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COMPARISON OF TWO DISPERSION ARTERIAL CANNULAS IN CORONARY ARTERY BYPASS GRAFT SURGERY

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Prestando orecchio alla sapienza e inclinando il cuore all'intelligenza; se la cerchi come l'argento e ti dài a scavarla come un tesoro, allora intenderai la giustizia, l'equità, la rettitudine. Perché la sapienza t'entrerà nel cuore, e la scienza sarà gradevole all'anima tua; la riflessione veglierà te e l'intelligenza ti proteggerà.

Proverbi 2:2,4,9-11.

So that thou incline thine ear unto wisdom and thou apply thy heart to understanding; if thou seekest her as silver and searchest for her as for hidden treasures, then shalt thou understand righteousness and judgment and equity: every good path. When wisdom entereth into thy heart and knowledge is pleasant unto thy soul, discretion shall keep thee, understanding shall preserve thee.

Proverbs 2:2,4,9-11.

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LIST OF ABBREVIATIONS

ACE	Angiotensin-converting enzyme
ACS	Acute coronary syndromes
ANOVA	Analysis of Variance
ASA	Acetylsalicylic acid
BFI	Big Five Inventory
BMS	Bare Metal Stents
BP	Blood Pressure
CABG	Coronary artery bypass graft
CAD	Coronary artery disease
CP	Cannulas pressure
CPB	Cardiopulmonary bypass
DES	Drug Eluting Stents
FR	Flow rate
Hb	Hemoglobine
Hct	Hematocrit
ITA	Internal thoracic artery
LAD	Left anterior descending coronary artery
LIMA	Internal mammary artery
LITA	Left internal thoracic artery
MCA	Middle cerebral artery
MES	Microembolic signals
MHC-SF	The Mental Health Continuum–Short Form
MMSE	Mini Mental State Examination
MSPSS	Multidimensional Scale of Perceived Social Support
NSTEMI	Non ST-elevation myocardial infarctions
NYHA	New York Heart Association
PANAS	Positive and Negative Affect Schedule
PCI	Percutaneous coronary intervention
plasma fHb	Plasma free hemoglobin
PR	Peripheral Resistance
RBCs	Red blood cells
SD	Standard deviation
SE	Standard error
SF-12	Item Short Form Health Survey
STEMI	ST-elevation infarctions
SVG	Saphenous vein graft
SWLS	Satisfaction With Life Scale
TCD	Transcranial Doppler
TIA	Transient ischemic attack
TEE	Transesophageal echocardiography
WSS	Wall shear stress

ABSTRACT

Neurological complications and cognitive dysfunctions in patients undergoing coronary artery bypass graft (CABG) surgery with cardiopulmonary bypass (CPB) remain common outcomes despite CPB machines advances and introduction of new technologies. Cerebral embolic load during CABG surgery is the main hypothesis for occurrence of those events. Embolization strongly relates to patients' characteristics and comorbidities, arterial cannulation site and tip shape of the arterial cannula. Based on cannula tip shape used during surgery, the jet stream of arterial tip cannula differently affects atherosclerotic plaques and could also damage endothelium in the area where the cannula jet hits the aortic wall. Desired features of arterial cannulas, to prevent those effects, are mainly high flow with low pressure. Dispersion cannulas were designed to achieve those features. Studies described dispersion cannulas performances *in vitro* and neurological and cognitive outcomes after CABG, but none compared dispersion flow arterial cannulas performances *in vivo* and their effects on neurological and cognitive outcomes after CABG.

This project aims to evaluate performances of two dispersion flow arterial cannulas, Optiflow (Sorin Group Italia S.r.l., Italy) and EZ Glide (Edwards Lifesciences LLC, USA), *in vivo* and to study any neurological complications and cognitive dysfunctions after CABG surgery seeking any possible correlations with microembolic events. To do so, we designed a prospective, randomized (1:1), non-blinded, monocentric study. A cohort of 23 patients (Optiflow group N=11; EZ Glide group N=12) was enrolled. First, we analyzed cannulas performances during CABG surgery in terms of hemodynamic and perfusion and investigated possible hemolytic effect. We analyzed microembolic events recorded during surgery and possible neurological complications and cognitive dysfunctions after CABG both monitoring any cardiovascular and neurological events occurred after surgery and cognitive assessments at three-month follow-up visit. Finally, we sought for any possible correlation between cognitive outcomes assessed at three-month follow-up visit and number of microembolic events recorded during surgery.

Results suggested that Optiflow cannula could guarantee a better peripheral perfusion because low cannula pressures were observed during the full procedure and even during high flow rates. This observation could imply reduced risk of possible endothelium damages in the area in which the cannula jet hits the aortic wall and reduced risk of atherosclerotic plaques dislodgement. Indeed, results showed that Optiflow cannula pressure is only influenced by changes in patients' blood pressure, that could be adapted during surgery, and not by flow rate, whereas EZ Glide cannula performance is only influenced by changes in flow rate; hence, for EZ Glide cannula, in the attempt of reaching optimal perfusion we cannot exclude possible complications due to high cannula pressure induced by high flow rates. No differences on hemolytic effects were found. When we studied the microembolic events recorded from aortic cannulation to decannulation phase, we found no differences between the two study groups, but in general the number of events was higher during specific 'surgical events' such cannulation, CPB onset and aortic cross clamp removal. Despite a higher number of microembolic events recorded during those 'surgical events', none of the patients had neurological complications or cognitive dysfunctions up to three months after surgery. Psychological and neuropsychological data showed no differences between the two study groups; when the analysis focused on single patient behavior, no deficit or worsening were observed at three-month follow-up visit compared to baseline. Finally, when we tested whether neuropsychological outcomes correlated with microembolic events we found no correlation.

This pilot study showed that both Optiflow and EZ Glide dispersion flow arterial cannulas have good performances and do not correlate with any neurological complications or cognitive dysfunctions after CABG surgery. Although the Optiflow cannula promises to guarantee higher performances than EZ Glide, future studies are needed to confirm our preliminary results.

Nonostante gli elevati progressi nelle macchine cuore-polmone e l'introduzione di nuove tecnologie, le complicanze neurologiche e le disfunzioni cognitive nei pazienti sottoposti a chirurgia di bypass aorto-coronarico (coronary artery bypass graft, CABG) con bypass cardiopolmonare (cardiopulmonary bypass, CPB) sono ancora molto frequenti. L'elevato carico embolico cerebrale durante gli interventi di CABG risulta essere l'ipotesi principale per la manifestazione di tali eventi. I fenomeni microembolici correlano con le caratteristiche e le comorbidità dei pazienti, con il sito di cannulazione arteriosa e con il design della punta della cannula arteriosa. In particolare, la tipologia di cannula utilizzata durante l'intervento influenza l'erogazione del flusso sanguigno che, se colpisce in modo non fisiologico la parete aortica, potrebbe immettere placche aterosclerotiche presenti sulla parete nel circolo sanguigno e danneggiare l'endotelio nella zona in cui il flusso colpisce la parete aortica. Per evitare tali effetti, la cannula arteriosa deve principalmente erogare elevata portata garantendo bassa pressione. Le cannule a dispersione di flusso sono state progettate con l'obiettivo di garantire queste condizioni. In numerosi studi *in vitro* sono state analizzate le caratteristiche di diverse cannule a dispersione di flusso e sono stati ampiamente riportati i deficit neurologici e cognitivi dopo interventi di CABG, ma nessuno ha studiato le prestazioni di cannule a dispersione di flusso *in vivo* e gli effetti sugli outcome neurologici e cognitivi dopo interventi di CABG.

Questo progetto si propone di valutare le prestazioni di due cannule arteriose a dispersione di flusso, Optiflow (Sorin Group Italia S.r.l., Italia) e EZ Glide (Edwards Lifesciences LLC, USA), *in vivo* e di studiare eventuali complicanze neurologiche e disfunzioni cognitive dopo intervento di CABG valutando possibili correlazioni con gli eventi microembolici registrati durante l'intervento. Per raggiungere questo obiettivo è stato disegnato uno studio prospettico, randomizzato (1:1), non in cieco e monocentrico. Una coorte di 23 pazienti (gruppo Optiflow N=11; gruppo EZ Glide N=12) è stata arruolata nello studio. Inizialmente, sono state valutate le prestazioni delle due cannule durante intervento di CABG in termini di emodinamica e perfusione, ed indagato possibili effetti emolitici. Sono stati successivamente analizzati gli eventi microembolici registrati durante la chirurgia e le possibili complicanze neurologiche e disfunzioni cognitive dopo CABG riportando gli eventi cardiovascolari e neurologici raccolti dopo l'intervento chirurgico e le valutazioni cognitive a tre mesi di follow-up. Infine, sono state valutate possibili correlazioni tra gli outcome cognitivi a tre mesi ed il numero di eventi microembolici registrati durante l'intervento chirurgico.

I risultati hanno suggerito che la cannula Optiflow potrebbe garantire una migliore perfusione periferica in quanto sono state osservate basse pressioni della cannula anche durante elevate portate. Questa osservazione potrebbe suggerire una riduzione del rischio di possibili danni all'endotelio nella zona in cui il flusso colpisce la parete aortica e del rischio di immissione di placche aterosclerotiche nel circolo sanguigno. I risultati mostrano che la pressione della cannula Optiflow è influenzata solo da variazioni della pressione sanguigna dei pazienti, regolabile durante l'intervento chirurgico, e non dalla portata, mentre la pressione della cannula EZ Glide è influenzata solo da cambiamenti nella portata; in quest'ultimo caso, non possiamo escludere eventuali complicanze legate alle elevate pressioni della cannula generate per garantire una ottimale perfusione periferica. Nessuna differenza tra i due gruppi di studio è emersa sugli effetti emolitici. L'analisi degli eventi microembolici registrati tra la fase di cannulazione aortica e la decannulazione, non ha mostrato alcuna differenza tra i due gruppi di studio, ma il numero di eventi è risultato maggiore durante specifiche fasi chirurgiche come la cannulazione, l'inizio della CPB e la rimozione del clamp aortico. Nonostante sia stato registrato un numero di eventi microembolici elevato durante tali fasi chirurgiche, nessuno dei pazienti ha sviluppato complicanze neurologiche o disfunzioni cognitive fino a tre mesi dopo l'intervento chirurgico. I dati psicologici e neuropsicologici non hanno evidenziato differenze tra i due gruppi di studio; inoltre le perfomance cognitive dei singoli pazienti, non hanno mostrato deficit o peggioramenti tre mesi dopo l'intervento chirurgico. Infine, l'analisi sulla possibile correlazione tra i dati di ciascun test neuropsicologico e gli eventi microembolici non ha mostrato alcun risultato significativo.

Questo studio pilota ha evidenziato che entrambe le cannule arteriose a dispersione di flusso, Optiflow e EZ Glide, hanno ottime prestazioni e non correlano con complicanze neurologiche o disfunzioni cognitive dopo interventi di CABG. Sebbene la cannula Optiflow prometta di garantire prestazioni superiori alla cannula EZ Glide, sono necessari ulteriori studi per confermare i nostri risultati preliminari.

INTRODUCTION

1 CORONARY ARTERY DISEASE (CAD)

1.1 Pathophysiology

Coronary artery disease (CAD) is characterized by a progressive reduction of the coronary arteries lumen due to atherosclerosis. This process results in inadequate myocardial perfusion to meet metabolic demand leading to ischemia. The progressive blockage of the coronary arteries usually produces a pattern of chronic stable angina. When the major adverse event of plaque rupture, with superimposed thrombosis, occurs it can result in unstable angina, myocardial infarction, non ST-elevation myocardial infarctions (NSTEMI) or ST-elevation infarctions (STEMI). Those are defined as acute coronary syndromes (ACS) that are symptomatic CAD. Traditional risk factors are hypertension, dyslipidemia, diabetes, smoking and obesity (Bojar, 2010).

1.2 Treatments

Symptomatic CAD is initially treated with medical therapy introducing statins to control dyslipidemia, antihypertensives to control hypertension, nitrates and β-adrenergic blockers to control angina and acetylsalicylic acid (ASA) as antiplatelet therapy.

Patients presenting ACS are candidate for percutaneous coronary intervention (PCI) as angioplasty and stenting (Drug Eluting Stents, DES; Bare Metal Stents, BMS) or coronary artery bypass graft (CABG). The justification for proceeding with an intervention is based primarily upon an assessment of whether the patient is at increased risk for a serious adverse cardiac event (Bojar, 2010).

The management strategies for patients with CAD are based on the current ACC/AHA recommendation (Amsterdam et al., 2014).

2 CORONARY ARTERY BYPASS GRAFT SURGERY (CABG)

Traditional CABG is performed through a median- or mini- sternotomy incision with use of cardiopulmonary bypass (CPB). The procedure involves bypassing the coronary blockages with grafts. The left internal thoracic artery (LITA) (or internal mammary artery, LIMA) is usually used as favorite graft to the left anterior descending coronary artery (LAD) and it is complemented by a second internal thoracic artery (ITA) graft or radial artery graft to the left system and/or saphenous vein graft

(SVG) interposed between the aorta and the coronary arteries (Bojar, 2010) (Figure 1). Concerns about CPB impact on organs' functions encouraged development of "off-pump" coronary surgery. This surgery allows complete revascularization without CPB, nevertheless the advantages are controversial.

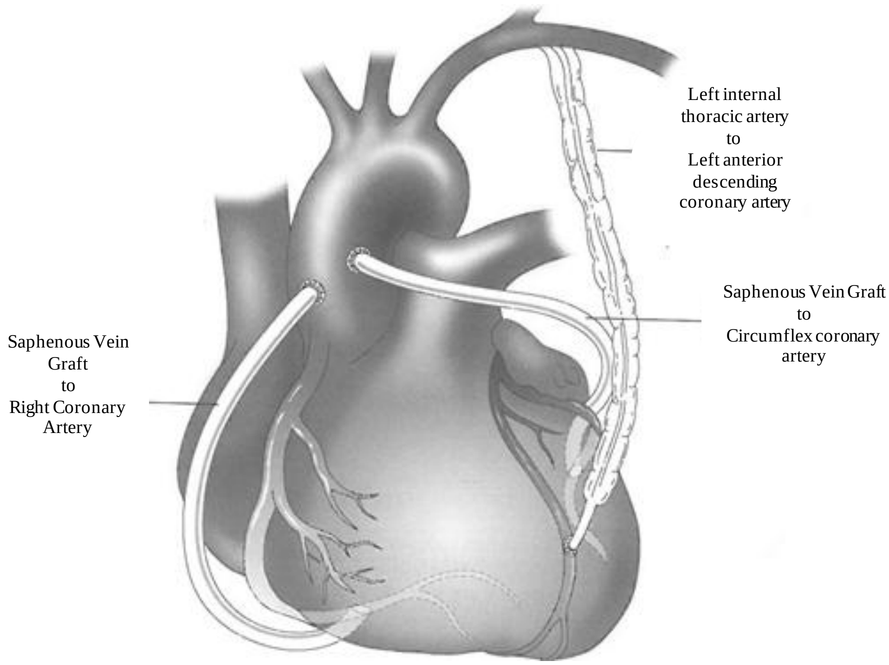


Figure 1: *Coronary artery bypass graft. A left internal thoracic artery placed to the left anterior descending coronary artery with saphenous vein grafts to the circumflex marginal and right coronary arteries*

2.1 Post-operative clinical outcomes

Operative mortality for elective CABG is in the range of 1-3%. Intraprocedural or early postoperative stroke affect 1-3% of patients (Head et al., 2013). Other important complications are postoperative myocardial infarction or injury, renal failure, deep sternal wound infection, mediastinitis, atrial fibrillation, delirium (Head et al., 2013) and cognitive dysfunction, including worsening in verbal memory and language comprehension, abstraction and visuospatial orientation, attention, psychomotor processing speed, concentration and visual memory, that affects 40% to 60% of patients in the early post-operative period (Patel at al., 2016). Re-exploration for bleeding is required in 2–6% of patients and increases the risk for all these complications.

Many of the procedural complications associated with CABG can be anticipated on the basis of the preoperative patient history, characteristics and demographics. The risk estimation is evaluated by the additive and logistic Euro-SCORE, used most frequently in Europe, and the Society of Thoracic Surgeons (STS) score, standard risk model in the USA (Head et al., 2013).

2.2 Long-term clinical outcomes

The SYNTAX and FREEDOM trials report a survival of 89%, rate of stroke from 2.5 to 5% and rates of myocardial infarction of 3.8% and 6.0% at 5-years (Mohr et al., 2013; Farkouh et al., 2012). Results from the FREEDOM trial suggest that strokes were severely disabling in 55% of diabetic patients with a stroke at any time during follow-up. In addition, results from the SYNTAX trial show that 68% of patients who suffered a stroke and survived had long-term residual deficits (Mack et al., 2013). Subjective improvement in quality of life was reported in 79.8% at 3-months and 54.8% patients were free from chest pain at 5 years (Booth et al., 1991). Cognitive dysfunction affects 20% to 40% of patients at 5 months (Patel at al., 2016).

Both post-operative and long-term clinical outcomes report neurological events as important complication after CABG. The importance of cerebral microemboli during cardiac surgery has been highlighted by several studies demonstrating a relationship between embolic load and neurological outcome (Pugsley et al., 1994; Braekken et al., 1998). Macroemboli in the arterial circulation reaching the brain can cause serious neurological damage such as stroke (Borger et al., 2001); microemboli, may cause more subtle neurological complications shown as postoperative cognitive dysfunction (Gerriets et al., 2010).

2.3 Correlation between neurological complications and cognitive dysfunctions after CABG and microembolic load during CPB

Neurological complications after CABG surgery is manifested as stroke in 1% to 3% of patients and cognitive dysfunction in 30% to 65% of patients 1 month after the surgery and in 20% to 40% of patients 5 months later (Patel at al.,2016). The finding of a relationship between early postoperative cognitive dysfunction and long-term cognitive decline highlights the importance of this complication and the need for strategies to reduce its occurrence (Newman et al., 2001). It is known that the risk for neurologic complications includes advanced patient age, systolic hypertension, prior stroke, diabetes, female gender, and atherosclerosis of the ascending aorta, but the finding of a relationship between cerebral microembolic load during CPB and cognitive dysfunction after CABG surgery supports the hypothesis that cerebral microembolism could be the cause of cognitive dysfunction (Clark et al.,1995; Sylivris et al.,1998; Fearn et al.,2001; Abu-Omar et al.,2004; Whitaker et al.,2004; Abu-Omar et al.,2006; Gerriets et al.,2010).

Since 1994 when Pugsley at al. have demonstrated a correlation between the number of microemboli recorded during surgery and the incidence of post-operative cognitive dysfunctions (Pugsley et al., 1994), numerous studies investigated this phenomenon in CABG surgery.

Cognitive dysfunction has been defined as impaired verbal or visual memory and language comprehension, impaired abstraction and spatial orientation, and decreased attention and psychomotor processing speed.

Clark et al. assessed cognitive functions in 120 patients after CABG; results showed that memory, comprehension, attention and constructional ability tests were significantly decreased 5-10 days after surgery and that patients with the highest number of emboli had the highest incidence of postoperative cognitive dysfunction (Clark et al.,1995). Also, Sylivris et al. assessed memory, psychomotor speed and mental flexibility in 32 patients after CABG showing a significant correlation between the early neuropsychological deficit and the total microembolic load during bypass (Sylivris et al., 1998). Fearn at al. used a standardized and computerized battery of tests to assess alertness, intensive vigilance and memory; results showed that the number of emboli is linked to the cause of memory deficits and that cerebral hypoperfusion impaired subsequent attention in postoperative tests 1 week, 2 and 6 months after surgery (Fearn et al., 2001). Abu-Omar et al. demonstrated that patients undergoing the use of CPB surgery have a significant reduction in prefrontal activation, which correlates with intraoperative cerebral embolic load 4 weeks after CABG. Two randomized trials, investigating the effects of neuroprotection solutions on neuropsychological functioning during CABG surgery, showed that a lower number of emboli causes a strong trend toward improving cognitive performance 6-8 weeks after surgery (Whitaker et al., 2004) and that embolization contributes to cognitive decline which is measurable 3 months after surgery (Gerriets et al., 2010).

3 ARTERIAL CANNULATION IN CARDIOPULMONARY BYPASS (CPB)

Despite the high development of the CPB circuit since its introduction by John H. Gibbon in 1953 (Gibbon, 1954), embolic dislodgements of atherosclerotic plaques pose a high risk for stroke and neurological dysfunctions (Roach et al., 1992; Blauth et al., 1992).

3.1 Aortic arch atheroma

Severe aortic arch atheroma is known to be an important risk factor for stroke and peripheral emboli with an increased risk in the elderly (Gardner al., 1985; Salomon et al., 1991). A study by Katz et al. demonstrated that patients with aortic arch atheroma had a significantly higher incidence of perioperative stroke compared to patients without significant disease during CPB. One of the patients in this study had an intraoperative stroke after the cannula going through an aortic arch plaque with a superimposed mobile thrombus was observed (Katz et al., 1992). When analyzed atheroembolic events in patients with and without severe disease of the ascending aorta after cardiac surgery a study showed that atheroembolic events occurred in 37.4% of patients in the first group and only in 2% of patients in the second group (Blauth et al., 1992). Mackensen et al. also showed that intraoperative embolic event was directly related to the atheromatous burden of the ascending aorta and aortic arch (Mackensen et al., 2003). Stern et al. studied 268 patients with severe aortic arch atheroma, showing that in 11.6% of the patients occurred stroke in the perioperative period and the in-hospital mortality, in those with intraoperative stroke, was 39%, and many of the survivors were severely disabled (Stern et al., 1999).

3.2 Arterial cannulation site

The choice of site for arterial cannulation also influences the numbers of cerebral emboli both gaseous and solid due to the aortic blood flow velocity profile (Kaufmann et al., 2009; Kaufmann et al., 2009). The distal aorta was associated with less cerebral emboli (Mullges et al., 2001; Borger et al., 1999).

3.3 Arterial cannula shapes

Another important component influencing the blood flow velocity profile in the aorta is the tip shape of the arterial cannula. The jet stream of the aortic tip cannula influences the wall shear stress (WSS) that consequently affects atherosclerotic plaques but also damages endothelium in the area where the cannula jet hits the aortic wall (Schnürer et al., 2011). Desired features of the arterial cannula are high flow with low pressure drop, low WSS, low hemolysis, non-kinking and easy insertion.

Dispersion cannulas have been designed to achieve the above features by reducing exit velocity during perfusion. Grooters et al. studied dispersion cannulas compared with a standard steel tip cannula showing a lower flow velocity profile (Grooters at al., 2003). Assmman et al. showed that dispersive cannula tips remarkably reduced the area of enhanced WSS and the absolute amount of WSS by approximately 50% compared with standard tips (Assmman et al., 2015). Schnurer et al. also concluded that dispersive aortic cannulas decrease aortic WSS and turbulence during extracorporeal circulation and may therefore reduce the risk of endothelial and blood cell damage as well as that of neurologic complications caused by atherosclerotic plaque mobilization (Schnürer et al., 2011).

3.4 Hemolysis and arterial cannulas

A well-known consequence of CPB use is the development of intravascular hemolysis (Vercaemst, 2008). It refers to hemoglobin release from ruptured red blood cells (RBCs) into the plasma characterized by an acute rise of circulatory plasma free hemoglobin (fHb).

The responsible forces for RBCs rupture during CPB have been identified in wall impact forces, blood-air interface, positive pressure, negative pressure, and shear stress, but it seems that RBCs have a high tolerance to most forces, except for shear stress (Wright, 2001). Leverett et al. also showed that the time of exposure seems to be another impacting factor related to RBCs rupture (Leverett et al., 1972). Hemolytic characteristics have been evaluated for each isolated CPB components and a high value with short duration of shear stress was found in arterial cannula and *vice versa* lower magnitudes of shear stress with longer duration was found in oxygenators and venous reservoirs.

In cannula, shear stress is in relation to the radius and length of the cannula and pressure drop over the cannula (Hessel, 1993). Pressure drop exceeding 100 mmHg are associated with excessive hemolysis and protein denaturation (Galletti, 1962). Studies showed that there is less shear stress in uniform sized cannulas and that straight tips are less hemolytic than angled tips (Ringgaard et al., 1997; Drewset al., 1974). Turbulent flow, reached at a specific fluid flow limit beyond which laminar flow becomes at first disturbed and then, at increasing flow rates, turbulent was showed to strongly contribute to blood trauma. At identical shear stress, turbulent flow produces far more blood trauma than laminar flow (Vercaemst, 2008).

4 TRANSCRANIAL DOPPLER (TCD) AND MICROEMBOLIC SIGNALS (MES) DETECTION

Transcranial Doppler (TCD) refers to a non-invasive technique, established by Aaslid et al in 1982, used to detect emboli during cardiac surgeries (Aaslid et al., 1982). The number of microembolic signals (MES) are commonly detected in the middle cerebral artery (MCA). In addition to counting the number of emboli, studies usually identify specific surgical manipulations that lead to emboli entering the cerebral circulation. A study by Barbut et al. showed that emboli detected following the removal of clamps are more likely to be clinically significant than emboli during other stages of surgery, as these have potential to contain pieces of aortic atheroma (Barbut et al., 1994).

With the technological advancement in the area of detecting emboli, it is still not possible to reliably distinguish the composition of emboli (fat, gaseous, atheroma particles, etc.). Differentiation of gaseous and solid emboli is based on principles that solid emboli would reflect more ultrasound at a higher frequency, and vice versa for gaseous emboli (Patel et al., 2016).

4.1 Gaseous emboli

Gaseous emboli contain oxygen or nitrogen; potential sources of gas entry include stopcocks, sampling and injection sites (Borger and Feindel, 2002), priming solutions, priming procedures, intravenous fluids, vents, the cardiotomy reservoir, tears or breaks in the perfusion circuit, loose purse-string sutures (especially during augmented venous return) (Willcox, 2002) rapid warming of cold blood (Geissler et al., 1997) cavitation, oxygenators, venous reservoirs with low perfusion levels (Jones et al.,2002; Mitchell et al., 1997) and the heart and great vessels. Aside from mistakes the cardiotomy reservoir is the largest source of gaseous emboli in membrane oxygenator perfusion systems.

4.2 Solid emboli

Solid emboli are related to blood products that result on clots, fibrin, platelet and platelet-leukocyte aggregation, hemolyzed red cells, cellular debris, and generation of chylomicrons, fat particles, and denatured proteins. Stored donor blood is also an important source of blood-generated particles (Lee et al., 1961). Other biologic emboli include atherosclerotic debris and cholesterol crystals and calcium particles dislodged by cannulation, manipulation for exposure, jet stream of the aortic inlet cannula, or the surgery itself.

5 OBJECTIVES

The aim of the project is to compare performances of two dispersion flow aortic cannulas, Optiflow (Sorin Group Italia S.r.l., Italy) and EZ Glide (Edwards Lifesciences LLC, USA), *in vivo* and to study any neurological complications and cognitive dysfunctions after CABG surgery.

The first objective is to compare the two study groups of patients *per* cannulas performances during CABG surgery.

- The absolute cannulas pressure values collected during the full procedure will be compared between the two groups first without taking into the analysis any patient or perfusion parameter.
- The analysis will then investigate possible influences of patients’ conditions on cannulas performances; cannulas pressure (CP) versus flow rate (FR) relationship will be analyzed under specific blood pressure (BP) and peripheral resistance (PR) conditions during CPB for both study groups.
- CP versus BP and CP versus PR relationships will be analyzed under fixed FR conditions during CPB:
- In order to include also the possible influence of patient factor on cannulas’ behavior, CP will be studied using a model including device, patient, FR, BP and PR as variables.

Second objective is to investigate possible hemolysis by studying blood samples for both study groups collected during the full procedure and 24 hours after surgery.

Third objective is to compare the two groups of patients *per* number of microembolic events recorded during the operative phase using TCD technique and investigate any possible correlation of the microembolic events with age, CPB time, carotid atheroma, aortic arch atheroma and number of grafts.

Forth objective is to compare possible neurological complications and cognitive dysfunctions after CABG in both groups of patients by monitoring any cardiovascular and neurological events occurred after surgery and cognitive assessments at three months follow-up visit.

Final objective is to analyze any possible correlation between cognitive outcomes of the two groups of patients assessed at follow-up visit and number of microembolic events recorded during the operative phase.

METHODS

6 ETHICS CONSIDERATIONS

This study was conducted in accordance with the latest version of the Declaration of Helsinki, Good Clinical Practices, ISO 14155 2011 and data protection laws and regulations.

6.1 Ethics Committee approval

The clinical study *'Eventi microembolici: confronto tra le cannule aortiche a dispersione di flusso Optiflow e EZ Glide (OPTIGLIDE)'* version 1.0 date 13/01/2015 was submitted to the *Comitato Etico degli IRCCS IEO e Monzino* and the study started only once ethical approval was obtained.

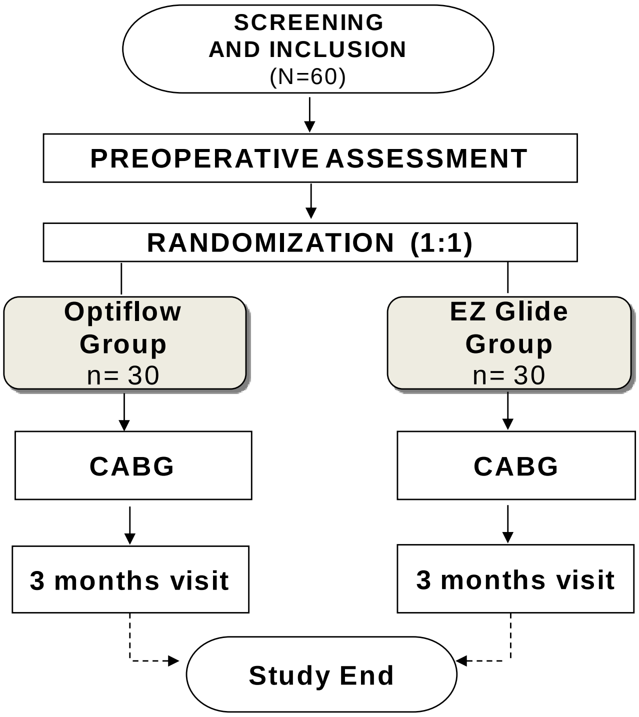
Ethics committee approval to conduct the study at Centro Cardiologico Monzino Istituto di Ricovero e Cura a Carattere Scientifico - Via C. Parea, 4 - 20138 Milano, with Prof. Gianluca Polvani as principal investigator, was obtained on February 25th, 2015 (Annex I)

6.2 Patients Informed Consent process

The patient's informed consent form (ICF) version 1.0 date 13/01/2015 (Annex II) approved by the Comitato Etico degli IRCCS IEO e Monzino contains in writing all relevant aspects pertaining to the clinical investigation, non-technical and understandable language.

The subject's informed consent was obtained and documented according to the principles of informed consent in the current version of the Declaration of Helsinki for Protection of Human Subjects, ISO 14155, and regulations. Date and time was clearly documented and always obtained before the patient's randomization.

7 GRAPHICAL STUDY DESIGN



8 STUDY FLOW CHART

Procedure	Before enrollment	Pre-operative	Operative	3 month follow-up
Screening	✓			
Informed consent	✓			
Medical history		✓		
Carotid assessment		✓		
Aortic arch atheroma assessment		✓		
Cognitive assessment		✓		✓
Surgical details			✓	
Hemodynamic and perfusion details			✓	
Transcranial doppler measurements			✓	
Blood tests			✓	
Cardiovascular and neurological events			✓	✓

9 IDENTIFICATION AND DESCRIPTION OF THE AORTIC CANNULAS

Dispersion flow aortic cannulas used in the study are described below.

9.1 EZ Glide Aortic Cannula

Cannulas designed with a tip able to disperse flow in a gentle, conical spray pattern that results in lowered exit force and line pressure, helping to prevent embolic release. Optimized tip angle provides centered flow inside the aorta, away from vessel wall. Characterized by an auto-dilating tip that enables fast and easy insertion (Figure 2A).

9.2 Optiflow Aortic Cannula

Cannulas designed with a basket-tip shape able to eliminates jet stream effects at low velocities and low pressure gradients. The tip is also designed to allow smooth insertion to reduce aortic wall shear stress affecting atherosclerotic plaque embolization (Figure 2B).

For both cannulas used, the tip size was 24Fr and the tip shape was curved.

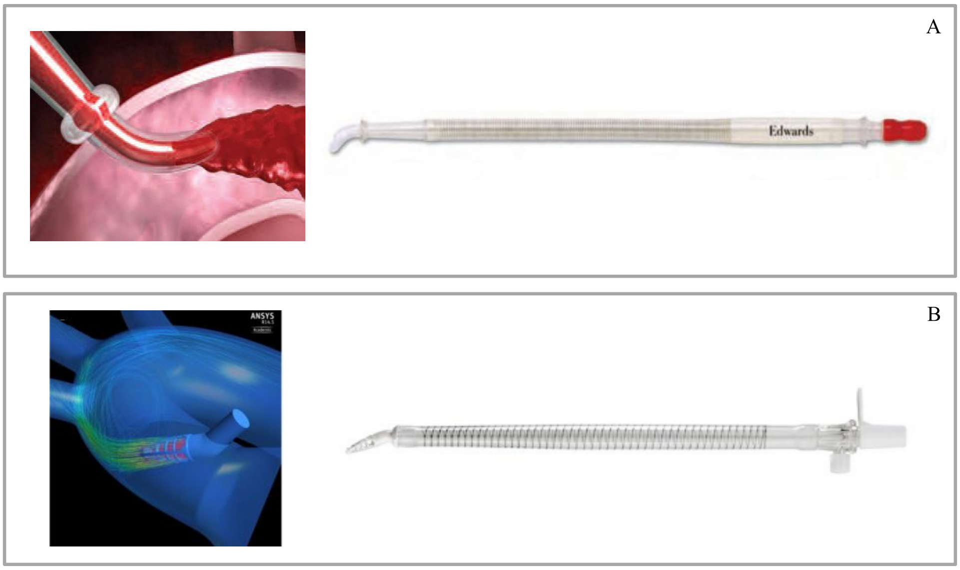


Figure 2: *Aortic cannulas. (A) EZ Glide aortic cannula (on the right), central web-like dispersion flow (on the left) (from Edwards Lifesciences website). (B) Optiflow aortic cannula (on the right), cannula outlet dispersion flow profile (on the left) (from Assmann et al., 2015)*

10 STUDY DESIGN

10.1 Trial Type

This is a prospective, randomized, non-blinded, monocentric and post market study. A total of 60 patients to be enrolled at Centro Cardiologico Monzino Istituto di Ricovero e Cura a Carattere Scientifico.

10.2 Patient Population

All subjects with CAD who are candidates for CABG according to current guidelines ACC/AHA (Amsterdam et al., 2014) in current medical practice are the intended population for inclusion in this randomized study.

10.3 Inclusion and Exclusion criteria

Inclusion criteria:

- Elective CABG surgery
- Patients' age: 50 years old ≤ age ≤ 75 years old
- Informed consent

Exclusion criteria:

- pre-existing psychiatric or neurological disease (in particular sequelae of cerebral infarction, cerebral trauma, dementia or depression)

11 STUDY PROCEDURES

11.1 Screening and Enrollment

Subjects were informed about the study including the potential risks and benefits prior to enrollment. Only those subjects who voluntarily provided written informed consent to participate, in a timely manner, were eligible for enrollment. After obtaining a written informed consent, an experienced neuropsychologist administered the following tests:

- MMSE - Mini Mental State Examination (Folstein et al., 1975; Measso et al., 1993);
- SF-12 - Item Short Form Health Survey (Ware et al., 1992; Apolone et al., 2005).

If the patients failed to obtain sufficient scores at the abovementioned tests, the patients were not enrolled in the study.

11.2 Pre-operative Assessments

11.2.1 Patients characteristics, comorbidities and therapy

Patients' characteristics, comorbidities and therapy were collected for each patient.

11.2.2 Carotid atheroma

Carotid echo-color doppler (Acuson Sequoia, Siemens Medical Solutions USA) was used for evaluating atherosclerosis of the carotid arteries. The extent of atherosclerosis was scored from 1 to 3 based on percentage of carotid stenosis:

- $0\% \leq$ score $1 \leq 30\%$
- $30\% <$ score $2 \leq 60\%$
- $60\% <$ score $3 \leq 90\%$

11.2.3 Aortic arch atheroma

Transesophageal echocardiography (TEE) was used for evaluating the presence of atheromatous disease of the aortic arch. The extent of calcification was scored as 'presence' or 'absence'.

11.2.4 Cognitive assessment

The cognitive assessment was determined by an experienced neuropsychologist, blinded to the type of cannula used during the surgery, using a battery of standardized validated neuropsychological tests.

Neuropsychological tests translated, adapted and validated in Italy:

- Visual Search (Spinnler and Tognoni, 1987);
- Phonemic verbal fluency (Novelli et al., 1986b);
- Semantic Fluency (Novelli et al., 1986b);
- Rey complex figure test (Rey, 1941; Osterrieth, 1944; Caffarra et al., 2002);
- Stroop test (Caffarra et al., 2002);
- Trail Making Test (Giovagnoli et al., 1996);
- Story recall test - "December 6th" (Spinnler and Tognoni, 1987);
- Words test (Novelli et al., 1986a);

- Digit Span Forward (Wechsler, 1939; Monaco et al., 2013);

Quality of life and subjective well-being:

- BFI - Big Five Inventory (John et al., 1991; Fossati et al., 2011).
- PANAS - Positive and Negative Affect Schedule (Watson et al.,1988; Terracciano et al., 2003).
- SWLS - Satisfaction With Life Scale (Diener et al., 1985; Lucas Carrasco et al., 2014).
- MHC-SF - The Mental Health Continuum–Short Form (Keyes, 2002; Petrillo et al., 2015).
- MSPSS - Multidimensional Scale of Perceived Social Support (Zimet et al., 1988; Prezza and Principato, 2002).

11.3 Randomization

For each patient, the randomization was assigned after enrollment and before surgery based on the randomization list (http://www.random.org). Subjects were randomly assigned in equal numbers (1:1) to one of two arms. The blocked randomization list was created before the study start.

11.4 Operative procedures

11.4.1 CABG surgery

All surgical procedures were elective. Before surgery patients were anticoagulated with anticoagulant therapy to prevent clotting in the CPB circuit.

The anesthetic technique consisted of a combination of low intermediate dose narcotics, inhalational agents, and neuromuscular-blocking drugs. Antibiotics were administered to prevent infection. Same surgeons performed the CABG operations using a median sternotomy for all patients. CABG was conducted either using the technique of constructing the distal anastomoses during a single period of aortic cross clamping and the proximal anastomoses through a single period of partial occlusion of the ascending aorta (dual-clamp technique); or both proximal and distal anastomoses were constructed using a single period of aortic cross clamping (single-clamp technique).

11.4.2 CPB settings

In order to avoid the introduction of bias related to the use of differed CPB components, the same surgical conditions were guarantee for all procedures. The CPB circuit was primed with electrolytic solution $NaHCO_3$ and heparin 25.000 U.I./5 ml (Epsoclar, Hospira italia S.r.l., Italia).

The CPB components used during the procedures were:

- Oxygenator system: Affinity Fusion (Medtronic Inc., USA);
- Centrifugal blood pump: Biomedicus (Medtronic Inc., USA);
- Venous cannula: cannula size 34/46 Fr (N.G.C. Medical S.r.l., Italia) and size 34/46 Fr (Edwards Lifesciences LLC, USA);
- Arterial cannula: randomly Optiflow (Sorin Group Italia S.r.l., Italy) or EZ Glide (Edwards Lifesciences LLC, USA);
- Cardioplegia cannula: retrograde size 14 Fr (Edwards Lifesciences LLC, USA) and antegrade size 12G (N.G.C. Medical S.r.l., Italia).

The cardioplegic solution used for all procedures was the modifies Buckberg (Edwards Lifesciences LLC, USA) delivered at 4°C temperature. This formulation serves as the crystalloid component, which is delivered 4:1 with oxygenated patient’s blood to crystalloid. Figure 3 shows the classical cannulation and aortic clamp for CABG surgery.

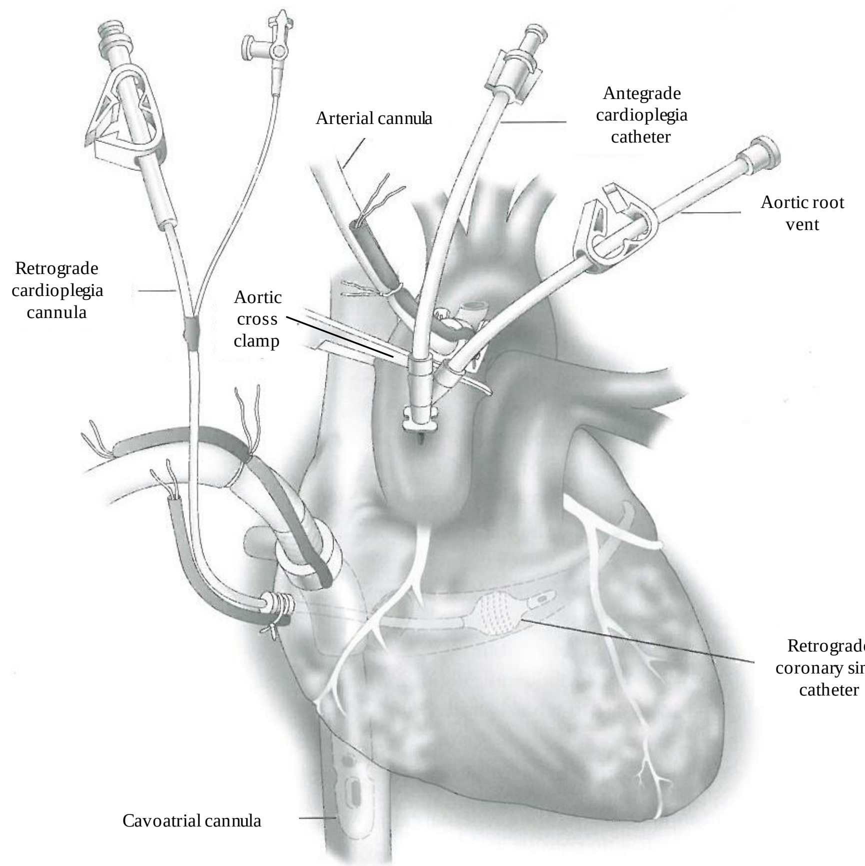


Figure 3: *Cannulation and clamping for CABG surgery. Aortic cannulation and aortic cross clamp proximal to the aortic cannulation site, venous cannulation, antegrade and retrograde cardioplegia catheters*

11.4.3 Arterial cannulation

The distal ascending aorta was the cannulation site for all procedures.

11.4.4 Patients' Temperature

Procedures were conducted in moderate hypothermic conditions. Patients' temperature was monitored at nasopharyngeal sites during the full procedure every 10 minutes.

11.4.5 Blood Tests

Blood tests including free plasma hemoglobin (mg/L), hemoglobin (g/dL) and hematocrit (%) were collected at baseline (T0) and during the full procedure 10 minutes after CPB (T1), 60 minutes after CPB (T2), CPB end (T3) and 24 hours after CABG (T4).

11.4.6 Hemodynamic and Perfusion

Perfusion line pressure (mmHg), CP (mmHg), FR (L/m) and BP (mmHg) were collected after the extracorporeal circulation start during the full procedure every 10 minutes. PR (mmHg/L/min) defined as resistance of the arteries to blood flow was calculated as mean arterial pressure*80/flow rate.

11.4.7 Surgical timing

Surgical timing (extracorporeal, cross clamp time) in minutes were recorded for all procedures.

12 TRANSCRANIAL DOPPLER

The TCD ultrasonography used was the Doppler Box X (Compumedics DWL Germany GmbH, Germany). Patients were monitored continuously beginning after aortic cannulation, 5 to 10 minutes before beginning bypass, and 10 to 15 minutes after discontinuing bypass. Two 2-MHz pulsed-wave probes, left and/or right, were used. An ad hoc helmet was placed on the patient's temple and the position was adjusted until signals were obtained from the MCA. Data were recorded continuously and stored on disk. Data was analyzed and reviewed off-line in order to detect the number of emboli, as MES, by two perfusionists.

The numbers of MES occurring during the following events were detected: cannulation, CPB onset, aortic cross clamp, aortic cross clamp off, side aortic clamp on, side aortic clamp off and decannulation. All MES occurring from cannulation to decannulation phases were recorded, difference between the total number of emboli recorded and the sum of emboli registered during the abovementioned 7 phases refers to not classified events.

13 FOLLOW-UP AT 3 MONTH

During the follow-up investigators collected information about any major cardiovascular or neurological events, especially strokes or transient ischemic attack (TIA), that occurred since the last visit.

Follow-up included cognitive assessments:

Neuropsychological tests translated, adapted and validated in Italy

- Visual Search (Spinnler and Tognoni, 1987);

- Phonemic verbal fluency (Novelli et al., 1986);
- Semantic Fluency (Novelli et al., 1986);
- Rey complex figure test (Rey, 1941; Osterrieth, 1944; Caffarra et al., 2002);
- Stroop test (Caffarra et al., 2002);
- Trail Making Test (Giovagnoli et al., 1996);
- Story recall test - "December 6th" (Spinnler and Tognoni, 1987);
- Words test (Novelli et al., 1986a);
- Digit Span Forward (Wechsler, 1939; Monaco et al., 2013);

Quality of life and subjective well-being

- PANAS - Positive and Negative Affect Schedule (Watson et al.,1988; Terracciano et al., 2003).
- SWLS - Satisfaction With Life Scale (Diener et al., 1985, Lucas Carrasco et al., 2014).
- MHC-SF - The Mental Health Continuum–Short Form (Keyes, 2002; Petrillo et al., 2015).
- MSPSS - Multidimensional Scale of Perceived Social Support (Zimet et al., 1988; Prezza & Principato, 2002).

14 STATISTICAL ANALYSIS

Differences between the Optiflow group and the EZ Glide group at baseline were studied applying the Fisher exact test using contingency table (dichotomous variables), or independent sample T-test or Kruskal-Wallis test (for continuous variables) for age, gender, New York Heart Association (NYHA) class, comorbidities, carotid atheroma, aortic arch atheroma, Logistic EuroSCORE, education, MMSE. The assumption of normal distribution was verified using the Kolmogorov-Smirnov test. If the assumptions of parametric statistics were not met, the non-parametric analyses were conducted.

To verify the first objective, the overall cannula pressure values collected during the full procedure were analyzed using a one-way Analysis of Variance (ANOVA) with independent factor 'group' and dependent variable 'cannula pressure'.

Theoretical flow rates and mean flow rates during CPB for each patient was compared using a two-way ANOVA with independent factor 'group' (Optiflow, EZ Glide) and dependent variable 'flow' (theoretical flow, CPB mean flow). CP versus FR relationship was analyzed under specific BP and PR conditions during CPB for both study groups using Pearson's linear regression model.

CP versus BP and CP versus PR relationships were analyzed under specific FR conditions during CPB for both study groups using Pearson's linear regression model.

To study possible influence of patient factor on cannulas' behavior, the dependent variable CP was studied using a linear regression mixed model with repeated measures; device, FR, BP and PR were set as fixed covariate variables and patient as random effect.

To verify the second objective, we first verified that the blood values at baseline (T0) were not significantly different between the two groups using a T-test, then we run a two-way repeated measures ANOVA with independent factor 'group' (Optiflow, EZ Glide) and dependent variable 'time' (T0, T1, T2, T3, T4).

To verify the third objective, we first studied any possible differences between the study groups among the surgery phases applying a two-way ANOVA with independent factor 'group' (Optiflow, EZ Glide) and dependent variable 'phase' (cannulation, CPB onset, aortic cross clamp, aortic cross clamp off, side aortic clamp on, side aortic clamp off and decannulation). Then we focused the analysis on each specific phase using a one-way ANOVA with independent factor 'group'. The total count of MES from cannulation to decannulation phases, as well as the total MES with no correlation, counted were also analyzed applying a one-way ANOVA with independent factor 'group'.

We investigated possible correlation of the total count of MES with age and CPB time using Pearson's linear regression model; possible correlation of the total count of MES with carotid atheroma, aortic arch atheroma and number of grafts were studied with Fisher exact test.

To verify the fourth objective, we applied a two-way ANOVA with independent factor 'group' (Optiflow, EZ Glide) and dependent variable 'time' (baseline, 3 months follow-up) to each neuropsychological test.

Finally, we investigated possible correlation of the total count of MES with each neuropsychological test result at three month follow-up.

Statistical analyses were conducted using the Statistica Software (version 5.5, StatSoft Inc.) and Prism (version 4.03 GraphPad Software Inc.).

Differences are considered significant at $p \leq 0.05$. Tukey's honest significance test was used for post hoc ANOVA analysis. If multiple comparisons, the Bonferroni correction was used.

RESULTS

15 BASELINE PATIENTS' CHARACTERISTICS

Baseline overall patient characteristics (Optiflow group N=11; EZ Glide group N=12) and baseline characteristics for patients who underwent neuropsychological assessment are summarized in Table 1. The number of patients planned was not reached because the study was approved on February 2015, but due to delay in the TCD ultrasonography supply - provided on December 2015 - the screening phase started only in January 2016. Due to inclusion/exclusion criteria and to failure to obtain patients' consent - several patients refused to participate either because of randomization or because they did not want to undergo neuropsychological assessment - the inclusion rate was 2 patients *per* month.

The Optiflow and the EZ Glide patients did not show any statistically significant difference for age (Fisher's exact test, 3 x 2 contingency table, age range 50-60, 60-70, 70-75 years, p=0.87), gender (Fisher's exact test, 2 x 2 contingency table, male and female, p>0.99), NYHA class (Fisher's exact test, 4 x 2 contingency table, NYHA class I/II/III/IV, p=0.83), Logistic EuroSCORE (one-way ANOVA: factor 'group', p=0.46), hypertension (Fisher's exact test, 2 x 2 contingency table, presence and absence, p=0.47), diabetes (Fisher's exact test, 2 x 2 contingency table, presence and absence, p=0.64), carotid atheroma (Fisher's exact test, 3 x 2 contingency table, score 1/2/3, p=0.82), aortic arch atheroma (Fisher's exact test, 2 x 2 contingency table, presence and absence, p=0.65). No patients in both groups had stoke, TIA or atrial fibrillation events in their medical history.

The sub-group of 12 patients (Optiflow N=7, EZ Glide N=5) who underwent both baseline and three month follow-up visit for psychological and neuropsychological assessment did not show any statistically significant difference for age (Fisher's exact test, 3 x 2 contingency table, age range 50-60, 60-70, 70-75 years, p>0.99), gender (Fisher's exact test, 2 x 2 contingency table, male and female, p=0.52), education (Fisher's exact test, 4 x 2 contingency table, low - medium - mid to high and high, p=0.73), MMSE (one-way ANOVA: factor 'group', p=0.87), NYHA class (Fisher's exact test, 4 x 2 contingency table, NYHA class I/II/III/IV, p=0.62), Logistic EuroSCORE (one-way ANOVA: factor 'group', p=0.78), hypertension (Fisher's exact test, 2 x 2 contingency table, presence and absence, p>0.99), diabetes (Fisher's exact test, 2 x 2 contingency table, presence and absence, p=0.46), carotid atheroma (Fisher's exact test, 3 x 2 contingency table, score 1/2/3, p>0.99), aortic arch atheroma (Fisher's exact test, 2 x 2 contingency table, presence and absence, p>0.99).

All patients were eligible to participate to the study because score for SF-12 did not show any particular condition and MMSE score was above 24, defines as pathological (Folstein et al., 1975; Measso et al., 1993).

Baseline overall patient characteristics

	Optiflow group (N=11)	EZ Glide group (N=12)
Age, years	64.3 ± 7.13	64.22 ± 7.63
Male/Female	10/1	10/2
Logistic EuroSCORE	1.23 ± 0.95	1 ± 0.53
NYHA Class I/II/II/IV	5/5/1/0	7/5/0/0
Comorbidities		
Hypertension	10 (90)	12 (100)
Diabetes	3 (27)	2 (16)
Carotid atheroma score 1/2/3 †	4/5/1	4/6/0
Aortic arch atheroma ‡	4 (57)	4 (66)
Prior stroke or TIA	0 (0)	0 (0)
Atrial fibrillation	0 (0)	0 (0)

Baseline sub-group patient characteristics

	Optiflow group (N=7)	EZ Glide group (N=5)
Age, years	62.75 ± 7.07	63.57 ± 7.16
Male/Female	6/1	3/2
Logistic EuroSCORE	1.16 ± 1.06	1.15 ± 0.64
NYHA Class I/II/II/IV	4/3/0/0	2/3/0/0
Education low/medium/mid to high/high	1/1/4/1	0/1/2/2
Comorbidities		
Hypertension	6 (85)	5 (100)
Diabetes	2 (28)	0 (0)
Carotid atheroma score 1/2/3 ††	2/3/1	1/3/0
Aortic arch atheroma ‡‡	1 (33)	1 (33)
Prior stroke or TIA	0 (0)	0 (0)
Atrial fibrillation	0 (0)	0 (0)
Neuropsychological screening test		
MMSE	28.71 ± 1.38	28.60 ± 0.89

Table 1: *Data are presented as mean ± standard deviation, N (%) or absolute numbers for each category. NYHA, New York Heart Association functional class; EuroSCORE, European System for Cardiac Operative Risk Evaluation; TIA, transient ischemic attack. MMSE, Mini Mental State Examination. † sub-group of patients underwent to carotid assessment (Optiflow group N=10; EZ Glide group N=7); ‡ sub-group of patients underwent to aortic assessment (Optiflow group N=7; EZ Glide group N=6). †† sub-group of patients underwent to carotid assessment (Optiflow group N=6; EZ Glide group N=4); ‡‡ sub-group of patients underwent to aortic assessment (Optiflow group N=3; EZ Glide group N=3)*

16 HEMODYNAMIC AND PERFUSION DURING CABG

16.1 Cannulas pressures

CP values collected during the full procedure, showed lower values for the Optiflow group compared to the EZ Glide group (mean ± standard deviation (SD), mmHg: 112.21 ± 15.80 vs 129.45 ± 19.42; one-way ANOVA, factor 'group', $p<0.00001$) (Figure 4).

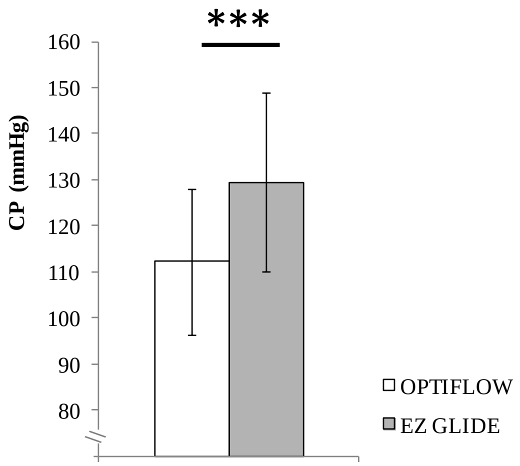


Figure 4: *Cannulas pressure (CP). Histograms represent the cannula pressure (CP, mmHg) for the Optiflow group (grand average N=119) and EZ Glide group (grand average N=100). Significantly lower CP values for Optiflow group compared to EZ Glide group. Error bars represent the standard deviation. ***p<0.00001*

16.2 Cannulas pressure versus flow rate

Theoretical flow rates and mean flow rates during CPB for each patient were compared. Data did not show any statistically significant difference between the theoretical flow rates and mean flow rates during CPB (mean ± SD, L/m: 4.57 ± 0.54 vs 4.70 ± 0.56; two-way ANOVA, factor 'flow', $p=0.09$), and any significant difference between the two study groups (two-way ANOVA, factor 'group', $p=0.59$; factors interaction, $p=0.76$).

In order to investigate possible influences of patients' conditions on cannulas performances, CP versus FR relationship was analyzed under fixed BP and PR conditions during CPB for both study groups.

16.2.1 Cannulas pressure versus flow rate under blood pressure conditions during CPB

CP versus FR relationship was analyzed under three BP conditions during CPB:

- hypotension ($40 \le BP \le 60$)
- standard BP ($60 < BP \le 75$)
- hypertensive peaks ($90 \le BP \le 110$)

Under standard patient BP condition during CPB, data showed a significant linear correlation for EZ Glide values and no correlation for Optiflow values (Pearson's correlation; EZ Glide values, r^2 =0.28, p=0.0007; Optiflow values, r^2=0.01, p=0.52) (Figure 5A).

Data did not show any statistically significant correlation for both EZ Glide and Optiflow values under hypotension condition (Pearson's correlation; EZ Glide values, r^2 =0.13, p=0.10; Optiflow values, r^2=0.02, p=0.48) neither hypertensive peaks condition during CPB (Pearson's correlation; EZ Glide values, r^2 =0.005, p=0.74; Optiflow values, r^2=0.035, p=0.33).

16.2.2 Cannulas pressure versus flow rate under peripheral resistance conditions during CPB

CP versus FR relationship was analyzed under three PR conditions during CPB:

- low ($800 \le PR \le 1200$)
- medium ($1200 < PR \le 1500$)
- high ($PR > 1500$)

Under low peripheral resistance condition during CPB, data showed a significant linear correlation for EZ Glide values and no correlation for Optiflow values (Pearson's correlation; EZ Glide values, r^2 =0.28, p=0.0003; Optiflow values, r^2=0.009, p=0.56) (Figure 5B).

Under medium peripheral resistance condition during CPB, data showed a significant linear correlation for both EZ Glide values and Optiflow values (Pearson's correlation; EZ Glide values, r^2 =0.32, p=0.0001; Optiflow values, r^2=0.35, $p<0.0001$) (Figure 5C).

Under high peripheral resistance condition during CPB, data showed no correlation for both EZ Glide values and Optiflow values (Pearson's correlation; EZ Glide values, r^2 =0.07, p=0.18; Optiflow values, r^2=0.04, p=0.19).

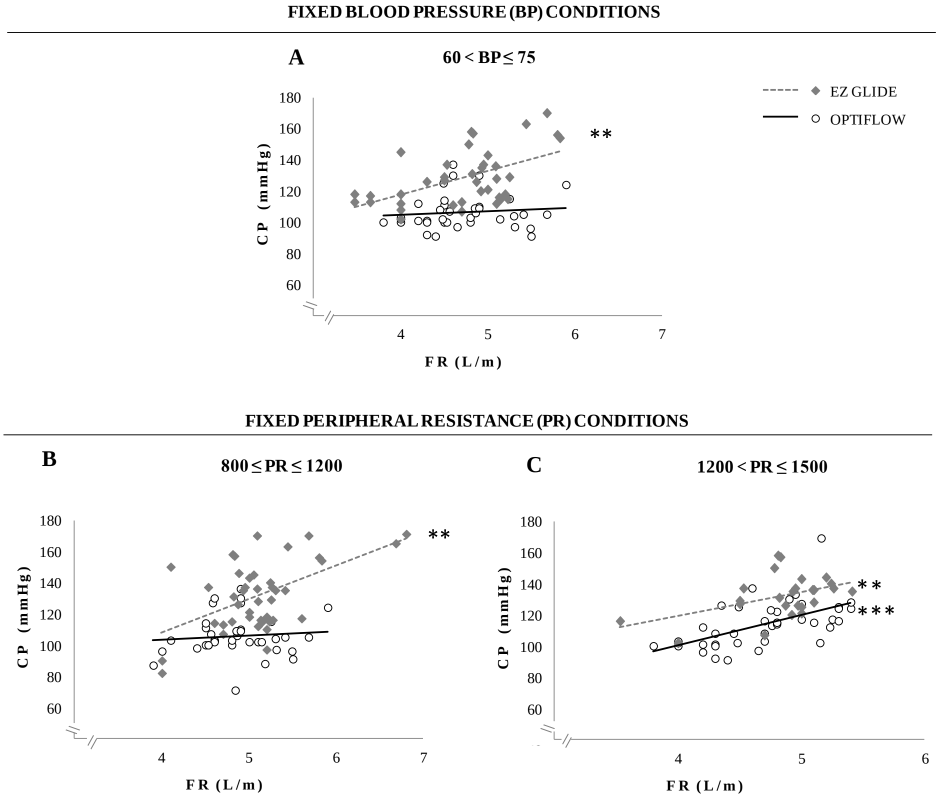


Figure 5: *Cannulas pressure (CP) versus flow rate (FR) plot. (A) CP vs FR plot under standard patient blood pressure during CPB (60 < BP ≤ 75); linear correlation for EZ Glide values was found. (B) CP vs FR plot under low peripheral resistance during CPB (800 ≤ PR ≤ 1200); linear correlation for EZ Glide values was found. (C) CP vs FR plot under medium peripheral resistance during CPB (1200 < PR ≤ 1500); linear correlation for both EZ Glide and Optiflow values was found. On the y-axis CP (mmHg) and on the x-axis FR (L/m). * p<0.05; ** p≤0.001;***p≤0.0001*

16.3 Cannula pressure versus blood pressure and versus peripheral resistance under fixed flow rate ranges

In order to investigate possible influences of patients’ characteristics on cannulas performances, CP versus BP and CP versus PR relationships were analyzed under three FR conditions during CPB:

- low (3 ≤ FR ≤ 4)
- medium (4 < FR ≤ 5)
- high (FR > 5)

16.3.1 Cannula pressure versus blood pressure under fixed flow rate ranges

Under low flow rate condition during CPB, data showed a significant linear correlation for both EZ Glide values and Optiflow values (Pearson's correlation; EZ Glide values, r^2 =0.73, p<0.0001; Optiflow values, r^2=0.53, p=0.0006) (Figure 6A).

Under medium flow rate condition during CPB, data showed a significant linear correlation for Optiflow values and no correlation for EZ Glide values (Pearson's correlation; Optiflow values, r^2 =0.23, p<0.0001; EZ Glide values, r^2=0.59, p=0.44) (Figure 6B).

Under high flow rate condition during CPB, data showed a significant linear correlation for both EZ Glide values and Optiflow values (Pearson's correlation; EZ Glide values, r^2 =0.24, p=0.004; Optiflow values, r^2=0.58, p<0.0001) (Figure 6C).

16.3.2 Cannula pressure versus peripheral resistance under fixed flow rate ranges

Under low flow rate condition during CPB, data showed a significant linear correlation for both EZ Glide values and Optiflow values (Pearson's correlation; EZ Glide values, r^2 =0.68, p<0.0001; Optiflow values, r^2=0.72, p<0.0001) (Figure 6D).

Under medium flow rate condition during CPB, data showed a significant linear correlation for Optiflow values and no correlation for EZ Glide values (Pearson's correlation; Optiflow values, r^2 =0.08, p=0.009; EZ Glide values, r^2=0.01, p=0.37) (Figure 6E).

Under high flow rate condition during CPB, data showed a significant linear correlation for Optiflow values and no correlation for EZ Glide values (Pearson's correlation; Optiflow values, r^2 =0.55, p<0.0001; EZ Glide values, r^2=0.06, p=0.17) (Figure 6F).

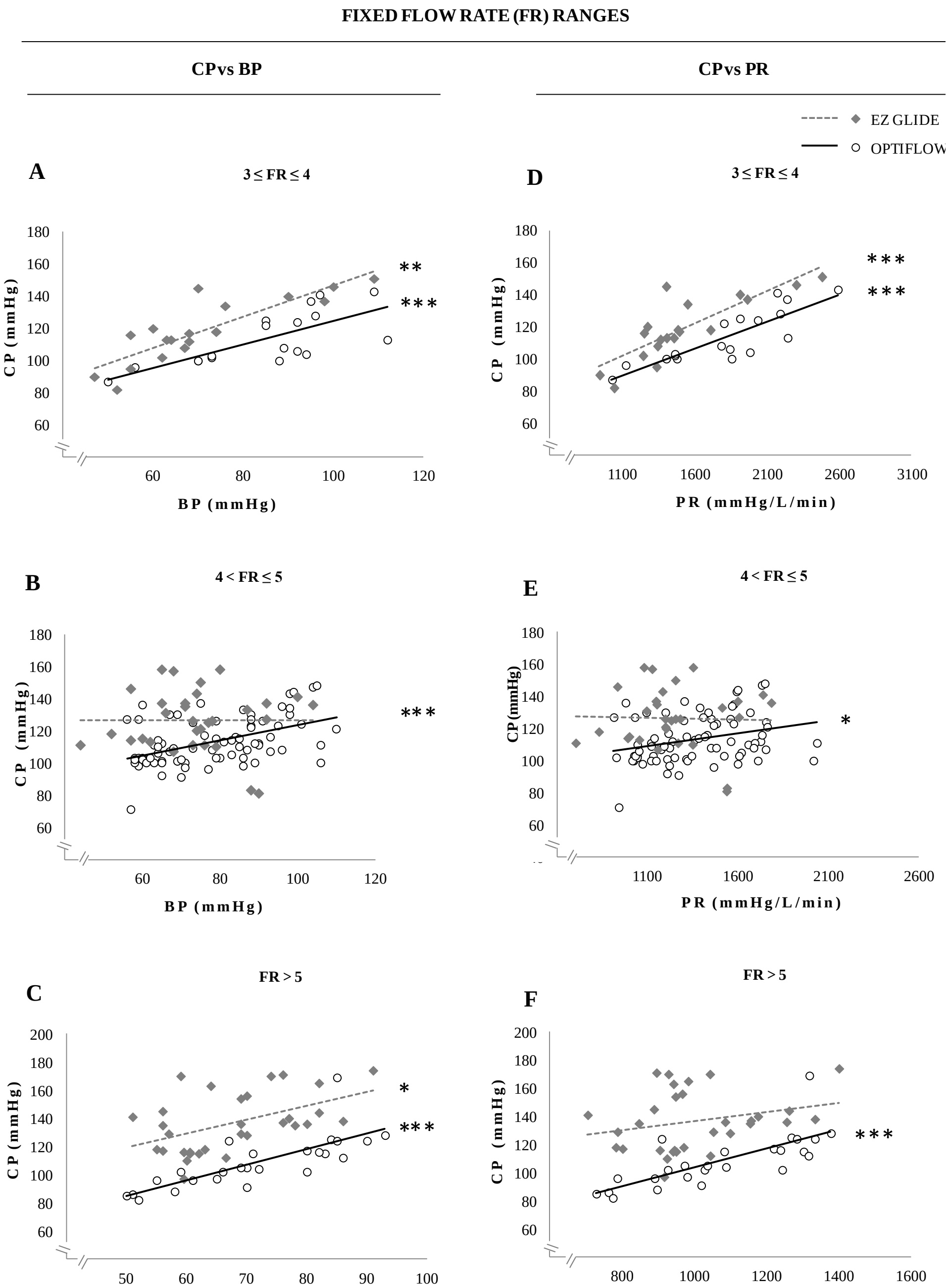


Figure 6: *Cannula pressure (CP) versus blood pressure (BP) and CP versus peripheral resistance (PR) under fixed flow rate (FR) ranges. (A) CP vs BP plot under low flow rate (3 < FR ≤ 4); linear correlation for both EZ Glide values and Optiflow values was found. (B) CP vs BP plot under medium flow rate (4 < FR ≤ 5); linear correlation for Optiflow values was found. (C) CP vs BP plot under high flow rate (FR > 5); linear correlation for both EZ Glide values and Optiflow values was found. On the y-axis the CP (mmHg) and on the x-axis the BP (mmHg). (D) CP vs PR plot under low flow rate (3 < FR ≤ 4); linear correlation for both EZ Glide values and Optiflow values was found. (E) CP vs*

*PR plot under medium flow rate ($4 < FR \leq 5$); linear correlation for Optiflow values was found. (F) CP vs PR plot under high flow rate ($FR > 5$); linear correlation for Optiflow values was found. On the y-axis the CP (mmHg) and on the x-axis the PR (mmHg/L/min). * $p<0.05$; ** $p\leq0.001$;***$p\leq0.0001$*

16.4 Summary of correlations for both EZ Glide and Optiflow under study conditions

Correlations studied for both EZ Glide and Optiflow groups under fixed study conditions are summarized in Table 2.

The Optiflow group showed significant correlation of CP vs FR relationship under medium PR condition during CPB. Correlations of CP vs both BP and PR under any fixed FR conditions were found.

The EZ Glide group showed significant correlation of CP vs FR relationship under standard BP condition during CPB and under low and medium PR conditions. Correlations of CP vs both BP and PR under low FR condition were found. Correlations of CP vs BP under high FR condition was also found.

GROUP	CORRELATIONS	CONDITIONS		
OPTIFLOW		BP (mmHg) conditions		
		40 ≤ BP ≤ 60	60 < BP ≤ 75	90 ≤ BP ≤ 110
	CP vs. FR	*Ns*	*ns*	*ns*
		PR (mmHg/L/min) conditions		
		800 ≤ PR ≤ 1200	1200 < PR ≤ 1500	PR > 1500
	CP vs. FR	*Ns*	***	*ns*
		FR (L/min) conditions		
		3 ≤ FR ≤ 4	4 < FR ≤ 5	FR > 5
	CP vs. BP	***	***	***
	CP vs. PR	***	*	***
EZ GLIDE		BP (mmHg) conditions		
		40 ≤ BP ≤ 60	60 < BP ≤ 75	90 ≤ BP ≤ 110
	CP vs. FR	*Ns*	**	*ns*
		PR (mmHg/L/min) conditions		
		800 ≤ PR ≤ 1200	1200 < PR ≤ 1500	PR > 1500
	CP vs. FR	**	**	*ns*
		FR (L/min) conditions		
		3 ≤ FR ≤ 4	4 < FR ≤ 5	FR > 5
	CP vs. BP	**	*ns*	*
	CP vs. PR	***	*ns*	*ns*

Table 2: *Correlations summary for both EZ Glide and Optiflow groups under study conditions. Cannula pressure (CP); flow rate (FR); blood pressure (BP); peripheral resistance (PR); ns, not significant, * p<0.05; ** p≤0.001; *** p≤0.0001*

16.5 Model estimation: a linear regression mixed model

The above described analyses have the limitation to use all the observations collected from each patient as independent variables. When we considered also the possible influences of patient factor for the cannulas' behavior evaluation we used a linear regression mixed model with repeated measures with device, FR, BP, PR as covariate fixed variables and the patients as random effect. The analysis showed that the dependent variable CP significantly correlates with device (p=0.001), FR (p=0.03), BP (p=0.003) and does not correlate with PR (p=0.69).

Because the device factor was significant we conducted a post hoc analysis stratified per device type. When we studied the Optiflow behavior, the analysis found a significant correlation with BP ($p<0.0001$) and no correlation with FR ($p=0.88$) and only a slight correlation with PR ($p=0.08$). When we studied the EZ Glide behavior, the analysis found a significant correlation with FR ($p=0.03$) and no correlation with BP ($p=0.79$) and PR ($p=0.48$).

17 BLOOD TESTS

17.1 Plasma free hemoglobin

Baseline plasma free hemoglobin (fHb) levels (T0) were comparable between Optiflow group and EZ Glide group (mean ± SE, mg/L: 58.91 ± 3.89 vs 76.42 ± 13.92, one-way ANOVA, factor 'group', $p=0.26$).

Plasma fHb significantly changed across the four time phases - T0, T1, T2, T3 and T4 - for both groups (two-way ANOVA, factor 'phase', $p <0.00001$), but no changes between groups (factor 'group', $p=0.59$; factors interaction, $p=0.54$). The post-hoc analysis showed that plasma fHb levels increased significantly in both groups soon 10 min after CPB start (T1) compared to T0 (mean ± SE, mg/L; Optiflow , 184.73 ± 17.31; EZ Glide, 147.17 ± 21.17, two-way ANOVA post-hoc factor 'phase', $p=0.004$), 60 minutes after CPB start (T2) (mean ± SE, mg/L; Optiflow , 245.36 ± 26.34; EZ Glide, 286.45 ± 44.59, two-way ANOVA post-hoc factor 'phase', $p=0.0001$), peaking at the end of CPB (T3) (mean ± SE, mg/L; Optiflow , 364.46 ± 54.80; EZ Glide, 391.40 ± 58.97, two-way ANOVA post-hoc factor 'phase', $p=0.0001$). After cessation of CPB, plasma fHb concentrations decreased in both groups showing no significantly differences at 24h after surgery (T4) compared to T0 (mean ± SE, mg/L; Optiflow , 60.11 ± 18.15; EZ Glide, 54.75 ± 9.91, two-way ANOVA post-hoc factor 'phase', $p=0.99$) (Figure 7A).

17.2 Hemoglobin

Baseline hemoglobin (Hb) levels (T0) were comparable between Optiflow group and EZ Glide group (mean ± SE, g/dL: 12.25 ± 0.41 vs 12.58 ± 0.50, one-way ANOVA, factor 'group', $p=0.61$).

Hb significantly changed across the four time phases - T0, T1, T2, T3 and T4 - for both groups (two-way ANOVA, factor 'phase', $p <0.00001$), but no changes between groups (factor 'group', $p=0.43$; factors interaction, $p=0.83$). During surgery, Hb levels decreased significantly in both groups 10 min (T1) after CPB start compared to T0 (mean ± SE, g/dL; Optiflow, 9.17 ± 0.45; EZ Glide, 9.29 ± 0.46, two-way ANOVA post-hoc factor ‘phase’, p=0.0001), 60 min after CPB start (T2) (mean ± SE, g/dL; Optiflow, 8.69 ± 0.32; EZ Glide, 8.29 ± 0.31, two-way ANOVA post-hoc factor ‘phase’, p=0.0001), at the end of CPB (T3) (mean ± SE, g/dL; Optiflow, 8.79 ± 0.36; EZ Glide, 9.58 ± 0.40, two-way ANOVA post-hoc factor ‘phase’, p=0.0001); after cessation of CPB, Hb concentrations increased slightly in both groups showing significantly differences still at 24h after surgery (T4) compared to T0 (mean ± SE, g/dL, Optiflow , 10.15 ± 0.63; EZ Glide, 10.27 ± 0.29, two-way ANOVA post-hoc factor ‘phase’, p=0.0001) (Figure 7B).

17.3 Hematocrit

Baseline hematocrit (Hct) levels (T0) were comparable between Optiflow group and EZ Glide group (mean ± SE, %: 35.98 ± 1.11 vs 35.92 ± 1.36, one-way ANOVA, factor ‘group’, p=0.97).

During surgery, Hct levels decreased significantly, showing the significant intraoperative hemodilution, in both groups 10 min (T1) after CPB start compared to T0 (mean ± SE, %; Optiflow , 26.46 ± 1.17; EZ Glide, 26.27 ± 1.33, two-way ANOVA post-hoc factor ‘phase’, p=0.0001), 60 min after CPB start (T2) (mean ± SE, %; Optiflow , 25.28 ± 0.83; EZ Glide, 25.62 ± 0.83, two-way ANOVA post-hoc factor ‘phase’, p=0.0001), at the end of CPB (T3) (Optiflow, mean ± SE, %: 25.80 ± 1.05; EZ Glide, 27.46 ± 1.13, two-way ANOVA post-hoc factor ‘phase’, p=0.0001); after cessation of CPB, Hct levels increased slightly in both groups showing significantly differences still at 24h after surgery (T4) compared to T0 (mean ± SE, %; Optiflow, 29.97 ± 1.94; EZ Glide, 29.58 ± 0.81, two-way ANOVA post-hoc factor ‘phase’, p=0.0001) (Figure 7C).

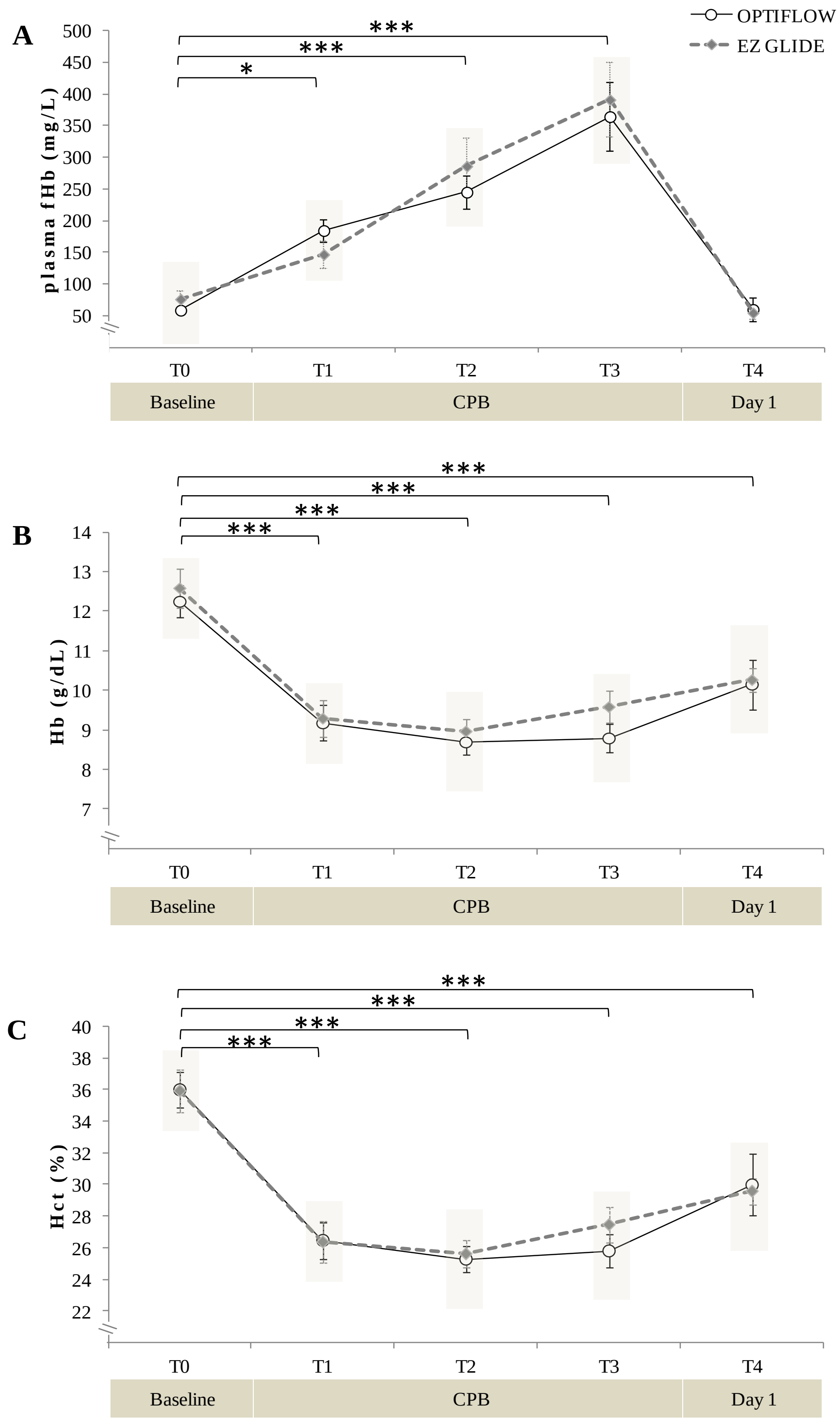


Figure 7: *Blood tests at baseline (T0), 10 minutes after cardiopulmonary bypass (CPB) start (T1), 60 minutes after CPB (T2), at CPB end (T3) and 24 hours after surgery (T4). (A) Changes of plasma free hemoglobin (plasma fHb,*

*mg/L) for both groups. During surgery, plasma fHb levels increased significantly in both groups at T1, T2 peaking at the end of CPB. Plasma fHb concentrations returned at baseline one day after surgery (T4). (B) Change of hemoglobin (Hb, g/dL) for both groups. During surgery, Hb levels decreased significantly in the CPB phase (T1, T2, T3) and only slightly increased one day after surgery (T4). (C) Hematocrit (Hct, %) showed the significant intraoperative hemodilution in the CPB phase (T1, T2 and T3) and only slightly increased one day after surgery (T4). Error bars represent the standard error (SE). Post hoc two-way ANOVA *$p<0.05$; **$p\leq0.001$; ***$p\leq0.0001$*

18 MICROEMBOIC SIGNALS (MES) REGISTERED BY TRANSCRANIAL DOPPLER

MES were recorded among 19 patients (Optiflow N=10, EZ Glide N=9) during the procedure. For 4 patients, it was not possible to register signals from MCA due to very low signal intensity.

Number of MES recorded from aortic cannulation to decannulation varied considerably (from 0 to 45). When we focused on possible differences between the study groups among the surgery phases studied (cannulation, CPB onset, aortic cross clamp, aortic cross clamp off, side aortic clamp on, side aortic clamp off and decannulation) the two-way ANOVA showed that the number of MES recorded differed significantly among the surgery phases (factor 'phase', p=0.02), but did not differed between the two study groups (factor 'group', p=0.29; factors interaction, p=0.45)

When we focused the analysis at each phase, we did not found any statistically significant difference between the two study groups except a slight tendency toward significance for the aortic cross clamp off phase (mean ± SE; Optiflow, 1.40 ± 0.78; EZ Glide, 11.33 ± 5.18; one-way ANOVA, p=0.06): aortic cannulation (mean ± SE; Optiflow, 9.33 ± 3.91; EZ Glide, 5.43 ± 2.48; one-way ANOVA, p=0.47), CPB onset (mean ± SE; Optiflow, 8.44 ± 3.73; EZ Glide, 5.50 ± 2.46; one-way ANOVA, p=0.55), aortic cross clamp (mean ± SE; Optiflow, 2.48 ± 1.04; EZ Glide, 2.75 ± 0.99; one-way ANOVA, p=0.97), side aortic clamp on (mean ± SE; Optiflow, 1.25 ± 0.28; EZ Glide, 1.89 ± 0.84; one-way ANOVA, p=0.50), side aortic clamp off (mean ± SE; Optiflow, 3 ± 0.93; EZ Glide, 1.33 ± 0.55; one-way ANOVA, p=0.16) and decannulation (mean ± SE; Optiflow, 4.90 ± 2.21; EZ Glide, 2.78 ± 0.60; one-way ANOVA, p=0.39) (Figure 8A).

When we studied the total count of MES from cannulation to decannulation phases, we did not find a statistically significant difference between the two groups (mean ± SE; Optiflow, 301.20 ± 65.91; EZ Glide, 391.63 ± 128.97; one-way ANOVA, p=0.53) as well as the total MES with no correlation counted (mean ± SE; Optiflow, 235.50 ± 56.02; EZ Glide, 260.56 ± 76.18; one-way ANOVA, p=0.79) (Figure 8B).

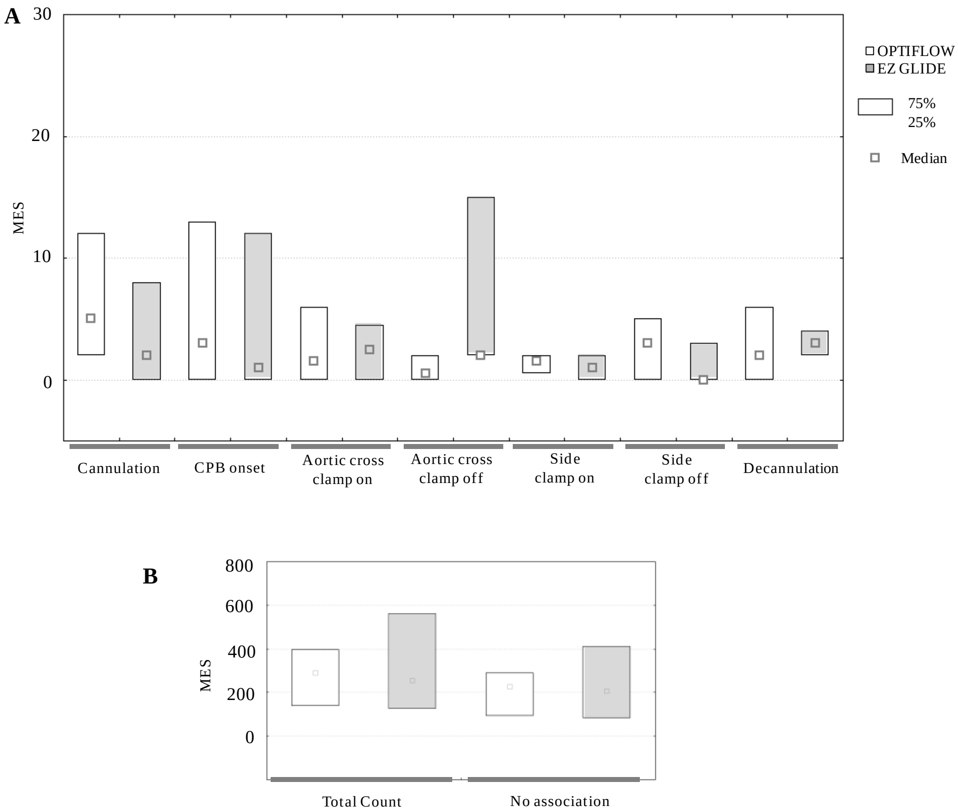


Figure 8: *Microembolic signals (MES). (A) MES recorder during the cannulation, CPB onset, aortic cross clamp, aortic cross clamp off, side aortic clamp on, side aortic clamp off and decannulation phases. Boxes represent the total count of MES for each phase for both Optiflow group and EZ Glide group expresses as median values (25th, 75th percentiles). (B) Total count of MES recorder during the surgery in the indexed phases and total count of MES registered during no specific phases for both Optiflow group and EZ Glide group expresses as median values (25th, 75th percentiles)*

When we investigated possible correlation of the total count of MES with age and CPB time, no correlation was found (age, r^2=0.06 p=0.30; CPB time, r^2= 0.0004 p=0.93).

We also verified if the carotid atheroma, aortic arch atheroma and number of grafts could correlate with the number of MES; we analyzed two groups of patients using the mean value calculated among the total number of MES registered for each patients, and no correlation was found (carotid atheroma, Fisher's exact test, 3 x 2 contingency table, score 1/2/3, p=0.13; aortic arch atheroma, Fisher's exact test, 4 x 2 contingency table, number of grafts 1-2-3-4, p>0.99; grafts, Fisher's exact test, 3 x 2 contingency table, score 1/2/3, p=0.68).

19 NEUROLOGICAL, NEUROPSYCHOLOGICAL AND PSYCHOLOGICAL OUTCOMES

19.1 Comparison between data collected at baseline and 3 months after CABG

Neurological outcomes

None of the patients in the study (Optiflow N=11, EZ Glide N=12) experienced neurological complications both in the early post-operative phase and three months after CABG surgery.

Neuropsychological and psychological outcomes

Neuropsychological and psychological analysis focused on a sub-group of 12 patients (Optiflow N=7, EZ Glide N=5) who provided data both at baseline (FU0) and at three month follow-up visit (FU1). Baseline neuropsychological sub-group patient characteristics are reported in Table 1, section 15.

With two-way ANOVA we did not find significant interaction between factor ‘group’ and ‘FU’ for all tests (p>0.05), except for phonemic verbal fluency (p=0.02) due to different trends of the two groups: Optiflow group showed increased values at FU1 compared to baseline (FU0, 26.71 ± 9.21; FU1, 30 ± 5.23) whereas EZ Glide showed decreased values at FU1(FU0, 33.60 ± 6.66; FU1, 31.60 ± 4.56). For both single factors ‘group’ and ‘FU’ the two-way ANOVA did not highlight significant interaction (p>0.05). Table 3 lists the results of neuropsychological tests raw scores for both Optiflow and EZ Glide patients at FU0 and FU1.

		Optiflow group (N=7)	EZ Glide group (N=5)	*p*-value *'time'*	*p*-value interaction
Mini Mental State Examination	FU0	28.71 ± 1.38	28.60 ± 0.89	0.37	0.44
	FU1	29.71 ± 0.76	28.80 ± 2.17		
Story recall test	FU0	9.61 ± 3.37	12.2 ± 0.67	0.64	0.1
	FU1	12.10 ± 1.60	11.64 ± 4.03		
Visual Search	FU0	47.14 ± 6.52	54.4 ± 3.36	0.1	0.82
	FU1	48.71 ± 9.27	56.60 ± 3.36		
Trail Making Test, A	FU0	58 ± 19.13	43 ± 16.93	0.3	0.72
	FU1	52.43 ± 12.18	41.20 ± 15.39		
Trail Making Test, B	FU0	146.14 ± 81.12	99.40 ± 37.17	0.28	0.67
	FU1	141.43 ± 58.55	86 ± 28.83		
Stroop test, time	FU0	19.79 ± 11.60	16.60 ± 9.82	0.6	0.45
	FU1	20.29 ± 11.32	13.60 ± 9.37		
Stroop test, error	FU0	1.14 ± 1.95	0.20 ± 0.45	0.88	0.32
	FU1	0.86 ± 1.21	0.40 ± 0.89		
Digit Span Forward	FU0	5.71 ± 1.11	6.40 ± 0.55	0.1	0.8
	FU1	6.14 ± 1.21	7 ± 1		
Rey complex figure test	FU0	31.79 ± 4.65	33.25 ± 2.50	0.52	0.14
	FU1	32.29 ± 4.86	35.40 ± 0.89		
Rey complex figure test, 10’	FU0	13.86 ± 4.88	19.75 ± 6.03	0.73	0.52
	FU1	15.57 ± 3.53	17.90 ± 5.07		
Phonemic verbal fluency	FU0	26.71 ± 9.21	33.60 ± 6.66	0.94	0.02*
	FU1	30 ± 5.23	31.60 ± 4.56		
Semantic Fluency	FU0	38.29 ± 10.37	44.20 ± 8.38	0.8	0.52
	FU1	38.71 ± 7.20	43.20 ± 8.32		
Words test	FU0	10.29 ± 3.50	13.60 ± 6.23	0.11	0.88
	FU1	11.71 ± 3.73	14.60 ± 4.16		

Table 3: *Results of neuropsychological tests (raw scores). p-value columns reports the two-way ANOVA factor ‘time’ and interaction between factor ‘group’ and ‘time’. *p≤0.05*

To complete this first analysis, we compared the equivalent scores[1] of the tests. Table 4 shows patients’ performances on the most difficult tests

[1] When raw scores are processed to obtain a corrected score, score is a standardized score. All tests used in this study have percentile ranks, based on age and education, with tables to transform the raw scores into equivalent scores: 0 indicates pathological score; 1 indicates score below the mean value; 2 indicates score within the mean value, 3 indicates score slightly above the mean value and 4 above mean value.

administered at FU0 and FU1. Some patients reported a score equal to 0 (highlighted in the table); this finding suggests that tests requiring high concentration were impacted in the pre-operative phase.

At the psychological level no differences emerged between the two study groups in any of the tests delivered. When we studied the two groups separately at FU0 and FU1 we did not find any statistically significant difference, except for the PANAS questionnaire showing that patients interviewed at baseline had higher emotional sensitivity, both for positive and negative emotions compared to FU1. Table 5 lists the results of psychological tests for both Optiflow and EZ Glide patients at FU0 and FU1.

	Rey complex figure test		Rey complex figure test, 10'		Phonemic verbal fluency		Words test	
	FU0	FU1	FU0	FU1	FU0	FU1	FU0	FU1
Optiflow group (N=7)	3	1	0	4	3	4	2	1
	4	4	2	1	2	1	3	1
	0	1	1	2	2	4	2	4
	2	0	4	4	3	4	4	4
	4	4	4	4	0	1	2	4
	4	4	4	4	4	4	4	4
		4	/	4	3	4	4	3
EZ Glide group (N=5)	4	4	4	4	4	4	0	1
	4	4	4	4	4	3	3	3
	4	4	4	4	4	4	2	3
	4	4	2	4	2	3	0	3
	3	4	4	3	4	3	4	4

Table 4: *Results of neuropsychological tests (equivalent scores).*

			Optiflow group (N=7)	EZ Glide group (N=5)	*p*-value '*time*'	*p*-value interaction
PANAS	positive affect	FU0	51,14 ± 8.45	51,4 ± 9.65	0.007*	0.27
		FU1	47.42 ± 7.82	43.80 ± 9,44		
	negative affect	FU0	34 ± 8.32	37.80 ± 10.35	0.002*	0.92
		FU1	22.71 ± 5.02	26 ± 3.6		
SF-12	physical health	FU0	40,3 ± 9,78	44,1 ± 9,03	0.67	0.92
		FU1	39,13 ± 9.02	42,28 ± 4.33		
	mental health	FU0	41.13 ± 11.09	40.06 ± 18.92	0.14	0.80
		FU1	49.08 ± 8.89	45.82 ± 4.90		
MHC-SF		FU0	37.66 ± 13,93	40,8 ± 12,79	0.52	0.61
		FU1	38.33 ± 14,09	46.40 ± 9.78		
SWLS		FU0	27.14 ± 2.79	28.20 ± 4.08	0.86	0.64
		FU1	26.85 ± 2,79	28.80 ± 4.14		
MSPSS	family	FU0	6.64 ± 0.57	6.67 ± 0.43	0.46	0.67
		FU1	6.82 ± 0.27	6.80 ± 0.44		
	friends	FU0	4.92 ± 1.74	6.5 ± 0.58	0.99	0.32
		FU1	5.32 ± 0.94	6.10 ± 0.54		
	other significant person	FU0	6.42 ± 0.64	6.70 ± 0.67	0.33	0.33
		FU1	6.75 ± 0.38	6.70 ± 0.67		

Table 5: *Results of psychological tests. p-value columns reports the two-way ANOVA factor 'time' and interaction between factor 'group' and 'time'.*p≤0.05*

19.2 Neuropsychological data and MES recorded during the operative phase

Out of the 15 patients with MES records during the procedure, 11 patients (Optiflow N=7, EZ Glide N=4) had neuropsychological data both at FU0 and at FU1.

We tested whether neuropsychological outcomes at FU1 correlate with the total number of MES recorded during the bypass time; we found no correlation for any neuropsychological test (Table 6).

	Goodness of Fit (r^2, p-value)
Mini Mental State Examination	0.02, 0.66
Story recall test	0.09, 0.37
Visual Search	0.08, 0.39
Trail Making Test, A	0.25, 0.12
Trail Making Test, B	0.26, 0.11
Stroop test, time	0.16, 0.22
Stroop test, error	0.01, 0.78
Digit Span Forward	0.12, 0.29
Rey complex figure test	0.10, 0.34
Rey complex figure test, 10 min	0.01, 0.78
Phonemic verbal fluency	0.07, 0.43
Semantic Fluency	0.01, 0.77
Words test	0.30, 0.08

Table 6: *Correlation of neuropsychological outcomes at FU1 with the total number of MES recorder during the bypass time for all tests*

DISCUSSION

20 HEMODYNAMIC AND PERFUSION DURING CABG

20.1 Cannulas pressures

When we studied the two aortic cannulas, Optiflow and EZ Glide, by comparing the pressure measured at cannula level during the full procedure for both patient groups, we found significantly lower values for the Optiflow cannula. Also, perfusion line pressure reflects cannula pressure with lower line pressure in the Optiflow group (mean ± SD, mmHg: 144.53 ± 21.09 vs 156.07 ± 26.24; one-way ANOVA, factor ‘group’, p=0.0003). This result is not affected by possible flow rates differences because in both groups we measured nearly the same rates (mean ± SD, L/m: 4.63 ± 0.53 vs 4.72 ± 0.65; one-way ANOVA, factor ‘group’, p=0.26). It is also important to highlight that Optiflow patients showed higher blood pressures during the full procedure compared to the EZ Glide patients with a slight tendency toward significance (mean ± SD, mmHg: 77.81 ± 15.15 vs 73.95 ± 15.12; one-way ANOVA, factor ‘group’, p=0.06) as well as peripheral resistances (mean ± SD, mmHg/L/min: 1372.76 ± 358.02 vs 1283.87 ± 349.47; one-way ANOVA, factor ‘group’, p=0.06). Those results can reflect the Optiflow cannula design with basket-tip shape able to guarantee low pressure during the full procedure.

20.2 Cannulas pressure versus flow rate

20.2.1 Cannulas pressure versus flow rate under blood pressure conditions during CPB

When we studied possible influences of patients’ blood pressure conditions during CPB on cannulas’ performances, we found that under standard CPB blood pressure ($60 < BP \leq 75$) EZ Glide cannula pressures significantly correlate with flow rate, whereas the Optiflow did not show specific correlation, confirming lower and stable pressure values under all conditions. This result may imply for Optiflow cannula a lower wall shear stress affecting atherosclerotic plaques as well as endothelium damages in the area where the cannula jet hits the aortic wall during standard CPB condition that corresponds to desired perfusion and to the longer CPB time during surgery.

20.2.2 Cannulas pressure versus flow rate under peripheral resistance conditions during CPB

When we studied possible influences of patients' peripheral resistance during CPB on cannulas' performances, EZ Glide cannula pressures significantly correlate with flow rates under both low and medium peripheral resistance whereas Optiflow only under medium peripheral resistance. This result shows that reaching high flow rates under low peripheral resistance the EZ Glide cannula pressure is affected reaching high cannula pressure.

20.3 Cannula pressure versus blood pressure and versus peripheral resistance under fixed flow rate ranges

When we focused on cannulas' pressures behavior under fixed flow rates, we found that the Optiflow cannula pressures significantly correlate with both blood pressure and peripheral resistance condition whenever the flow rate condition. The EZ Glide showed correlation with both blood pressure and peripheral resistance only for low flow rates and high flow rates. This result shows that the EZ Glide cannula pressure is significantly influenced by flow rate changes whereas the Optiflow behavior is constant whatever the flow rate during CPB.

20.4 Model estimation: a linear regression mixed model

When we estimated a model for cannulas performances taking into the analysis all factors, we analyzed singularly in the above paragraphs, we found a global picture of the variables influencing cannulas pressures behavior. We found that the dependent variable *cannula pressure* significantly correlates with the type of cannula, flow rate, blood pressure and does not correlate with peripheral resistance (Figure 9A).

In particular, the post-hoc analysis on type of cannula showed that Optiflow cannula performance is significantly influenced by changes in blood pressure, and it is not surprising that the analysis showed a slight correlation also with peripheral resistance regardless the flow rate (Figure 9B), as partially described at section 20.3.

EZ Glide cannula performance is significantly influenced by changes in flow rate, regardless the blood pressure and peripheral resistance (Figure 9B), as partially described at section 20.3.

Those results could suggest that the Optiflow cannula could guarantee a correct peripheral perfusion with safeguard of peripheral organs, applying high flow rates, reducing possible damages to the endothelium in the area in which the cannula jet hits the aortic wall and reduced risk of atherosclerotic plaques dislodgement because the cannula pressure is not influenced by flow rate.

The EZ Glide cannula is able to guarantee a correct peripheral perfusion only reaching high cannula pressure, because its performances are significantly influenced by flow rates; in this case, we cannot exclude possible damages to the endothelium in the area where the cannula jet hits the aortic wall or risk of atherosclerotic plaques dislodgement due to the high cannula pressure.

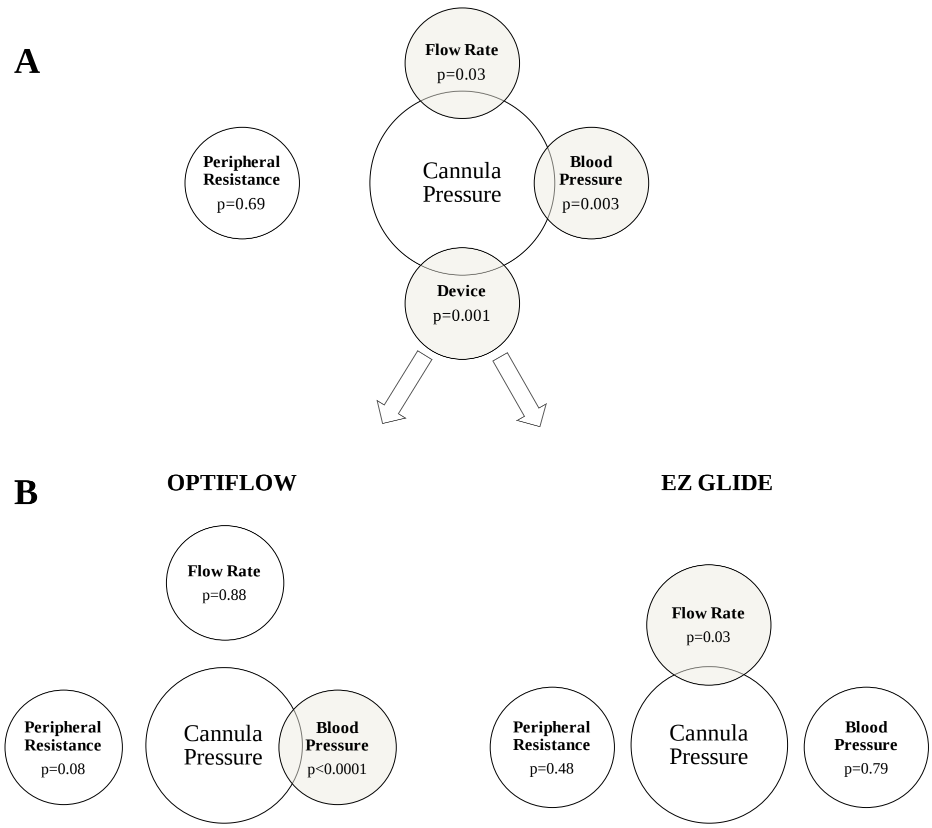


Figure 9: *Graphic representation of cannula pressure model estimation. (A) The graph shows that the dependent variable 'cannula pressure' significantly correlates with 'device', 'flow rate', 'blood pressure' and does not correlate with 'peripheral resistance'.(B) Optiflow - the analysis found a significant correlation only with 'blood pressure'. EZ Glide - the analysis found a significant correlation only with 'flow rate'*

21 BLOOD TESTS

21.1 Plasma free hemoglobin

Analysis of hemolytic effect showed a significant increase of plasma fHb in both study groups since the first phase of CPB, 10 min after CPB start, peaking at the end of CPB, with no difference between Optiflow and EZ Glide groups. Both groups returned at baseline one day after surgery.

In order to avoid the introduction of bias related to the use of differed CPB components, the same surgical conditions were guaranteed for all procedures (section 11.4.2) and both cannulas were used according to the manufacturer's instructions.

Together with aortic cannula, studies identified different areas where RBC damage can occur in CPB system: Tubing/Connectors, Venous Cannula, Venous Reservoirs, Pumps and Oxygenators (Vercaemst, 2008). In particular, pump hemolytic effect is primarily caused by shear stress, time, negative pressure, and turbulent flow and can differ according to different designs (Lawson et al., 2005), but for all patients we used one specific pump design (section 11.4.2). Also, the suction phase, increasing negative pressures led to an increase in the number of microbubbles, and this air- blood contact, causing high shear stresses, is a major factor in the damage to the RBCs.

21.2 Hemoglobin

The analysis of Hb showed a significant decrease in both study groups since the first phase of CPB, 10 min after CPB start, keeping significant low values up to one day after surgery. No difference between Optiflow and EZ Glide groups was found. Those results are in line with the normal pattern of Hb levels after cardiac surgery; in fact, postoperative Hb levels tend to drift downward during the first four postoperative days and then drift upward during the next six postoperative days (George et al., 2012).

21.3 Hematocrit

During surgery, Hct levels decreased significantly, showing the significant intraoperative hemodilution, keeping significant low values up to one day after surgery, as previously observed (Harrison et al., 2006), in both Optiflow and EZ Glide groups. Success perfusion strategy were applied to guarantee Hct values above 20% identified as trigger value for complications (Harrison et al., 2006; Karkouti et al., 2005;

Habid et al., 2003); none of the patients in both Optiflow group and EZ Glide group had Hct below this level during CPB.

22 MICROEMBOIC SIGNALS (MES) REGISTERED BY TRANSCRANIAL DOPPLER

When we studied the MES recorded from aortic cannulation to decannulation, the analysis showed that the number of MES recorded differed significantly among the surgery phases (cannulation, CPB onset, aortic cross clamp, aortic cross clamp off, side aortic clamp on, side aortic clamp off and decannulation), but did not differed between the two study groups. This is in line with findings that MES mainly occur during specific ‘surgical events’ such aortic cannulation (Stroobant et al., 2005), start of CPB (Fearn et al., 2001), aortic cross clamp off (Barbut et al., 1995) side clamp-off (Liu et al., 2009; Motallebzadeth et al., 2007; Barbut et al., 1995), manipulation of heart and aorta or no associated with any maneuver (Stump et al., 1996). In our experience we confirm the majority of MES registered during the surgery was during aortic cannulation (22.39% of total MES), CPB onset (22.02% of total MES) and aortic cross clamp removal (21.28% of total MES). We did not find high MES during the side clamp events (side clamp on, 4.95% and side clamp off, 6.61% of total MES).

When we investigated possible correlation of the total count of MES with age, CPB time, carotid atheroma, aortic arch atheroma and number of grafts, no correlation was found. This can be related to the number of patients (Table 1section 15), indeed we have to highlight that only 19 patients (Optiflow N=10, EZ Glide N=9) have the MES acquisition and that a sub-group of patients underwent to carotid assessment (Optiflow group N=10; EZ Glide group N=7) as well as only a sub-group of patients underwent to aortic assessment (Optiflow group N=7; EZ Glide group N=6).

23 NEUROLOGICAL, NEUROPSYCHOLOGICAL AND PSYCHOLOGICAL OUTCOMES

23.1 Comparison between data collected at baseline and 3 months after CABG

<u>Neurological outcomes</u>

None of the patients in the study experienced neurological complications both in the early post-operative phase and three months after CABG surgery. This result shows that both surgical settings, including devices under investigation, and perfusion technique are safe.

Neuropsychological and psychological outcomes

Both neuropsychological and psychological assessments did not highlight significant changes in the post-operative follow-up (3 months after surgery) compared to baseline evaluation. This result, together with the evidence that none of the patients had neurological complications, further attests to the safety of the devices under investigation, surgical settings and perfusion technique.

Psychological data showed a significant result only for the PANAS evaluation; in particular, patients reported higher emotional sensitivity before surgery, especially for negative emotions. This is not particularly surprising, because PANAS was administered to patients the day before surgery (sometimes few hours before surgery), following a long neuropsychological evaluation

This interpretation is also supported by neuropsychological performances. Cognitive tests that may be influenced by emotional state (anxiety) showed pathologically low performances at baseline (equivalent score was 0) and higher ones at T1 (score equal to or greater than 1; Table 4, section 19.1). This finding suggests that the emotional state, immediately before surgery, affected performances requiring an interaction between attention and language and/or praxis and/or memory. The greater the emotional activation, the lower may be performances requiring high concentration.

23.2 Neuropsychological data and MES recorded during the operative phase

When we tested whether neuropsychological outcomes correlated with MES recorded during bypass time, we found no correlation for any neuropsychological test. This can be related to the low number of patients, in fact neuropsychological data collection both at baseline and at 3-month follow-up involved only 11 patients (Optiflow N=7, EZ Glide N=4). It is also worth noting that the current literature is inconsistent as concerns the impact of embolization during cardiac surgery on cognition. These findings suggest that the goal of improving cognitive safety could involve further careful consideration of multifactorial events (Patel et al., 2016).

24 LIMITATIONS

The first limitation of this research is the number of patients, much lower compared to what we planned in the clinical plan approved by the ethics committee (patient planned: 60; patient studied: 23). The number of patients planned was not reached because the study was approved on February 2015, but due to delay in the TCD ultrasonography supply - provided on December 2015 - the screening phase started on January 2016. Due to inclusion/exclusion criteria and to failure to obtain patients' consent - some patients refused to participate due to randomization or because they did not want to undergo to neuropsychological assessment - the rate of inclusion was 2 patients *per* month (section 15). Number of patients with three month follow-up was limited. Only a sub-group of 12 patients (Optiflow N=7, EZ Glide N=5) underwent both baseline and three month follow-up visit for neuropsychological assessment. Moreover, some studies showed that longer follow-up can show further evidences on neuropsychological outcomes, hence having information only up to three months could hide information.

Another limitation refers to lack of complete information: TCD acquisitions were obtained sometimes only from left or right MCA and sometimes from both; also, we did not have information on carotid and aortic assessment for some patients.

A limitation related to hemodynamic and perfusion of the two cannulas is the lack of information on flow velocity profile in the aorta. This could help to investigate if the laminar flow reached turbulent flow status by exceeding the Reynolds number, hence inducing hemolysis as well as information on the shear stress.

Finally, we identify as last limitation the lack of information on renal functions. Possible hypoperfusion inducing renal dysfunctions could have been detected in the post-operative phase by analyzing serum creatinine, urine output and glomerular filtration rate.

CONCLUSION

This pilot study showed that both Optiflow and EZ Glide dispersion flow aortic cannulas have good performances in terms of hemodynamic and perfusion and do not induce any neurological complications or cognitive dysfunctions after CABG surgery.

Evidence that the Optiflow cannula showed significant lower pressures during the full procedures, together with the finding that this cannula is able to guarantee optimal perfusion under standard CPB patient blood pressure condition, because it is able to reach high flow rates ensuring stable low cannula pressures, may suggest to recommend this cannula to prevent possible endothelium damages, dislodgment of atherosclerotic plaques and peripheral damages. Because the standard CPB patient blood pressure condition is the longer phase during surgeries, this finding can suggest to use this device during procedures requiring a long time surgery. This idea is further supported by the finding that the Optiflow cannula performance only correlate with patient blood pressure, not with the flow rate; hence it could guarantee a correct peripheral perfusion with safeguard of peripheral organs, through high flow rates.

On the contrary, the EZ Glide cannula performance only correlate with flow rate, hence in the attempt of reaching optimal perfusion, we cannot exclude possible damages to the endothelium in the area in which the cannula jet hits the aortic wall due to the high cannula pressure; *vice versa* preventing aortic wall damages we could experience hypoperfusion with damages of peripheral organs.

Data from TCD acquisitions could support the above-mentioned results but did not show any differences between the two study devices as well as any correlation with neuropsychological tests. Given the evidence that the current literature is divided as to whether the impact of embolization during cardiac surgery has any adverse impact on cognition, this finding can further suggest that the goal of improving cognitive safety could involve further careful consideration of multifactorial events (Patel et al., 2016).

Although the Optiflow cannula promises to guarantee higher performances than EZ Glide, future studies are needed to confirm our preliminary results. This pilot study gathered the necessary information to design a large, prospective, randomized study investigating the performances of those devices in long time surgeries by studying possible device-induced dysfunctions related to possible hypoperfusion like postoperative renal failure (Head et al., 2013).

Above the possible influence of the type of cannula, another important result, that takes into account also surgical approach and perfusion technique, is that all patients underwent to CABG surgery at this center, did not experience any neurological complications and cognitive dysfunctions up to three months after surgery.

In conclusion, our experience, studying two dispersion flow aortic cannulas *in vivo*, highlighted different performances in terms of hemodynamic and perfusion between Optiflow and EZ Glide cannulas.

These findings should help us and others in studying in deep the mechanism of embolization and consequent impact on cognition in a large cohort of patients, during long time surgery to further investigate early and late post-operative outcomes in patients requiring cardiac surgery in on-pump procedures. Optiflow arterial cannula could prove being very effective in reducing patient’s complications and meet procedural needs.

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ANNEX I

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AN/dt

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CCM sede

Oggetto: CCM 233 – Eventi microembolici: confronto tra le cannule aortiche a dispersione di flusso Optiflow e EZ Glide (OPTIGLIDE)

In riferimento alla richiesta di valutazione di quanto in oggetto, si informa che questo Comitato etico nella seduta del 18 febbraio 2015 ha espresso parere favorevole. Nell'informativa per il paziente per quanto riguarda l'intervento è più opportuno fare riferimento al modulo di informativa e consenso previsto dal centro cardiologico per i fini assistenziali che andrebbe allegato.

Lo studio viene registrato con il n. R217/15-CCM 233 (n. di riferimento da citare sempre nella corrispondenza).

Si allega l'elenco dei componenti del Comitato etico, partecipanti alla seduta.

Distinti saluti.

Dott Atanasio Nonis
Responsabile Segreteria Tecnico-Scientifica

IEO
Istituto Europeo di Oncologia

Centro Cardiologico
Monzino

Comitato Etico degli IRCCS
Istituto Europeo di Oncologia e Centro Cardiologico Monzino

RIUNIONE 18/02/2015

COMPONENTI PRESENTI E QUALIFICHE

BERTI Ferruccio Farmacologo, (Vice Presidente)
CASTOLDI Massimo Direttore Sanitario CCM
CERUTTI Sergio Esperto in dispositivi medici e Ingegnere clinico
DE CARLI Luigi Esperto in genetica
DELLE PIANE Susanna Farmacista CCM
GALLUS Giuseppe Biostatistico
GASTALDI Stefano Rappresentante del volontariato e dell'associazionismo di tutela dei pazienti
MICCINELLI Pasquale Esperto in materia giuridica e assicurativa
NONIS Atanasio Farmacologo, esperto regolatorio
PELLEGRINI Maurizio Medico di Medicina Generale
REZZONICO Rossano Pediatra

ANNEX II

FOGLIO INFORMATIVO E CONSENSO PER IL SOGGETTO
Versione 1.0 del 13/01/2015

Eventi microembolici: confronto tra le cannule aortiche a dispersione di flusso Optiflow e EZ Glide (Studio OPTIGLIDE)

Gentile Signore/a,
Il Suo Dottore ha dato indicazione all'intervento di innesto di bypass aortocoronarico che permetterà di superare il condotto vascolare ostruito. Tale intervento chirurgico prevede l'utilizzo di cannule per la circolazione extracorporea ed in questo ospedale si sta svolgendo uno studio clinico approvato dal Comitato Etico del Centro Cardiologico Monzino S.p.A. Con questo foglio vogliamo spiegarLe perché riteniamo che Lei possa prendere parte a questo studio e cosa dovrà fare se Lei decidesse di partecipare.
Il medico responsabile del progetto ed i suoi collaboratori sono a Sua disposizione per rispondere a tutte le Sue domande. La preghiamo di leggere attentamente quanto riportato di seguito e di decidere in assoluta libertà se partecipare a questo studio.

Obiettivo dello studio:
La procedura chirurgica a cui si sottoporrà si esegue in circolazione extracorporea che permette di sostituire la funzione di pompa del cuore e di ossigenazione del polmone durante l'intera procedura. Questa funzione è garantita dell'impiego di dispositivi dedicati, tra cui cannule aortiche in grado di deviare il flusso sanguigno nella macchina cuore-polmone. Lo studio ha lo scopo di valutare gli effetti neuroprotettivi di due cannule comunemente utilizzate nella pratica clinica di questo istituto: la cannula aortica Optiflow (Sorin Group Italia S.r.l., Italia) e la cannula aortica EZ Glide (Edwards Lifesciences Corporation, USA).

Che cosa comporta la partecipazione allo studio?

Se Lei decide di partecipare a questo studio, sarà invitato a partecipare alle sessioni riportate di seguito.

Prima dell'intervento chirurgico di innesto di bypass aortocoronarico:

- avrà un colloquio con un Neuropsicologo che le farà una serie di domande per la valutare alcuni aspetti neuropsicologici;
- sarà assegnato in modo random ad uno dei due gruppi, gruppo Otiflow o gruppo EZ Glide.

Durante l'intervento chirurgico di innesto di bypass aortocoronarico, per ciascun gruppo (Optiflow e EZ Glide) sarà valutata:

- la resistenza della cannula attraverso la misurazione della caduta di pressione;
- la Free plasma hemoglobin (FPH);
- la presenza di eventi microembolici a livello cerebrale, mediante doppler transcranico utilizzato in fase preoperatoria.

Dopo intervento chirurgico di innesto di bypass aortocoronarico, per ciascun gruppo (Optiflow e EZ Glide) sarà eseguita:

- valutazione neurologica. Solo in caso di sospetto clinico di infarto cerebrale sarà eseguita una valutazione mediante risonanza magnetica per immagini (tecnica di indagine non invasiva). Se controindicazioni alla RMI verrà eseguita una tomografia computerizzata (tecnica di indagine non invasiva);
- valutazione neuropsicologica, come al preoperatorio, a 2 settimane (+/- 4 giorni);
- valutazione neuropsicologica, come al preoperatorio, a 3 mesi (+/- 2 settimane).

Quali sono i possibili rischi/disagi legati alla partecipazione a questo studio?
Lei non verrà esposto/a a rischi in quando saranno impiegati solo dispositivi utilizzati nella pratica clinica.

Lei è obbligato/a a partecipare allo studio?
La sua partecipazione allo studio è volontaria. Inoltre in qualsiasi momento Lei dovesse cambiare idea e volesse ritirarsi dallo studio, è libero/a di farlo.

A chi chiedere ulteriori informazioni?
Se desidera ulteriori informazioni su questo studio può contattare il responsabile dello studio:

Prof. Gianluca Polvani	*gianluca.polvani*@ccfm.it	02 58002311
		02.58002626

MODULO DI CONSENSO INFORMATO

Il/la sottoscritto/a____ _____ dichiara di partecipare allo studio clinico di ricerca descritto nel presente documento. Il mio consenso è espressione di una libera decisione, non influenzata da promesse di benefici economici o di alta natura né da obblighi nei confronti del medico responsabile dello studio. Sono consapevole di essere libero/a di ritirarmi dallo studio in qualsiasi momento io lo desideri. Sono consapevole inoltre di non aver l'obbligo di motivare la mia decisione di ritirarmi dallo studio, a meno che essa non derivi dalla comparsa di disturbi, effetti indesiderati o non previsti.

Prendo atto che lo studio è stato sottoposto all'attenzione ed approvato da un comitato per degli studi clinici ed accetta che lo studio venga condotto in ottemperanza alle normative ministeriali e secondo i principi sanciti della dichiarazione di Helsinki.

Mi è stata data l'opportunità di leggere le informazioni contenute nella parte informativa di questo documento (pagine 1-2) e di porre domande circa gli scopi e le metodiche dello studio, i benefici ed i possibili rischi, gli effetti indesiderati ed i miei diritti come partecipante alla ricerca.

Ho compreso tutte le informazioni ed i chiarimenti che mi sono stati dati e ho avuto il tempo sufficiente per prendere in considerazione la mia partecipazione a questo studio.

Acconsento che il trattamento dei miei dati personali, ivi compresi quelli inerenti allo stato di salute venga effettuato per gli scopi specifici della ricerca nei limiti e con le modalità indicatemi nel presente documento di informazione e consenso.

Qualora io lo desideri, il Mio medico di famiglia, o altro medico da me indicato, sarà informato circa la mia partecipazione a questo studio.

Confermo che mi è stata consegnata copia del presente documento informativo e di consenso.

Firma del soggetto ________________________data__________________

DICHIARAZIONE DELLO SPERIMENTATORE

Dichiaro di aver fornito al soggetto informazioni complete e spiegazioni dettagliate circa la natura, le finalità, le procedure e la durata di questo studio. Dichiaro inoltre di aver fornito al soggetto il foglio informativo ed una copia datata e firmata del modulo di consenso informato.

Nome dello sperimentatore (in stampatello) ____

Firma dello sperimentatore _____________________________________

data ____________________

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Prof. Roberto Weinstein for giving me the opportunity to carry out this research project.

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