treatment.

### 8.2.2 TCD monitoring of ECP response

At their first ECP session of subjects (treatment pressure 150mmHg), we monitored their cerebral blood flow changes on bilateral MCAs using ST3 Transcranial Doppler system (Spencer Technologies, Seattle, USA). Bilateral M1 segments of MCA were insonated at the depth of highest flow velocity between 50 to 60mm. The mean flow velocity data were recorded before and during ECP respectively for 3 minutes. Meanwhile beta-to-beat continuous blood pressures were monitored using Task Force Monitor system during the procedure.

### 8.2.3 Data analysis

TCD data were analyzed based on whether it was the infract side or contralateral side. Cerebral augmentation index was calculated as the change percentage of mean flow velocity caused by ECP, in order to evaluate the hemodynamic effects of ECP. We divided patients into good and poor outcome groups according to with 3-month mRS. mRS score between 0~2 was considered as good outcome, whereas mRS between 3~6 was regarded as poor outcome. We compared the differences between two outcome groups in terms of clinical data and hemodynamic response during ECP. Then we investigated if hemodynamic responses were the predictors for good outcome using multivariate logistic regression. Continuous data was analyzed by T-test if normally distributed and by non-parametric tests if skew distributed. Category data was analyzed by Chi-square. Significance lever was generally inferred at $p<0.05$.

**8.3 Results**

There were 36 patients with recent ischemic stroke and stroke relevant large artery occlusive disease recruited into this study. The completed ECP sessions of these patients varied from 10 to 35 times. At 3 months after ECP treatment, 21 patients were followed up with good outcome (mRS 0~2) and 15 patients poor outcome. (**Table 8.1**)

No difference except admission NIHSS, was found between two groups when comparing clinical characteristics. NIHSS on admission in good outcome group was significantly lower than poor outcome group (median 5 versus 9).

**Table 8.1** Clinical characteristics of two outcome groups

| | mRS 0~2 (21) | mRS 3~6 (15) | P value |
|---|---|---|---|
| Age (year) | 69 (43 ~ 81) | 72 (51 ~ 81) | 0.712 |
| Gender, Male (n, %) | 20, 95.2% | 12, 80% | 0.287 |
| HT (n, %) | 18, 85.7% | 12, 80% | 0.677 |
| DM (n, %) | 8, 38.1% | 7, 46.7% | 0.607 |
| Chronic heart disease (n, %) | 2, 9.5% | 2, 13.3% | 1.000 |
| Hyperlipidaemia (n, %) | 8, 38.1% | 7, 46.7% | 0.607 |
| Previous CVA history (n, %) | 10, 47.6% | 5, 33.3% | 0.391 |
| Smoking (n, %) | 8, 38.1% | 5, 33.3% | 0.322 |
| Stroke onset to exam (day) | 5 (1 ~ 19) | 5 (1 ~ 14) | 0.893 |
| NIHSS on admission | 5 (2 ~ 11) | 9 (4 ~ 24) | 0.003* |
| Left side infarct (n, %) | 15, 71.4% | 8, 53.3% | 0.265 |
| Right side infarct (n, %) | 6, 28.6% | 7, 46.7% | 0.265 |
| ECP duration (hour) | 35 (11 ~ 35) | 35 (10 ~ 35) | 0.207 |
| Baseline HR (times/min) | 69.13 ± 12.36 | 65.77 ± 8.81 | 0.640 |
| Baseline systolic BP (mmHg) | 142.40 ± 15.31 | 143.49 ± 22.73 | 0.785 |
| Baseline diastolic BP(mmHg) | 86.88 ± 11.04 | 89.29 ± 15.69 | 0.511 |
| ECP systolic BP (mmHg) | 143.69 ± 18.96 | 150.37 ± 19.49 | 0.232 |
| ECP diastolic BP (mmHg) | 94.36 ± 11.15 | 97.69 ± 16.33 | 0.462 |
| Systolic increase (%) | 3.81 ± 5.51 | 4.81 ± 5.89 | 0.818 |
| Diastolic increase (%) | 11.73 ± 16.64 | 9.17 ± 8.03 | 0.462 |

Notes: * $p<0.05$.

**Table 8.2** Mean flow velocities at baseline and ECP in two groups

| Velocity (cm/s) | mRS 0~2 (21) | mRS 3~6 (15) | P value |
|---|---|---|---|
| ipsilateral | | | |
| Baseline MV | 64.08 ± 23.07 | 53.93 ± 21.72 | 0.092 |
| ECP MV | 65.93 ± 23.16 | 54.75 ± 20.24 | 0.069 |
| Ipsilateral CAI | 3.43 ± 6.16 | 3.01 ± 5.99 | 0.614 |
| contralateral | | | |
| Baseline MV | 60.34 ± 31.02 | 59.50 ± 21.37 | 0.688 |
| ECP MV | 62.99 ± 31.92 | 60.83 ± 19.96 | 0.760 |
| Contralateral CAI | 4.57 ± 4.34 | 3.42 ± 5.99 | 0.344 |

Notes: Mann-Whitney test was used to compare differences between good outcome and poor outcome group.

Mean flow velocity of both groups increased during ECP on both cerebral sides compared with baseline. Mean flow velocity of good outcome group on the ipsilateral infarct side was relatively higher than that of poor outcome groups either at baseline or under ECP, but the difference did not research the significant level. (**Table 8.2**) The flow velocity data were similar on contralateral sides of two groups. There was no difference of CAI on ipsilateral or contralateral side of two groups.

**8.4 Discussion**

Patients in good outcome group have relative higher flow velocity at baseline and during ECP on the ipsilateral side. But CAI, the increase percentage induced by ECP, is similar in two outcome groups. And the cerebral flow velocities at baseline or during ECP do not correlate with the clinical outcome of counterpulsation-treated ischemic stroke patients. During the investigation of ECP on cerebral circulation of ischemic stroke patients, we observe different hemodynamic responses to ECP. Some patients may have dramatic changes on TCD waveform but the mean flow velocities just slightly increase. On the other side, some patients may perceive more than 20% remarkable increase of mean flow velocities. According to current results, we can not tell the prognosis of patients after treatment from the flow changes during the first session of ECP. Ischemic stroke events may cause fluctuant systemic stress reaction during acute phase both physically and mentally. The admission blood pressure of ischemic stroke patients also varies in a wide range. This result is based on the investigation of first ECP session. The cerebral hemodynamics at this early stage may be not stable enough to provide reliable information of ECP responses to predict clinical outcome. Further studies

in a more steady recovery phase rather than acute hospitalization phase may discover promising hemodynamic parameters.

Admission NIHSS is the only predictor found for good outcome after ECP treatment based on these 36 ischemic stroke patients. Lower NIHSS score, better clinical outcome. Higher NIHSS indicates severe neurologic deficits caused by stroke attack, which strongly influences the development, recovery and prognosis of ischemic stroke.

There are several limitations in this pilot study. First, this is a study of relative small group of patients. Second, we mainly focus on the changes of mean flow velocity during ECP. Maybe other parameters, such as peak augmentation diastolic velocity, pulsatility index or resistance index, could reveal more interesting findings in the prediction of clinical outcome after external counterpulsation.

In summary, we do not find specific hemodynamic response pattern to predict outcome of ECP treatment based on flow data of first ECP session. Further exploration of promising hemodynamic parameters of ECP-treated stroke patients may help to identify good responders to ECP treatment.

# CHAPTER 9 LONGER TREATMENT DURATION IS ASSOCIATED WITH BETTER FUNCTIONAL OUTCOME OF COUNTERPULSATION-TREATED ISCHEMIC STROKE PATIENTS

## 9.1 Background

External counterpulsation is a noninvasive and effective method for augmenting cerebral perfusion. In ECP system, there are three pairs of pneumonic cuffs applied to the calves, lower thighs, and upper thighs. ECG triggers cuff inflation sequentially from distal to proximal during diastole and releases cuff pressure before the start of systole. Diastolic pressure on the lower extremities improves venous return and cardiac output, while deflation before systole leads to increased systolic unloading. Therefore, ECP could help to increase perfusion of vital organs, such as brain, liver and kidney. [6, 217] Nowadays ECP is widely accepted as a safe and highly beneficial treatment for angina pectoris [4], and it is investigated for ischemic stroke patients with large artery stenosis. [5] The standard duration of external counterpulsation is generally several weeks (5 daily 1 hour sessions each week for 7 weeks, for a total of 35 sessions), based on empiric data from studies in China. [156] However, there is limited published data about the influence of the duration of the ECP treatment on treatment effects. Lawson WE et al. reported that 21.7% angina patients in an incomplete treatment course group had at least one Canadian Cardiovascular Society (CCS) class reduction compared with 83.4% responders in a complete treatment group. [167] Data from the International EECP Patient Registry showed that additional extended therapy (more than 35 hours) or even repeat treatment

was proved to help patients achieve further symptom improvement. [156, 166-167] A short course of 10 sessions ECP therapy before high risk coronary artery bypass graft could improve myocardial perfusion and left ventricular function. [141] But the effect of ECP treatment duration on stroke patients is not known.

Our previous study found that ECP may help the recovery of ischemic stroke patients with large artery occlusive disease. [6] Previous reports have used the data from ECP registry to investigate various effects of ECP. [7-8] We aim to explore the effects of ECP on ischemic stroke from our ECP registry, which includes stroke patients only. The purpose of this study is to discover the predictors of good functional outcome for ECP-treated ischemic stroke patients. We proposed to retrospectively find out the significant predictors in different outcome groups then identify those independent factors in the multivariate model.

## 9.2 Methods

### 9.2.1 Subjects

We recruited consecutive patients with recent ischemic stroke and cerebral large artery stenosis, and treated them with ECP at the Prince of Wales Hospital, Hong Kong. Acute ischemic stroke was diagnosed according to WHO criteria, and subjects were included in the study if there was at least moderate carotid or cerebral large artery stenosis (>50% stenosis) by magnetic resonance angiography (MRA), computed tomography angiography (CTA), transcranial Doppler (TCD) [218], or Doppler duplex. Those with cardioembolic stroke and a history of intracranial haemorrhage were excluded. Contraindication for ECP (such as sustained hypertension, aortic aneurysm, severe

peripheral artery disease, carotid dissection, and so on), severe systemic diseases, and malignancy were also in the exclusion criteria. Among those stroke patients recruited from May 3, 2004, to April 15, 2010, 226 patients received ECP treatment using the Enhanced External Counterpulsation system, Vamed Medical Instrument Company device, model number MC2 or MC3 (Guangdong, China) with cuff inflation pressure of 150~225mmHg. The standard protocol involved 35 one-hour sessions (at least 5 times per week), but some failed to complete the whole course treatment for reasons described below. All patients agreed ECP treatment as an additional therapy for their routine medical treatment and signed informed consent.

Since most patients (188 cases, 83.2%) completed at least 10 hourly sessions of ECP and 10 sessions of ECP therapy were suggested beneficial to surgical outcome after coronary artery bypass graft [141], we included these 188 patients in the next step, as shown in **Figure 9.1**. We followed up patients at three months after treatment with the modified Rankin Scale at clinic visits; 155 patients successfully completed the three-month follow up while 33 did not. Among those 155 patients, 112 completed all 35 sessions, and 43 did not. Of the 43 patients who did not receive all 35 sessions of ECP treatment, 16 patients (37.2%) failed to complete whole course because of lack of social support for outpatient attendance after discharge from the hospital (**Table 9.1**). Eight patients (18.6%) declined further treatment after their neurological deficit improved. Four patients (9.3%) discontinued treatment because they could not tolerant the adverse effects, such as leg pain, haematuria, and skin abrasion. Seven patients (16.3%) stopped treatment because of other comorbidities such as depression, ventricular ectopic beat, and renal failure. Six patients (14%) declined to continue treatment for personal reasons and two

for 4.6% reasons unknown. At the three-month follow-up, one patient had developed cardiovascular disease, and one patient had died from renal failure (neither complete 35 sessions of ECP). No patients had TIA or recurrent stroke.

9.2.2 Data Analysis

We dichotomized the three-month follow-up modified Rankin Scale (mRS) into good (mRS 0~2) and bad (mRS 3~6). We compared the demographics differences (e.g., age, gender and vascular risk factors) between two groups, as well as medical history, medications used, and the number of ECP sessions completed. Continuous data was analyzed by independent-samples T tests when there was a normal distribution and by Mann-Whitney Test if there was a skewed distribution. Category data was analyzed by Chi-Square test. We used multivariate logistic regression to identify the independent predictors for a better outcome among these ECP-treated stroke patients. Significance level was defined as $p<0.05$.

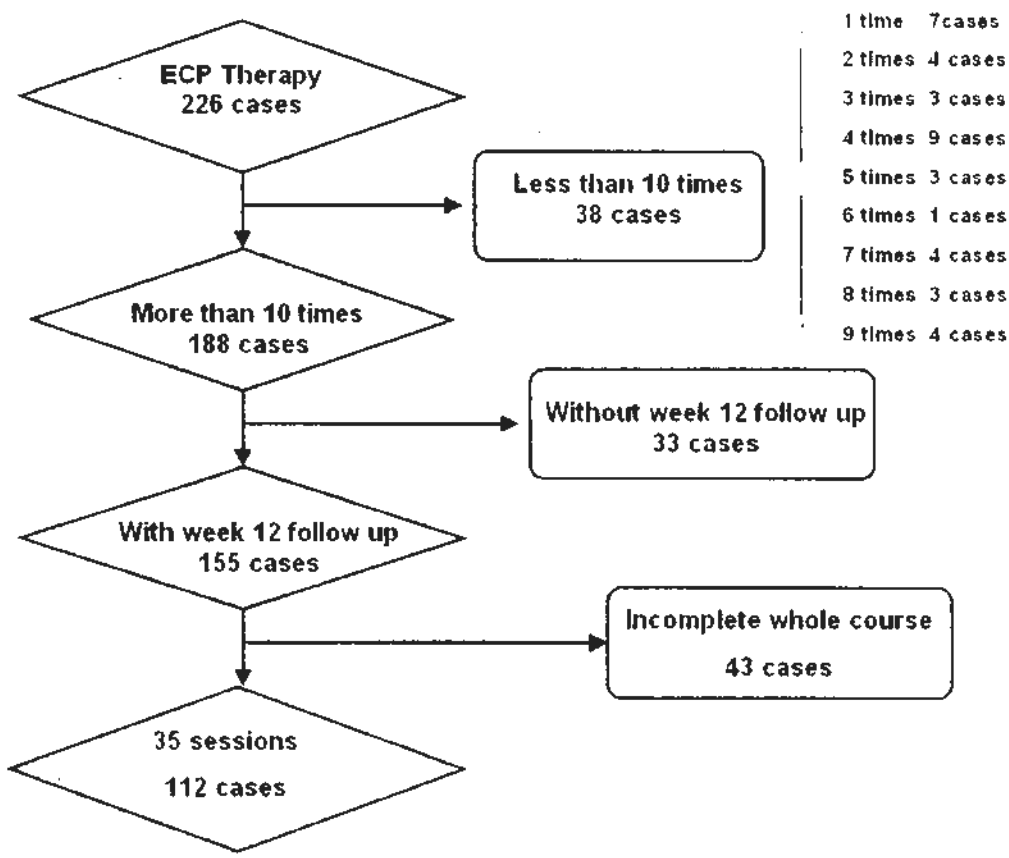


**Figure 9.1** Flow chart of Patient selection from ECP registry

**Table 9.1** Distribution of reasons for incomplete ECP treatment

| Reason | Subject numbers (43) | Percentage |
|---|---|---|
| Lack supports for outpatient treatment | 16 | 37.2% |
| Decline further treatment after neurological improvement | 8 | 18.6% |
| Intolerant adverse effect | 4 | 9.3% |
| Other upcoming clinical events | 7 | 16.3% |
| Personal reason | 6 | 14% |
| Unknown | 2 | 4.6% |

## 9.3 Results

### 9.3.1 Subject characteristics

For these 155 patients, 65.8% of them were male. The median of admission NIHSS scores of all subjects was 6. There were 94 patients (60.6%) received ECP treatment within 7 days after stroke onset, and 144 patients (92.9%) within 1 month. The median interval from stroke onset to start of ECP was 6 days. For 70.2% of these 155 patients, the relevant ischemic infarct sites were located in anterior circulation, and for 29.8% of the sites were located in posterior circulation. There were 119 (76.8%) patients verified with intracranial large artery stenosis, 6 patients with extracranial carotid stenosis and 30 (19.3%) patients with both extracranial and intracranial stenosis. Good outcome was found in 99 (63.9%) patients and bad outcome in 56 patients at the 3-month follow-up. The good outcome group patients were younger, with lower admission NIHSS, higher admission systolic blood pressure, lower total cholesterol and longer duration of ECP therapy. Patients with good outcome also had more TIA history and a longer interval from stroke onset to start of ECP treatment. Patients with bad outcome tended to use more aspirin and statin. (**Table 9.2**)

### 9.3.2 Multivariate analysis

Multivariate logistic regression showed that ECP duration (OR 1.073, 95%CI 1.012~1.137, p=0.018), admission NIHSS (OR 0.744, 95%CI 0.658~0.841, p<0.001) and admission systolic blood pressure (OR 1.024, 95%CI 1.008~1.041, p=0.003) were independent predictors for a favorable functional outcome after ECP treatment (**Table**

**9.3**). In the multivariate model, the adjusted odds ratio associated with one hour increase in ECP duration is 1.073.

The distribution of median ECP duration according to 3-month mRS scores was shown in **Figure 9.2**. In 112 patients who completed 35 hours of ECP treatment, 87.3% of patients with baseline mild stroke severity (admission NIHSS $\leq 5$) had favourable outcome at 3 months while only 49.0% of patients with moderate or severe neurologic impairment at baseline achieved better outcome ($p<0.001$, **Table 9.4**).

**Table 9.2** Clinical characteristics of subjects in two groups

| | Patient (155) | mRS 0~2 (99) | mRS 3~6 (56) | P value |
|---|---|---|---|---|
| Age (year) | 69 (43~88) | 67.28 ± 9.41 | 71.30 ± 10.75 | 0.016 * |
| Gender, Male (n, %) | 102, 65.8% | 69, 69.7% | 33, 58.9% | 0.175 |
| HT (n, %) | 119, 76.8% | 78, 78.8% | 41, 74.5% | 0.436 |
| DM (n, %) | 70, 45.2% | 40, 40.4% | 30, 53.6% | 0.114 |
| Chronic heart disease (n, %) | 18, 18.2% | 18, 18.2% | 7, 12.5% | 0.356 |
| LDL-C (mmol/L) | 2.98 ± 1.06 | 2.86 ± 1.04 | 3.21 ± 1.08 | 0.060 |
| HDL-C (mmol/L) | 1.20 (0.60~1.40) | 1.28 ± 0.33 | 1.28 ± 0.33 | 0.998 |
| Triglycerids (mmol/L) | 1.40 (0.40~11.70) | 1.50 (0.40~5.40) | 1.40 (0.60~11.70) | 0.289 |
| Total cholesterol (mmol/L) | 4.95 ± 1.19 | 4.80 ± 1.20 | 5.21 ± 1.15 | 0.042* |
| Previous TIA (n, %) | 22, 14.2% | 19, 19.2% | 3, 5.4% | 0.018* |
| Previous ischemic stroke (n, %) | 42, 27.1% | 23, 23.2% | 19, 33.9% | 0.150 |
| Smoking (n, %) | 49, 31.6% | 32, 32.3% | 17, 30.4% | 0.800 |
| Alcoholism (n, %) | 19, 12.3% | 13, 13.1% | 6, 10.7% | 0.659 |
| Stroke onset to ECP (day) | 6 (0~95) | 7 (0~95) | 5 (0~21) | 0.018* |
| NIHSS on admission | 6 (0~22) | 4 (0~22) | 10 (3~20) | <0.001* |
| Admission systolic BP (mmHg) | 164.70 ± 29.57 | 168.24 ± 33.03 | 158.94 ± 21.94 | 0.046* |
| Admission diastolic BP (mmHg) | 85.86 ± 15.91 | 86.94 ± 17.19 | 84.09 ± 13.54 | 0.302 |
| ECP duration (hour) | 35 (10~35) | 35 (11~35) | 35 (10~35) | 0.019* |
| Aspirin (n, %) | 129, 83.2% | 78, 78.8% | 51, 91.1% | 0.049* |
| Clopidogrel (n, %) | 24, 15.5% | 16, 16.2% | 8, 14.3% | 0.756 |
| ACE Inhibitor (n, %) | 74, 47.7% | 45, 45.5% | 29, 51.8% | 0.448 |

| | | | | |
|---|---|---|---|---|
| Beta.Blocker (n, %) | 19, 12.3% | 12, 12.1% | 7, 12.5% | 0.945 |
| Calcium channel blocker (n, %) | 28, 18.1% | 22, 22.2% | 6, 10.7% | 0.074 |
| Statin (n, %) | 115, 74.2% | 68, 68.7% | 47, 83.9% | 0.037* |

Notes: * $P<0.05$. Continuous data was presented as median as well as maximum and minimum if data was not normally distributed, and its intergroup analysis was performed by Mann-Whitney Test.

**Table 9.3** Predictive factors for 3-month outcome in multivariate logistic regression

| | OR | 95% CI | | P value |
|---|---|---|---|---|
| | | Lower | Upper | |
| Admission NIHSS | 0.744 | 0.658 | 0.841 | <0.001* |
| Admission systolic BP (mmHg) | 1.024 | 1.008 | 1.041 | 0.003* |
| ECP duration (hour) | 1.073 | 1.012 | 1.137 | 0.018* |
| Age (year) | | | | 0.165 |
| Total cholesterol | | | | 0.082 |
| Previous TIA | | | | 0.071 |
| Stroke onset to ECP time (day) | | | | 0.151 |
| Aspirin | | | | 0.409 |
| Statin | | | | 0.566 |

Notes: * P<0.05. ECP duration was the session times of ECP treatment.

**Table 9.4** Baseline stroke severity and 3-month clinical outcome in prolonged treatment group

| | mRS 0~2 | mRS 3~6 | Total |
|---|---|---|---|
| Admission NIHSS ≤ 5 | 55 | 8 | 63 |
| Admission NIHSS > 5 | 24 | 25 | 49 |
| Total | 79 | 33 | 112 |

Notes: Analysis was performed using Chi-square Test, $p<0.001$.

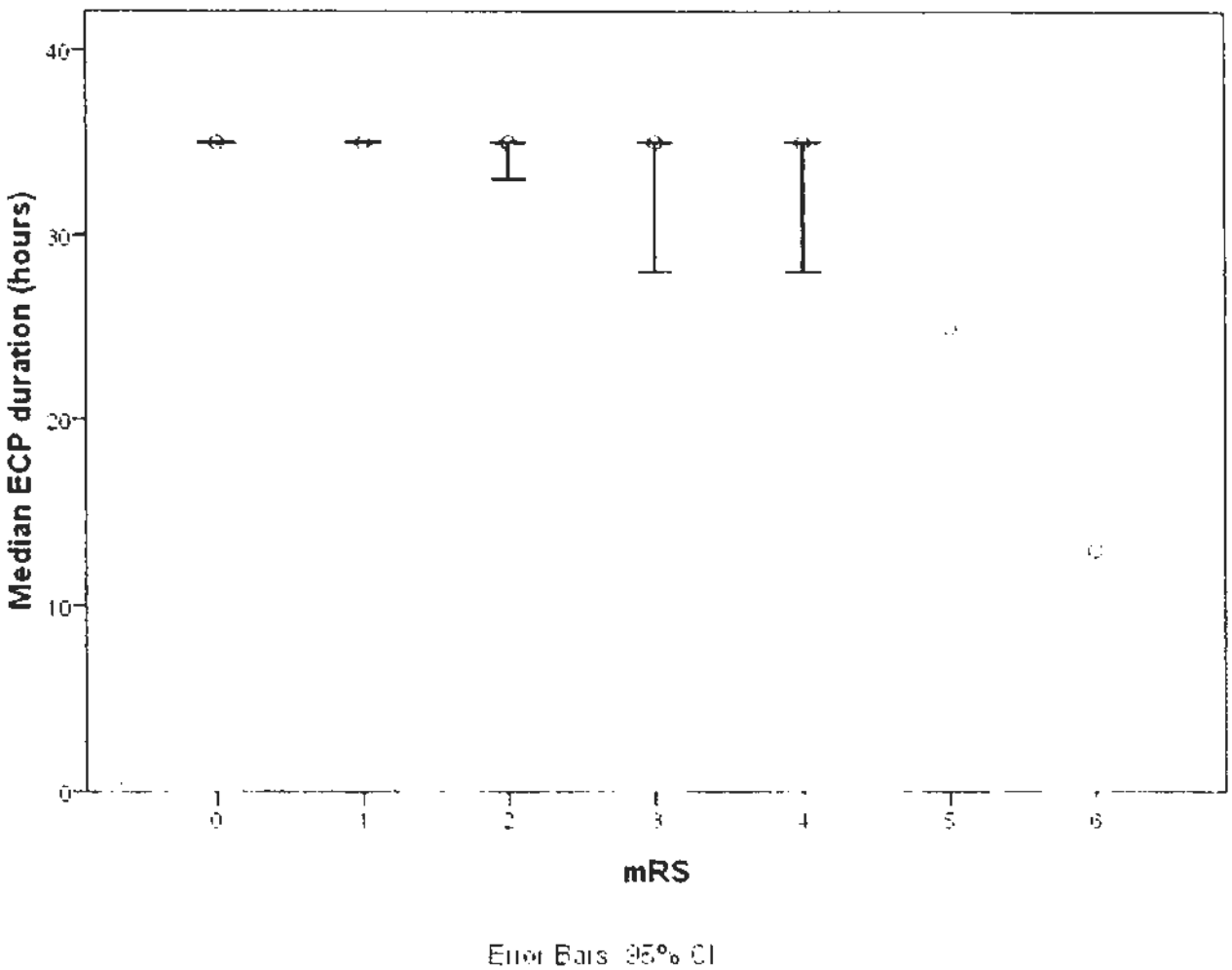


**Figure 9.2** Distribution of median ECP duration according to 3-month mRS scores. The median and its error bar are shown. Only one patient had mRS=5 and one patient had mRS=6.

### 9.4 Discussion

For the first time, the duration of ECP therapy is found to be an important predictor of stroke recovery after ECP treatment. ECP duration is independently associated with clinical outcome after adjustment for well-known predictors such as age, gender and admission NIHSS. Patients with longer treatment duration tend to have better functional outcome three months after stroke. This finding is consistent with experiments in angina pectoris, which showed that patients who completed treatment had better GCS reduction than did patients who did not complete treatment. [167] The importance of ECP duration may depend on the mechanisms of external counterpulsation. The increased arterial wall shear stress in a pulsatile manner induced by ECP improves vascular endothelial function, which is essential to the effectiveness of ECP treatment. [219] The improvement in systemic endothelial function may promote collateral recruitment and angiogensis, which may be affected by longer external counterpulsation through the activation and expression of vascular endothelial growth factor (VEGF) after 30 hours of ECP sessions, as reported. [9, 170] The modified shear stress also inhibits intimal hyperplasia and atherosclerosis progression after seven weeks of ECP, which may due to effects of ECP on proinflammatory gene expression. [185-186] Angiogenesis promotion and atherosclerosis regression caused by ECP do not occur rapidly or immediately; a long duration of treatment may be required to make a difference in these factors.

Admission NIHSS and systolic BP are also independent predictors of clinical outcome after treatment. A higher NIHSS score indicates a more severe stroke and usually indicates a poor prognosis. Patients with baseline mild stroke severity are more likely to benefit from a prolonged course of ECP treatment compared with those more severely

impaired at baseline. The prognostic value of admission blood pressure is more uncertain. Meta-analysis and some studies of all stroke subtypes found that high admission blood pressure is a predictor of bad functional outcome after stroke. [220] However, other studies have found that high admission blood pressure may be a predictor of good outcome. [221] For patients with arterial occlusive disease, high blood pressure may be beneficial. [222] Since our patients all had large artery occlusive disease, our finding that higher admission systolic blood pressure is associated with better outcome is consistent with the findings of other studies. Interestingly, ECP could reduce systolic BP in patients during the treatment and follow-up period. [196] Although other variables (TIA history, the use of Aspirin and Statin, and the interval from stroke onset to start of ECP) were shown to be significant in the univariate analysis, none of them was found to be independently associated with the clinical outcome in multivariate model. Therefore, they are considered as confounders due to relatively small sample size.

Our study has several limitations needed to be discussed. First, it is a retrospective study of a registry. Second, the number of patients included is relatively small, although this is the first report from an ECP registry for stroke patients. Third, this study lacks supports from the comparison with control group. However, there is a randomized clinical trial with conventional medical treatment group as a control to investigate ECP effects on ischemic stroke patients, which is ongoing now in our center. The results from this randomized controlled study will be more convincing. Patients with poor outcome may find it difficult to attend ECP after discharge from the hospital, so they may be less likely to complete all 35 sessions, although our finding of the importance of treatment

duration was independent of admission NIHSS. A small group of patients with progressive stroke may have different NIHSS scores at admission and start of ECP. Our study population includes a mixed group of ischemic stroke patients, such as artery-to-artery embolism, small vessel disease, and watershed strokes. It is interesting to analyze the effects of ECP on different stroke categories in future large sample size study.

In summary, we found that longer ECP duration may be associated with better clinical outcome for ischemic stroke patients.

# SECTION VI

# CHAPTER 10 AUGMENTATION REPONSES OF EXTERNAL COUNTERPULSATION ON DISTAL INTERNAL CAROTID ARTERY

## 10.1 Background

External counterpulsation (ECP) is a noninvasive, highly beneficial and long term effective treatment for ischemic heart disease. [3, 150] ECP inflates pressure on three pairs of pneumonic cuffs applied to the lower extremities during diastole and releases cuff pressure before the start of systole, resulting in diastolic augmentation. Previous studies found ECP induced hemodynamic changes of cerebral circulation through monitoring of blood flow velocity of middle cerebral artery using transcranial Doppler. [188, 200] ECP could help the recovery of ischemic stroke patients with large artery occlusive disease accompanied by the increase of cerebral blood flow. [5] The report of hemodynamic effects of ECP on cerebral circulation is rare and mainly unknown, especially in ischemic stroke patients.

Transcranial Doppler is a useful and convenient tool for cerebrovascular disease on diagnosis, prevention and prognosis. TCD monitoring is also commonly used in clinical works. However, the use of TCD to investigate vessels of anterior circulation is limited by acoustic temporal bone window of subjects. Insufficient or absent of temporal window restricts the utility of TCD. A significant number of elder subjects, particularly female elders, failed to complete TCD exam due to lack of good temporal window. To solve the problem of the limitations of TCD on anterior circulation, we are wondering if we could

investigate cerebral blood flow based on the distal internal carotid artery (ICA) from neck instead of MCA from temporal window.

We do a pilot study of ICA monitoring and aim to explore hemodynamic effects of ECP to ischemic stroke patients on distal ICA. Then further test the feasibility of ICA monitoring.

## 10.2 Methods

### 10.2.1 Subjects

We recruited recent ischemic stroke patients with large artery occlusive disease into this study. Patients were hospitalized into Acute Stroke Unit, Prince Wales of Hospital, The Chinese University of Hong Kong. They were verified with ischemic stroke due to large artery occlusive disease. The enrolled patients had good temporal windows for TCD monitoring. Exclusion criteria included intracranial hemorrhage, cardioembolic stroke, and contraindications of ECP treatment (such as sustained hypertension, aortic aneurysm and deep vein thrombosis). The clinical characteristics of all subjects were documented for analysis.

### 10.2.2 Procedure

All subjects underwent ECP treatment using the Enhanced External Counterpulsation system, model number MC3 (Vamed Medical Instrument Company device, Foshan, China) with treatment pressure 150mmHg. TCD monitoring was performed before and during ECP respectively for 3 minutes using EMS-9UA TCD system (Delica, Shenzhen,

China). In this pilot study, we just monitored MCA and ICA on the right side at the same time. M1 segment of MCA was insonated from temporal window at depth of 50~60mm through a 1.6MHz probe on a fixed headframe. Distal ICA was insonated from neck at the depth of 50~55mm through another 1.6MHz probe on a novel developed carotid frame. Mean flow velocities of MCA and ICA were recorded for analysis. We also monitor the beat-to-beat blood pressure using Task Force Monitor system during the whole procedure.

10.2.3 Data analysis

CAI was calculated as change percentage of flow velocity during ECP compared with baseline. CAIs on MCA and ICA were respectively calculated. We performed the Spearman's Correlation analysis between MCA CAI and ICA CAI. The correlations between CAI and BP changes were also analyzed using Spearman's correlation. Significance level was inferred at $p<0.05$.

**10.3 Results**

We recruited 14 cases in to this preliminary study of ICA monitoring. Mean age of these patients was 60.93 and 78.6% of patients were male. Median of admission NIHSS for these patients was 3. Baseline systolic and diastolic blood pressures were generally within normal range. The systolic blood pressure increased 6.85mmHg during ECP and diastolic blood pressure increased 4.75mmHg. (**Table 10.1**)

**Table 10.1** Subject characteristics

| | Subjects (14) |
|---|---|
| Age (year) | 60.93 ± 9.99 |
| Gender, Male (n, %) | 11, 78.6% |
| Hypertension (n, %) | 12, 85.7% |
| Diabetes Mellitus (n, %) | 5, 35.7% |
| Dyslipidaemia (n, %) | 7, 50.0% |
| History of IHD (n, %) | 1, 7.1% |
| Previous CVA history (n, %) | 6, 42.9% |
| Smoking (n, %) | 4, 28.6% |
| Alcoholism (n, %) | 2, 14.3% |
| NIHSS on admission | 3 (0~9) |
| Baseline systolic BP (mmHg) | 128.96 ± 15.69 |
| Baseline diastolic BP (mmHg) | 83.88 ± 10.23 |
| Systolic Bp increase (%) | 6.85 (-5.89 ~ 12.64) |
| Diastolic Bp increase (%) | 4.75 (-7.21 ~ 19.09) |

Mean flow velocity of MCA and ICA both decreased after ECP in this study. (**Table 10.2**) Median of CAI on MCA was -5.08 and median of CAI on ICA was -4.79. There was not significant correlation between cerebral augmentation of MCA and ICA induced by ECP. Analysis of CAI values and blood pressure changes during ECP had no significant findings. (**Table 10.3**)

**10.4 Discussion**

In this preliminary study, we newly explore the observation of cerebral blood flow from distal internal carotid artery using a novel TCD probe and neck frame. This study shows the flow velocities of MCA and ICA both decrease during ECP, and it is inconsistent with our previous studies, which reveal MCA flow velocities increase after ECP. Meanwhile MCA is the major branch of ICA, and MCA blood flow reduces when the ipsilateral ICA is compressed. However, we do not detect any correlation between the hemodynamic responses of two arteries to ECP. There are several reasons and limitations leading to this result.

**Table 10.2** Changes of mean flow velocity on MCA and ICA

| Velocity (cm/s) | MCA | ICA | Correlation P value |
|---|---|---|---|
| Baseline MV | 50.83 ± 13.94 | 32.75 ± 4.23 | |
| ECP MV | 48.42 ± 13.25 | 30.83 ± 3.92 | |
| CAI | -5.08 | -4.79 | 0.392 |
| | (-11.94 ~ 3.61) | (-17.59 ~ 9.27) | |

**Table 10.3** CAI of MCA or ICA and blood pressure changes

| | Systolic BP change (%) | Diastolic BP change (%) |
|---|---|---|
| MCA CAI | 6.85 (-5.89 ~ 12.64) | 4.75 (-7.21 ~ 19.09) |
| -5.08 (-11.94 ~ 3.61) | | |
| Correlation P value | 0.352 | 0.736 |
| ICA CAI | 6.85 (-5.89 ~ 12.64) | 4.75 (-7.21 ~ 19.09) |
| -4.79 (-17.59 ~ 9.27) | | |
| Correlation P value | 0.651 | 0.693 |

Anatomic variations of internal carotid artery exist in normal population. The location of bifurcation of carotid artery varies individually. We can not sure if the insonated ICA is the distal proportion of internal carotid artery when ICA is insonated at depth of 50~55mm. The extracranial or intracranial proportion of internal carotid artery also can't be directly distinguished from TCD waveform. The mechanism of cerebral autoregulation regulates intracranial cerebral blood flow, and the hemodynamic responses to ECP are possibly different in intracranial and extracranial system. Decrease of MCA blood flow does correlate with ICA decrease. It may due to the uncertain proportion of distal ICA.

We use 1.6MHz probe in TCD monitoring. It is distinct from conventional 2 MHz probe with more powerful penetration. Since the observation of MCA response is contrary with our previous findings using 2MHz probes. The stability and accuracy of this 1.6MHz probe system need further validation.

Due to the limited sample size, we recruited different subtypes of ischemic stroke, including atherosclerotic stroke, small vessel diseases and transient ischemic attack. The cerebral hemodynamic response of ECP on ischemic stroke patients may differ between various etiologies. This may be one of the confounding factors resulting in negative findings.

It is experimental to use a neck frame for monitoring in this study. Since there is no steady basis for ICA probe even on a carotid frame, it is quite different from MCA probe

with steady bone basis. It is easy to produce artifacts by spontaneous breathe and external intervention. Meanwhile the inflation and deflation of cuff pressures during ECP may induce slight body movement, and it may enlarge the probability of presence of artifacts during ICA monitoring.

In summary, we explore the hemodynamic response of internal carotid artery to external counterpulsation using carotid frame. We fail to observe consistent responsive changes on MCA and ICA. The use of ICA to substitute MCA in patients without good temporal window cannot be supported.

# SECTION VII

# CHAPTER 11 GENERAL DISCUSSION

External counterpulsation is a novel method to improve the perfusion of vital organs including brain, which may benefit ischemic stroke patients with large artery occlusive disease from our previous studies. Reperfusion of ischemic tissue plays an important role in the development, recovery and prognosis of ischemic stroke. This noninvasive method to improve cerebral perfusion is valuable if its applications and indications in cerebrovascular disease are approved and established. Since diastolic augmentation of ECP induces significant systemic hemodynamic changes and the corresponding cerebral hemodynamic changes are mainly unknown, this research reveals some new insights on cerebral hemodynamic effects of ECP in ischemic stroke. It explore the clinical application of this promising treatment option to ischemic stroke and further discloses some important mechanisms of potential benefits of ECP on ischemic stroke.

Results of this research show that cerebral blood flow velocities of patients with recent ischemic stroke markedly increase not only on infarct affected but also on the contralateral side. The augmentation effects of ECP on cerebral circulation in stroke patients possibly work via impaired cerebral autoregulation rather than cerebral vasoreactivity. Cerebral autoregulation is globally impaired after ischemic stroke both in the infarct ipsilateral and contralateral side. Autoregulation may be increasingly impaired over the first five days of ischemic stroke [105] and recovery over the next three months [114]. This indicates the application of ECP in the first few days after stroke may have more pronounced benefits rather than in the recovery phase of stroke events. Parallel

improvement of cerebral blood flow by ECP suggests collateral blood supply is enhanced bilaterally to ischemia territories. Due to the important role of collaterals in prognosis of stroke, collateral promotion effect of ECP may bring better clinical outcome and diminish residual neurological deficit after ischemic stroke.

The increased cerebral blood flow by ECP in patients received cerebral angioplasty of stenting is less than in patients with medical treatment at follow-up. Except its therapeutic application, ECP could be used as a new measurement tool to assess cerebral autoregulation by cerebral augmentation index. This use of ECP in cerebral circulation could noninvasively provide information of cerebral hemodynamic changes from the ability to accommodate blood flow augmentation. Cerebral augmentation index may be applied to evaluate autoregulation condition in future clinical practice.

Under treatment pressure from 150 to 262.5mmHg, mean blood flow velocities significantly increase from baseline but they do not differ from each other. Interestingly, peak diastolic augmentation velocity continuously increases as the pressure is elevated but peak systolic velocity and end diastolic velocity reversely decreases. This result discloses the real time cerebral hemodynamic changes in instant response to ECP. The optimal pressure of ECP treatment in ischemic stroke is obtained in the balance of maximal blood flow augmentation effects and minimal adverse influences. However, it just preliminarily starts in the view of cerebral blood flow velocity increase. Other endpoints, like ischemic penumbra reperfusion, neurological function, recurrent events

and long-term outcome, could be added to evaluate optimal treatment pressure in further studies.

Based on findings of this research, longer ECP treatment duration, higher admission systolic blood pressure and lower admission NIHSSS are associated with better clinical outcome at three months after stroke. Benefits of longer duration are consistent with other studies of ECP in ischemic heart disease. [166-167] These results remind us some precautions, such as appropriate blood pressure control, when we performed ECP treatment in patients with recent stroke. Current data do not suggest any predictive hemodynamic parameters correlated with favorable outcome after stroke. The cross-section observation of first ECP session therapy may not fully demonstrate the whole picture of relationships between hemodynamic response pattern and stroke outcome. Further investigations will deliver more messages on good responders of ECP treatment in cerebrovascular disease.

The pilot study of ICA monitoring does not satisfactorily explore cerebral hemodynamic effects of ECP in ischemic stroke patients. However, the exploration of new methods to handle with the limitation of insufficient acoustic window in TCD studies will not stop. A well-designed study with better equipped TCD systems and powerful analytic software is warranted in this field.

# CHAPTER 12 SUMMERY AND FUTURE WORK

In summary, the extent of cerebral blood flow augmentation during ECP in patients with recent stroke appears to be the same in the infarct side and contralateral side. The potential circulation may be enhanced by ECP to improve the collateral blood supply of ischemic territories both from the infarct ipsilateral side and contralateral side. Dynamic augmentation effects of ECP as measured by cerebral augmentation index were different from the well established vasomotor reactivity. ECP, this novel treatment concept used in ischemic stroke, may also become a new measurement tool to assess the cerebral autoregulation. This may expand its applications to evaluation benefits of interventions. 150mmHg appears to be the optimal and safe pressure to be used to reach the maximal augmentation effect. Further increase in pressure does not increase cerebral blood flow velocity. The duration of ECP therapy is found to be an important predictor for stroke recovery on ECP-treated acute ischemic stroke patients, in addition to the well-known prognostic factors such as admission NIHSS and blood pressure. These predictors help us to choose suitable ischemic stroke patients to perform ECP treatment.

In this study, we investigate the hemodynamic effects of ECP on cerebral circulation using TCD, which indirectly reflect changes of cerebral blood flow by flow velocity. The clinical application of cerebral augmentation index of ECP needs further exploration. We may apply neuroimaging techniques, such as perfusion MRI or PET scan, to more directly observe the effects of ECP on cerebral perfusion in the future.

Since ECP increases cerebral blood flow and improves cerebral perfusion through diastolic augmentation, its applications in other subtype of ischemic stroke could be explored in the future, such as hemodynamic stroke. Hemodynamic stroke is caused by hypoperfusion rather than artery-to-artery embolism or local vascular atherosclerotic disease, which can be resulted from heart failure and hypoperfusion. ECP has been proven to benefit heart failure and left ventricular dysfunction as well as improvement of systolic blood pressure. [143, 196, 223] ECP may be a promising treatment to manage hemodynamic stroke.

The beneficial mechanisms of ECP to ischemic stroke need further discussion. In addition to cerebral blood flow, other aspects like endothelial function, angiogenesis and focal atherosclerosis regression would be the future directions to investigate the application of ECP in stroke. Moreover, the complex but delicate relationship between ECP and blood pressure, this important risk factor of stroke and predictor for clinical outcome, could be further defined in later studies. Research questions, like the appropriate range of blood pressure to benefit from ECP, are interesting to be interpreted.

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# APPENDIX I NATIONAL INSTITUTES OF HEALTH STROKE SCALE (NIHSS)

| STROKE SCALE ITEM | Scoring Definitions | |
|---|---|---|
| **1a.** LOC | 0=alert and responsive<br>1=arousable to minor stimulation<br>2=arousable only to painful stimulation<br>3=reflex reponses or unarousable | |
| **1b.** LOC Questions--Ask pt's age and month. Must be exact. | 0=Both correct<br>1=One correct (or dysarthria, intubated, foreign lang)<br>2=Neither correct | |
| **1c.** Commands--open/close eyes, grip and release non-paretic hand, (Other 1-step commands or mimic ok) | 0=Both correct (ok if impaired by weakness)<br>1=One correct<br>2=Neither correct | |
| **2.** Best Gaze--Horizontal EOM by voluntary or Doll's. | 0=Normal<br>1=partial gaze palsy; abnl gaze in 1 or both eyes<br>2=Forced eye deviation or total paresis which cannot be overcome by Doll's. | |
| **3.** Visual Field--Use visual threat if nec. If monocular, score field of good eye. | 0=No visual loss<br>1=Partial hemianopia, quadrantanopia, extinction<br>2=Complete hemianopia<br>3=Bilateral hemianopia or blindness | |
| **4.** Facial Palsy--If stuporous, check symmetry of grimace to pain. | 0=Normal<br>1=minor paralysis, flat NLF, asymm smile<br>2=partial paralysis (lower face=UMN)<br>3=complete paralysis (upper & lower face) | |
| **5.** Motor Arm--arms outstretched 90 deg (sitting) or 45 deg (supine) for 10 secs. Encourage best effort. Circle paretic arm in score box | 0=No drift x 10 secs<br>1=Drift but doesn't hit bed<br>2=Some antigravity effort, but can't sustain<br>3=No antigravity effort, but even miminal mvt counts<br>4=No movement at all<br>X=unable to assess due to amputation, fusion, fx, etc. | L or R |
| **6.** Motor Leg--raise leg to 30 deg supine x 5 secs. | 0=No drift x 5 secs<br>1=Drift but doesn't hit bed<br>2=Some antigravity effort, but can't sustain<br>3=No antigravity effort, but even miminal mvt counts<br>4=No movement at all<br>X=unable to assess due to amputation, fusion, fx, etc. | L or R |
| **7.** Limb Ataxia--check finger-nose-finger ; heel-shin; and score only if out of proportion to paralysis | 0=No ataxia (or aphasic, hemiplegic)<br>1=ataxia in upper or lower extremity<br>2= ataxia in upper AND lower extremity<br>X=unable to assess due to amputation, fusion, fx, etc. | L or R |
| **8.** Sensory--Use safety pin. Check grimace or withdrawal if stuporous. Score only stroke-related losses. | 0=Normal<br>1=mild-mod unilateral loss but pt aware of touch (or aphasic, confused)<br>2=Total loss, pt unaware of touch. Coma, bilateral loss | |
| **9.** Best Language--Descr ibe cookie jar picture, name objects, read sentences. May use repeating, writing, stereognosis | 0=Normal<br>1=mild-mod aphasia; (diff but partly comprehensible)<br>2=severe aphasia; (almost no info exchanged)<br>3=mute, global aphasia, coma. No 1 step commands | |
| **10.** Dysarthria--read list of words | 0=Normal<br>1=mild-mod; slurred but intelligible<br>2=severe; unintelligible or mute<br>X=intubation or mech barrier | |
| **11.** Extinction/Neglect--simultaneously touch patient on both hands, show fingers in both vis fields, ask about deficit, left hand. | 0=Normal, none detected. (vis loss alone)<br>1=Neglects or extinguishes to double simult stimulation in any modality (vis, aud, sens, spatial, body parts)<br>2=profound neglect in more than one modality | |

# APPENDIX II MODIFIED RANKIN SCALE (mRS)

**MODIFIED RANKIN SCALE (MRS)**

Patient Name: _____

Rater Name: _____

Date: _____

| Score | Description |
|---|---|
| 0 | No symptoms at all |
| 1 | No significant disability despite symptoms; able to carry out all usual duties and activities |
| 2 | Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance |
| 3 | Moderate disability; requiring some help, but able to walk without assistance |
| 4 | Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance |
| 5 | Severe disability; bedridden, incontinent and requiring constant nursing care and attention |
| 6 | Dead |

TOTAL (0–6): _____

## APPENDIX III PUBLICATIONS AND PRESENTATIONS

Publication:

1. WH Lin, Q Hao, B Rosengarten, WH Leung, KS Wong. Impaired Neurovascular Coupling in Ischaemic Stroke Patients with Large or Small Vessel Disease. Eur J Neurol. 2011 May; 18(5):731-6.

2. Q Hao, KS Wong, WH Lin, Thomas Leung, M Kaps, B Rosengarten. Ethnic Influences on Neurovascular Coupling: A Pilot Study in Whites and Asians. Stroke. 2010 Feb; 41(2):383-4.

Presentations:

1. WH Lin, L Xiong, JH Han, XY Chen, Thomas Leung, KS Wong. **Poster Presentation** at the 20th European Stroke Conference, Hamburg, Germany. May 25, 2011. Poster Number 315. Hemodynamic Effects of External Counterpulsation on Cerebral Circulation Is A Different Measure of Impaired Cerebral Autoregulation from Vasomotor Reactivity.

2. WH Lin, Thomas Leung, Yannie Soo, Vincent Ip, Lisa Au, LC Chan, KS Wong. **Poster Presentation** at the 20th European Stroke Conference, Hamburg, Germany. May 25, 2011. Poster Number 631. Stenting Improves Cerebral Autoregulation in Stroke Patients with Intracranial Large Artery High-grade Stenosis

3. Xin Wang, WH Lin, YD Zhao, Thomas Leung, Christopher Chen, KS Wong. **Oral Presentation** at the 20th European Stroke Conference, Hamburg, Germany. May 26, 2011. The effectiveness of Dual Antiplatelet Treatment in Acute Ischemic Stroke Patients with Purely Intracranial Stenosis: a Subgroup Analysis of CLAIR Study.

4. WH Lin, L Xiong, JH Han, XY Chen, Thomas Leung, KS Wong. **Poster Presentation** at the 16th Meeting of the ESNCH 2011. Munich, Germany. May 21-22, 2011. Poster Number 52. Cerebral Hemodynamic Effect of External Counterpulsation Is A Different Measure From Vasoreactivity.

5. WH Lin, Thomas Leung, Yannie Soo, Vincent Ip, Lisa Au, LC Chan, KS Wong. **Poster Presentation** at the 16th Meeting of the ESNCH 2011. Munich, Germany. May 21-22, 2011. Poster Number 82. Stenting Improves Cerebral Autoregulation in Stroke Patients with Intracranial Large Artery Stenosis

6. WH Lin, JH Han, L Xiong, Thomas Leung, Yannie Soo, Vincent Mok, XY Chen, KS Wong. **Poster Presentation** at International Stroke Conference 2011, Los Angeles, California. Feb 9, 2011. Poster Number P19. Longer Treatment Duration is Associated with Better Functional Outcome of Counterpulsation-treated Ischemic Stroke Patients.

7. WH Lin, L Xiong, JH Han, Thomas Leung, Yannie Soo, Vincent Mok, XY Chen, KS Wong. **Poster Presentation** at International Stroke Conference 2011, Los Angeles, California. Feb 9, 2011. Poster Number P231. Optimal Pressure of External Counterpulsation for Cerebral Blood Flow Augmentation in Ischemic Stroke Patients.

8. JH Han, L Xiong, WH Lin, Yannie Soo, Thomas Leung, KS Wong. **Poster Presentation** at International Stroke Conference 2011, Los Angeles, California. Feb 9, 2011. Poster Number P223. Effects of External Counterpulsation in Patients with Progressing Ischemic Stroke and Large Artery Occlusive Disease.

9. WH Lin, JH Han, L Xiong, Thomas Leung, Yannie Soo, Vincent Mok, XY Chen, KS Wong. **Poster Presentation** at Brain 2011, Hong Kong. Jan 7-8, 2011. Poster Number

P03. Longer Treatment Duration is Associated with Better Functional Outcome of Counterpulsation-treated Ischemic Stroke Patients.

10. WH Lin, L Xiong, JH Han, Thomas Leung, Yannie Soo, Vincent Mok, XY Chen, KS Wong. **Poster Presentation** at Brain 2011, Hong Kong. Jan 7-8, 2011. Poster Number P04. Optimal Pressure of External Counterpulsation for Cerebral Blood Flow Augmentation in Ischemic Stroke Patients.

11. WH Lin, L Xiong, JH Han, Thomas Leung, Yannie Soo, Vincent Mok, XY Chen, KS Wong. **Poster presentation** at 19th European Stroke Conference, Barcelona, Spain. May 26, 2010. Poster Number 298. Flow Velocities Increases by the Same Extent in the Ischemic Hemisphere When Compared with the Contralateral Side During External Counterpulsation.

12. WH Lin, L Xiong, JH Han, Thomas Leung, Yannie Soo, Vincent Mok, XY Chen, KS Wong. **Oral Presentation** at 15th Meeting of the European Society of Neurosonology and Cerebral Hemodynamics, Madrid, Spain. May 24, 2010. Different Hemodynamic Responses to Enhanced External Counterpulsation on Ischemic Stroke Patients.

13. WH Lin, L Xiong, JH Han, Thomas Leung, Yannie Soo, Vincent Mok, XY Chen, KS Wong. **Poster Presentation** at 15th Meeting of the European Society of Neurosonology and Cerebral Hemodynamics, Madrid, Spain. May 23, 2010. Poster Number P52. Flow Velocity Increase by the Same Extent in the Ischemic Hemisphere when Compared with the Contralateral Side during Enhanced External Counterpulsation.

14. WH Lin, Q Hao, B Rosengarten, KS Wong. **Poster Presentation** at 14th Meeting of the European Society of Neurosonology and Cerebral Hemodynamics, Riga, Latvia. May

25, 2009. Poster Number P34. Impaired Cerebrovascular Reactivity in Patients with Large Intracranial Artery Stenosis.

15. Q Hao, Thomas Leung, WH Lin, KS Wong. **Oral Presentation** at 14th Meeting of the European Society of Neurosonology and Cerebral Hemodynamics, Riga, Latvia. May 25, 2009. TCD Evaluation Before and After Middle Cerebral Artery Stenting.

# APPENDIX IV IMPAIRED NEUROVASCULAR COUPLING IN ISCHEMIC STROKE PATIENTS WITH LARGE OR SMALL VESSEL DISEASE

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# Impaired neurovascular coupling in ischaemic stroke patients with large or small vessel disease

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**Keywords:**
cerebrovascular disease, functional transcranial Doppler, neurovascular coupling

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**Background and purpose:** There is limited data of neurovascular coupling on stroke patients, especially on comparison of different etiologies. We aim to test the hypothesis that patients with small vessel disease (SVD) are impaired on neurovascular coupling rather than stroke patient with large intracranial artery stenosis (LIAS), because small vessel is more associated with microcirculatory function. To assess microcirculatory integrity of stroke patients, we performed a functional transcranial Doppler test using a standardized visual stimulation test.

**Methods:** The neurovascular coupling was measured in the asymptomatic occipital cortex in ischaemic stroke patients with LIAS, SVD, and healthy elder controls. Bilateral posterior cerebral arteries were monitored to measure evoked flow velocity during resting and visual stimulation phase. Peak systolic flow velocity responses were recorded, and time course of hemodynamic response was modeled according to a control system analysis with the parameters gain, natural angular frequency, attenuation, and rate time.

**Results:** Reproduced for both sides, the functionally induced flow velocity changes (gain) were significantly lower in LIAS and SVD compared with controls ($P < 0.001$). Reductions in both stroke groups were in the same order. Neurovascular coupling in LIAS group did not show difference at the side of vessel stenosis compared with non-stenosis side or at different stenotic degrees.

**Conclusions:** Interestingly, both LIAS and SVD showed an uncoupling of the blood supply of active neurons. This points to an additional small vessel dysfunction in patients with LIAS.

## Introduction

In acute ischaemic stroke, cerebral hemodynamics plays a critical role in influencing severity and duration of symptoms, stroke progression, and even its outcome. Adequate blood supply of neurons does not only depend on the patency of the cerebral vessels and collateral systems but also on compensative mechanisms in the microcirculation. Stroke patients or patients at high cerebrovascular risk have reported with impaired cerebrovascular reactivity by transcranial Doppler (TCD) [1], positron emission tomography [2], $^{133}$Xenon single-photon emission computed tomography ($^{133}$Xe SPECT) [3], or functional magnetic resonance imaging (fMRI) method [4]. The impairment of cerebrovascular reactivity could predict the risk of stroke in patients with large artery occlusive disease [5]; meanwhile, it is also a risk marker for small vessel disease (SVD) [6].

The neurovascular coupling mechanism is a brain intrinsic regulative principle of the microcirculation that adapts local cerebral blood flow in accordance with the underlying neuronal activity [7]. Using a Doppler technique and applying a standardized visual stimulation task, the functionally coupled hemodynamic responses can be readily assessed in both posterior cerebral arteries (PCA) [8].

There is limited data of neurovascular coupling on stroke patients, especially on comparison of different stroke etiologies. In this study, we aim to compare the integrity of the neurovascular coupling in patients with large intracranial artery stenosis (LIAS) versus patients with SVD. Because the neurovascular unit defines the close structural and functional relationships between

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neurons and the cerebral microcirculation [9], the functional Doppler test reflects mainly on a function of small arterioles. Based on these statements, we hypothesized that patients with SVD are impaired on neurovascular coupling rather than patients with LIAS.

## Methods

### Subjects

We recruited 13 patients with LIAS and 21 with SVD. They were hospitalized because of acute ischaemic stroke in Acute Stroke Unit of The Prince of Wales Hospital, The Chinese University of Hong Kong, from July 2007 to March 2009. All patients underwent a computed tomography (CT) and MRI as well as magnetic resonance angiography (MRA) or computed tomography angiography (CTA). Patients with LIAS were verified with moderate or severe LIAS of the middle or anterior cerebral artery. The severity of artery stenosis was graded as mild (< 30%), moderate (30–70%), and severe (> 70%) according to radiological imaging by a neurologist professional on neuroimaging. Patients with PCA stenosis or signs of SVD on a MRI scan were excluded. Patients with SVD did not have signs of LIAS in the angiography, but had lacunar infarct lesions on MRI, with or without periventricular white matter change. The severity of SVD was classified by the modified Fazekas's score [10]. Patients with cardiac arrhythmias or suspected cardioembolic stroke were excluded as well as those patients with extracranial carotid or vertebrobasilar artery stenosis confirmed by carotid duplex ultrasonography and TCD. Subjects with a fetal type of supply of the P2-segment via the posterior communicating artery present on the CTA or MRA and validated by transcranial Duplex sonography were also excluded. Patients presenting with clinical symptoms related to the visual cortex or with uncorrectable vision deficits were also excluded. Functional transcranial Doppler (fTCD) test was performed during follow-up clinic visit in stroke group. All ischaemic stroke patients' clinical data were documented for study once recruited. Seventeen healthy elders were recruited as control, and they had no risk factors or history of cerebrovascular events. All subjects had sufficient cognitive function, mobility, and education level to cooperate with the fTCD tests.

For assessing the vascular risk factor profile of patients, the current blood pressure, medical history of arterial hypertension and antihypertensive medication, smoking habit, presence of a diabetes mellitus or hyperlipidaemia were obtained from medical records.

The study was approved by the institutional review committee of the Prince of Wales Hospital, The Chinese University of Hong Kong, and each volunteer gave written informed consent.

### Procedure

Two 2-MHz probes were mounted on a headframe, which was fitted individually. During tests, subjects were sitting on chairs in a quiet room wearing the headframe, and bilateral $P_2$ segments of PCA were insonated on both sides at a depth of 60 mm from temporal acoustic window. PCA blood flow velocity (including peak systolic and end-diastolic) was recorded using a Multidop T2 Doppler device (DWL, Sipplingen, Germany).

We applied a visual reading stimulation paradigm in functional TCD tests, as described previously [11]. We used a text-dominated news magazine, and subjects were instructed to read the text columns silently. The stimulation protocol consisted of 10 cycles with each cycle 1 min. Each cycle started with a 20-s resting phase followed by a 40-s stimulation phase. During the resting phases, subjects had to close their eyes and to open eyes and read silently during stimulation phases. Phase changes were signalized by a tone signal.

### Functional TCD data record and analysis

Beat-to-beat data of 10 times repeated rest-activation phases were interpolated linearly with a 'virtual' time resolution of 50 ms for averaging procedures. Data were transformed to relative data using the resting flow velocity level averaged for a time span 5 s before the beginning of the stimulation as a baseline. The baseline was set to zero. The typical hemodynamic responses in relation to stimulation can be seen in Fig. 1.

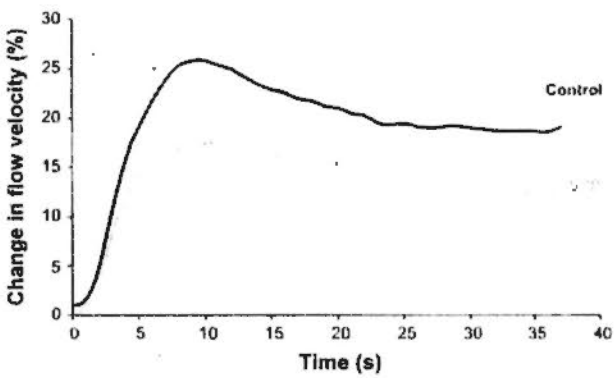


**Figure 1** Group averaged hemodynamic response curves after start of stimulation (zero) for the control group (black), small vessel disease (SVD) group (gray), and large intracranial artery disease (LIAS) group (light gray).

The method and algorithm for analyzing the data sets in terms of a control system have been described in detail previously [7]. The blood flow curve was transformed into terms of a second-order linear system with the following form G(s): $G(s) = K\ (1 + T_v\ s)/(s^2/\omega^2 + 2s\zeta/\omega + 1)$, where $K$ represents the gain; $T_v$, the rate time; $\omega$, the undamped natural angular frequency (natural frequency); and $\zeta$, the attenuation parameter. The gain is the flow velocity change from resting to visual stimulation under stable hemodynamics. The rate time specifies the steepness of initial increase in flow velocity. The undamped natural angular frequency describes the oscillatory features of the system, such as tonus and speed, and the attenuation parameter represents the dampening of the system, such as elastic properties of vascular wall.

### Statistical analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Blood pressure data were expressed as means ± standard deviations. Age, month from stroke onset to exam, degree of vessel stenosis, and Fazeka's score were expressed as median and range. Category data (gender, medical history, and medications) were expressed as frequency and percentage and tested by chi-square test. Age difference among three groups was checked using Kruskal–Wallis test. Month from stroke to exam was tested by Mann–Whitney. Doppler data were analyzed through one-way ANOVA comparing Doppler data between the different groups (LIAS; SVD; control). Fisher's protected least significant difference was applied as *post hoc* test when statistical significance occurred. In LIAS group, Mann–Whitney was performed to detect difference between stenosis side and non-stenosis side as well as different stenotic degrees. Stenosis side was defined as the relevant side with cerebral large artery stenosis. If the patient had both sides stenosis, both left-side and right-side data were recruited to stenosis side. Statistical significance level was inferred at $P < 0.05$. To assess possible side differences between the right and left cortex, the data from control and SVD were analyzed accordingly. We also did Pearson correlations (data were respectively normally distributed in three subject groups) of resting velocity and gain on account that gain was peak velocity change based on resting velocity.

## Results

### Clinical characteristics

The clinical characteristics of each group were shown in Table 1. There was no significant difference between subjects' age. In LIAS group, seven patients had left-side stenosis (7/13, 53.8%), four right-side stenosis (4/13, 30.8%), and two cases stenosis of both sides (2/13, 15.4%). Fazeka's score of SVD group was evaluated by MRI review as the group median is 1. Blood pressure during test was measured, and no significant blood pressure difference was found among different groups. Risk factors and medications did not show any distinct distribution (Table 1).

### Functional Doppler data

The results of functional TCD data between different subject groups were shown in Table 2. The resting flow

**Table 1** Clinical characteristics of subjects

| | Control | LIAS | SVD | *P* |
|---|---|---|---|---|
| Age | 59 (55–66) | 58 (44–81) | 64 (50–77) | 0.555 |
| Male | 6/17 (35.5%) | 10/13 (76.9%) | 17/21 (81.0%) | 0.552* |
| Hypertension | | 11/13 (84.6%) | 18/21 (85.7%) | 0.647* |
| Diabetes mellitus | - | 3/13 (23.1%) | 8/21 (38.1%) | 0.301* |
| Hyperlipidaemia | - | 8/13 (61.5%) | 12/21 (57.1%) | 0.800* |
| TIA | | 3/13 (23.1%) | 2/21 (9.5%) | 0.274* |
| Smoker | - | 5/13 (38.5%) | 6/21 (28.6%) | 0.549* |
| Anti-HT agent | - | 10/13 (76.9%) | 15/21 (71.4%) | 0.525* |
| Left-side stenosis | - | 7/13 (53.8%) | - | |
| Right-side stenosis | - | 4/13 (30.8%) | - | |
| Both sides stenosis | | 2/13 (15.4%) | - | |
| Fazeka's score | | | 1 (1–3) | |
| Month from stroke onset to exam | - | 7 (2–19) | 18 (2–85) | 0.228* |
| Systolic BP | 128.00 ± 2.83 | 122.67 ± 21.22 | 126.86 ± 6.77 | 0.749 |
| Diastolic BP | 76.00 ± 5.66 | 72.00 ± 13.45 | 76.14 ± 8.71 | 0.771 |

BP was expressed as means ± standard deviations; while age, month from stroke onset to exam and Fazeka's score were expressed as median and range. Category data were expressed as frequency and percentage; SVD, small vessel disease; LIAS, large intracranial artery stenosis. *comparison between LIAS and SVD.

**Table 2** Functional TCD data analysis of different subject groups

| Group | Resting velocity, cm/s | Gain, $K$ | Natural frequency, $\omega$(1/s) | Attenuation, $\zeta$ | Rate time, $T_r$ (s) |
|---|---|---|---|---|---|
| Control | 52.093 ± 12.022 | 18.803 ± 6.117 | 0.220 ± 0.056 | 0.532 ± 0.121 | 3.868 ± 3.236 |
| LIAS | 45.165 ± 10.914 | 11.487 ± 5.842 | 0.191 ± 0.062 | 0.581 ± 0.179 | 3.956 ± 2.596 |
| SVD | 45.809 ± 9.178 | 11.290 ± 6.014 | 0.192 ± 0.058 | 0.526 ± 0.245 | 2.556 ± 2.320 |
| ANOVA; *P*-value | 0.030* | < 0.001* | 0.100 | 0.544 | 0.093 |
| Control vs. LIAS | 0.024* | < 0.001* | | | |
| Control vs. SVD | 0.022* | < 0.001* | | | |
| LIAS vs. SVD | 0.827 | 0.905 | | | |

Values were expressed as means ± standard deviations. Fisher's PLSD was used in *post hoc* test; SVD, small vessel disease; LIAS, large intracranial artery stenosis; *statistical significance was inferred at $P < 0.05$.

**Table 3** Comparison between stenosis side and non-stenosis side in LIAS as well as right versus left side in SVD and controls

| Side | Resting velocity, cm/s | Gain, $K$ | Natural frequency, $\omega$(1/s) | Attenuation, $\zeta$ | Rate time, $T_r$ (s) |
|---|---|---|---|---|---|
| *LIAS* | | | | | |
| Stenosis | 48.264 ± 11.155 | 10.618 ± 6.713 | 0.189 ± 0.064 | 0.572 ± 0.162 | 3.789 ± 2.553 |
| Non-stenosis | 40.688 ± 9.374 | 12.742 ± 4.358 | 0.193 ± 0.064 | 0.594 ± 0.211 | 4.198 ± 2.792 |
| *P* value | 0.049* | 0.243 | 0.712 | 0.947 | 0.713 |
| *SVD* | | | | | |
| Right side | 46.357 ± 10.156 | 11.849 ± 7.336 | 0.189 ± 0.050 | 0.571 ± 0.226 | 2.909 ± 2.725 |
| Left side | 45.294 ± 8.436 | 10.763 ± 4.613 | 0.194 ± 0.065 | 0.484 ± 0.262 | 2.224 ± 1.886 |
| *P* value | 0.943 | 1.000 | 0.942 | 0.304 | 0.834 |
| *Control* | | | | | |
| Right side | 50.898 ± 8.924 | 18.839 ± 6.670 | 0.229 ± 0.063 | 0.517 ± 0.114 | 3.779 ± 3.697 |
| Left side | 53.458 ± 15.058 | 18.761 ± 5.668 | 0.209 ± 0.045 | 0.550 ± 0.132 | 3.969 ± 2.750 |
| *P* value | 0.934 | 0.967 | 0.392 | 0.533 | 0.662 |

Mann–Whitney was used for analysis; SVD, small vessel disease; LIAS, large intracranial artery stenosis; *$P < 0.05$.

velocity was relatively higher in elderly control than stroke patients either in LIAS or SVD, $P = 0.030$. Gain was significantly reduced in ischaemic stroke patients (both LIAS and SVD) compared with control ($P < 0.001$) and did not differ between both groups. There were no significant results comparing natural frequency, attenuation, and rate time parameter.

Comparison between stenosis side and non-stenosis side in LIAS group was given in Table 3. Resting velocity of stenosis side was higher than that of non-stenosis side ($P = 0.049$). Those parameters in the control system (gain, natural frequency, attenuation, and rate time) had unremarkable difference between two sides. Meanwhile, statistical evaluation of side differences in the control group and the SVD group did not reveal in any significant result. Moreover, there was no significant finding between moderate and severe stenotic degrees within LIAS stenosis side (Table 4).

From the correlation analysis (Table 5), resting velocity was negatively associated with gain (−0.552) in control group. However, LIAS and SVD did not present this kind of relationship.

**Table 5** Pearson correlations of resting velocity and gain in subject groups

| Group | Control | LIAS | SVD |
|---|---|---|---|
| Resting velocity (cm/s) | 52.093 ± 12.022 | 45.165 ± 10.914 | 45.809 ± 9.178 |
| Gain, $K$ | 18.803 ± 6.117 | 11.487 ± 5.842 | 11.290 ± 6.014 |
| Pearson correlation | −0.552 | 0.019 | 0.292 |
| *P* value | 0.002* | 0.932 | 0.099 |

LIAS, large intracranial artery stenosis; SVD, small vessel disease; *$P < 0.05$.

**Table 4** Comparison between different stenotic degrees in LIAS

| Stenotic degree | Resting velocity (cm/s) | Gain, $K$ | Natural frequency, $\omega$(1/s) | Attenuation, $\zeta$ | Rate time, $T_r$ (s) |
|---|---|---|---|---|---|
| Moderate stenosis | 42.603 ± 5.523 | 11.558 ± 3.679 | 0.160 ± 0.047 | 0.615 ± 0.255 | 3.598 ± 1.503 |
| Severe stenosis | 52.590 ± 13.140 | 11.407 ± 8.147 | 0.209 ± 0.073 | 0.561 ± 0.169 | 4.109 ± 3.375 |
| *P* value | 0.131 | 0.571 | 0.252 | 0.705 | 1.000 |

Mann–Whitney was used for analysis; LIAS, large intracranial artery stenosis.

## Discussion

The cerebral circulation is very important for an intact brain function. Because of lacking substrate and oxygen stores and a high metabolic rate, the brain is highly dependable on the current cerebral blood flow. It receives 15% of the cardiac output. The resistance is mainly determined by the large cerebral arteries [12]. On the microcirculatory level, two effective brain intrinsic vasoregulative principles, the cerebral autoregulation and the neurovascular coupling, govern the fine-tuned blood flow adaptation. This study innovatively uses functional TCD to evaluate the neurovascular coupling in a clinically asymptomatic cortical area in two distinct ischaemic stroke patients groups. Interestingly, we found an impaired neurovascular coupling in LIAS patients pointing to an additional dysfunction of the neurovascular coupling and therefore microcirculation. The decline in evoked flow velocity responses in the LIAS group was in the same order when compared with patients with SVD. Because the atherosclerotic disease process had affected not only the large arteries but also in the parallel small vessels, a generalized atherosclerotic disease process in patients with LIAS might be assumed. Because the MRI scans lacked signs of SVD, the disturbance might indicate the incidental risk of patients with LIAS developing SVD at the same time. It might be followed that patients with LIAS suffer from a dual cerebrovascular risk concerning of stenosis in the basal cerebral arteries and an impaired neurovascular coupling in the microcirculation. Our data might in part explain the clinical evidence that patients with LIAS have relatively severe neurological deficits, high early recurrent stroke, and worse long-term outcome [5,13–15].

Based on our present results and that of the literature, a decreased gain parameter indicates an inappropriate flow adaptation to metabolic needs of neurons, which increases the ischaemic risk of patients. It is also supported by disappeared correlations between resting flow velocity and gain in LIAS or SVD. The cerebrovascular reactivity impairment extended to clinically asymptomatic vascular territories outside the infarct site or stenotic artery area. Our data nicely match with results of Zhao *et al.* [16], who also found a generalized impairment of vasoreactivity in stroke patients using MRI continuous arterial spin labeling imaging. These findings agree with current concepts assuming atherosclerosis as a generalized disease with a focus on distinct organ systems in different patients groups [9].

The current investigation may also give a hint on therapy strategy. Recently, therapeutic regimen with either a statin therapy [17] or application of angiotensin type 1 (AT1) receptor blocker [18] showed to induce vasoreactivity recovery, which improves through pathway of nitric oxide system. Further investigations might be encouraged to investigate treatment effects on microcirculation.

The sample size of this study is small but appropriate. Because of the differences in the parameter between groups, the power of the statistical test was close to 1. Although the Doppler technique measures flow velocity rather than flow, relative flow velocity changes correlate very well with flow changes. This correlation exists because the basal cerebral vessels do not contribute to the neurovascular coupling and therefore remain constant in diameter [11].

Large intracranial artery stenosis may change global cerebral hemodynamics with collateral flow and compensation via neighboring basal arteries. The slightly higher resting velocities on the stenosis side in LIAS group might be reasonable owing to collateralization because such side-differences were not presented in the control and SVD group. No effect on the neurovascular coupling response could be found between stenosis side and non-stenosis side supporting our assumption of a rather additional microcirculatory dysfunction in this patient group. However, further research in larger study samples and patients with more severe intracranial stenosis has to be followed to investigate the role of possible collateral circulation or the relevance of distinct risk factors on the neurovascular coupling response in more details.

In conclusion, our study demonstrated an impaired neurovascular coupling in ischaemic stroke patients independent from suffering from a LIAS or SVD supporting the hypothesis of atherosclerosis as a disseminated disease. The combined large as well as SVD in patients with LIAS might reduce compensative properties in this patient group suffering stroke and therefore possibly explains their worse outcome in clinical studies.

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## Disclosure of conflict of interest

The authors declare no conflict of interest.

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