UNIVERSITY OF MEDICINE AND PHARMACY
"CAROL DAVILA", BUCHAREST
DOCTORAL SCHOOL
MEDICINE

PERIOPERATIVE TRAJECTORY AND SOURCES OF VARIATION OF PLASMA VISCOSITY IN CARDIAC SURGERY

SUMMARY OF THE DOCTORAL THESIS

PhD Coordinator:
PROF. UNIV. DR. TOMESCU DANA-RODICA

PhD Student:
VĂLEANU LIANA

2022

Table of contents

Introduction

The haemodynamic profile of patients in shock is determined by the interactions between macrocirculation, microcirculation and cellular component and may change depending on the time point at which it is analysed. Most recommendations for resuscitation in patients with shock are directed towards assessment and optimization of macrocirculatory parameters, with the end purpose of restoring tissue perfusion and maintaining cellular homeostasis [1]. However, the dissociation of the three compartments - macrocirculatory, microcirculatory and cellular - is present in shock, and the optimization and restoration of physiological parameters of microcirculation and blood flow at this level do not always depend on macrocirculatory optimization [2].

Microcirculatory changes in cardiac surgery are the result of the haemodilution and the inflammatory syndrome associated with cardiopulmonary bypass (CPB) [3]. As in shock, changes observed in the microcirculation may persist irrespective of the correction of macrocirculatory parameters [2-3], as they are dependent on complex and incompletely described interactions between endothelium, glycocalyx and local rheological conditions.

Among the factors that determine and regulate blood flow at the microcirculatory level, haemorheology remains probably one of the most neglected areas in clinical literature today, particularly in the case of shock or cardiac surgery.

General Part

Chapter 1 of the PhD thesis reviews the main concepts related to haemorheology, focusing on the determinants of haemorheological parameters (Subchapter 1.1) and on the relationship between their changes and the risk of cardiovascular events (Subchapter 1.2). Blood exhibits characteristics similar to a non-Newtonian fluid, and whole blood viscosity (WBV) values change with shear rate and depend on cellular and plasma factors [4]. The aggregation and deformability of red blood cells are the main determinants of blood flow characteristics in large vessels, and plasma viscosity (PV) is the main determinant of blood flow resistance in the microcirculation [5-6]. Unlike blood, plasma has the characteristics of a Newtonian fluid [4]. The main factors influencing the PV value are total protein concentration and fibrinogen concentration [4]. The PV value is modulated by the presence of chronic and acute pathophysiological conditions. In subjects with altered

haemorheological parameters, the increased stress applied to the vascular wall induces endothelial injury, vascular remodelling and triggers the process of atherosclerosis [7]. Chronically elevated WBV and PV values are associated with the presence of cardiovascular risk factors and the occurrence of adverse cardiovascular events [8]. Chronic effects of exercise can be considered a haemorheological fitness that protects against cardiovascular disease [9].

Chapter 2 describes the relationship between haemorheological parameters and microcirculation, focusing on changes in microcirculation and haemorheological parameters in patients in shock (Subchapter 2.1) and in patients undergoing cardiac surgery (Subchapter 2.2). The microcirculation is a complex vascular network involved in the regulation of blood flow, tissue perfusion and the exchange of oxygen, carbon dioxide, nutrients and metabolites between blood and surrounding tissues [10]. Short-term control of blood flow in the microcirculation is achieved through a self-regulatory phenomenon, determined by the interaction between the neural response, myogenic response and local metabolic and rheological conditions [11]. Endothelial cells respond to local rheological conditions mainly by increasing nitric oxide production and release, which induces local vasodilation [11-12], and PV-induced shear stress on the capillary wall can influence local blood flow through changes in cytoskeleton and cytoplasmic viscosity of endothelial cells [13]. In critically ill patients, alteration of the balance between locally released vasodilators and vasoconstrictors, the presence of proinflammatory mediators and the occurrence of intravascular microthrombosis lead to endothelial barrier disruption, decreased self-regulatory capacity of microcirculation and tissue hypoperfusion [14-15].

In shock, microcirculation is characterized by the presence of vascular dysfunction associated with heterogeneous blood flow distribution (coexistence of capillaries with blocked, intermittent, normal or increased flow) [16], along with shock-induced endotheliopathy, altered glycocalyx and increased capillary permeability [17]. The literature describes four phenotypes of microcirculatory dysfunction (distributive, anaemic, obstructive and hypoperfusion) that characterize or may coexist in different types of shock [2] and are associated with mortality [18]. Together, these macrocirculatory and microcirculatory changes form the basis of altered haemodynamic parameters, resulting in uncoupling of macrocirculation and microcirculation and worsening of organ dysfunction despite correction of macrocirculatory parameters [2]. In addition, therapies that are directed at correcting macrocirculatory parameters in shock, such as fluid administration

and use of vasoactive medication may also lead to worsening of microcirculatory shock [19-20]. Haemodilution following fluid administration also results in a decrease in WBV and PV, resulting in a decreased release of vasodilator mediators by endothelial cells [13, 21]. The return to normal values of rheological parameters, irrespective of increased oxygen-carrying capacity secondary to transfusion, is more important for maintaining microcirculatory parameters due to stimulation of local nitric oxide and prostacyclin release [21]. Fluid resuscitation with high viscosity solutions leads to the progressive improvement of capillary blood flow and maintenance of functional capillary density (FCD) consequent to increased PV values [22], but their use is limited by the risk of renal injury [23]. Vasoconstrictor medication can cause arteriolar vasoconstriction and microcirculatory derecruitment by the stagnation of blood flow at the capillary level, with the occurrence of microcirculatory tamponade phenomena [2, 20].

In cardiac surgery, haemodynamic changes induced by CPB can lead to tissue hypoperfusion. The microcirculation has a profile independent of the macrocirculation profile, the most common phenotypes being type II (anaemic) and IV (hypoperfusion) [3]. The two major events associated with CPB are the change of the circulatory profile from pulsatile to non-pulsatile blood flow and the induction of a systemic inflammatory response. These two events can acutely decrease the PV value and reduce the FCD [2] in CPB, leading to the onset of organ dysfunction. Non-pulsatile blood flow leads to reduced perfusion in the microcirculation [24] and it is associated with decreased nitric oxide activity [25], suggesting a deterioration of microcirculatory conditions. The change in circulatory profile is associated with varying degrees of hypotension, haemodilution, anaemia, hypothermia and hyperoxia. Fluid administration may result in the recruitment of the microcirculation [26], but excessive administration and hypervolemia may cause tissue oedema, particularly in the presence of the inflammatory syndrome, endothelial barrier disruption and increased capillary permeability [17]. Normovolemic haemodilution caused by the use of CPB circuit priming solutions and the fluid administration for maintaining adequate flow during CPB has two distinct consequences resulting in decreased tissue oxygen-carrying capacity. First, normovolemic haemodilution is associated with the development of dilution anaemia and increased oxygen diffusion distance between capillaries with continuous blood flow and tissues [2]. Second, a decrease in the value of rheological parameters, and in particular PV, results in decreased flow-dependent self-regulatory capacity of the microcirculation through decreased nitric oxide production and release by endothelial cells [22, 27]. The anaemia caused by cardiac surgery-associated

haemodilution is one of the factors involved in the development of postoperative acute kidney injury [28], but the mechanism of this association is unclear. Increased haematocrit following red blood cell transfusion improves local microcirculatory conditions, leading to an increased number of perfused capillaries and increased tissue oxygenation, the likely mechanism being the increase in WBV [22]. However, the maintenance of the PV value, even in extreme anaemia, is associated with maintenance of tissue perfusion by maintaining blood flow and FCD in the microcirculation [5, 22, 28], and the adverse effects of reduced FCD on tissue perfusion in the context of haemodilution are counteracted by an increase in PV [5]. The heterogeneity of microcirculatory blood flow distribution is the most probable factor associated with tissue hypoperfusion during CPB [5-6], since FCD is independent of oxygen-carrying capacity [21]. The systemic inflammatory syndrome results from blood exposure to major components of the CPB system, and its presence contributes to organ dysfunction in the postoperative period, particularly in acute kidney injury and acute respiratory distress syndrome [29-30]. Endothelial dysfunction, impaired glycocalyx structure and increased capillary permeability play a central role in inflammatory syndrome-induced microcirculation changes [31-32]. Microcirculation changes described in cardiac surgery are the result of complex interactions between endothelium, glycocalyx and local haemorheological conditions. Currently, there is no study evaluating preoperative, intraoperative and postoperative changes in PV, the main determinant of capillary blood flow, in cardiac surgery.

Personal contributions

The importance of PV for microcirculation regulation is documented in detail in the experimental literature [5-6]. Several important observations extracted from the review of this literature are relevant for clinical practice:

- the PV values vary very little between different animal species and between individuals of the same species and at different times [33], suggesting that PV has physiological regulatory mechanisms ensuring a tight control;
- the PV value maintenance is associated with FCD maintenance even when haemoglobin values are low in various models of normovolemic haemodilution [5-6];

- decreasing PV values below the 1.2 cP limit can lead to decreased FCD and organ hypoperfusion [6, 34].

These experimental observations stress the importance of PV in controlling and regulating microcirculation. However, few data in the literature investigate PV changes in the clinical setting, with most studies being conducted more than 25 years ago [35].

The main objective of this thesis was to investigate the time-dependent profile of PV, its main determinants (total protein and fibrinogen) and haemoglobin in cardiac surgery with CPB, being the first study of its kind.

The secondary objectives of this thesis were the following:

- The description of the coefficient of variation and percentage changes in perioperative PV values in a cohort of cardiac surgery patients, since the literature describes small interindividual variations of PV values compared to other parameters such as total protein, fibrinogen or haemoglobin concentrations;
- The description of the sources of variation of PV values in the preoperative setting, at the end of CPB and on day 6 postoperatively;
- The identification of possible differences in postoperative outcome between patients with PV values lower than 1.2 cP and patients with PV values higher than 1.2 cP.

The prospective, observational studies included in the PhD thesis were conducted in the Emergency Institute for Cardiovascular Diseases Prof. Dr. C. C. Iliescu, Bucharest, between 1st 2020 and 31st May 2021 and included 50 patients undergoing cardiac surgery with CPB. The studies were approved by the Ethics Committee of the Emergency Institute for Cardiovascular Diseases Prof. Dr. C. C. Iliescu (registration number 22927 dated 1.10.2018) and patients were included in the study after obtaining the informed consent. The exclusion criteria were patient refusal to participate in the study and absence of sinus rhythm. The choice of cardiac surgery patients was based on the fact that two stimuli of similar amplitude occur during cardiac surgery in all patients. The first stimulus is the acute normovolemic haemodilution at the time of CPB initiation due to the priming volume of the circuit, and its presence allowed us to assess the impact of acute haemodilution on PV values. The second stimulus, the presence of which allowed for the assessment of PV changes in the postoperative period, is represented by the inflammatory syndrome associated with the surgery itself and CPB in particular that may appear as early as intraoperatively and persist in the first postoperative days. Intraoperatively, the patients

received standard and invasive monitoring, intravenous anaesthetic induction was performed with fentanyl, propofol and rocuronium, and the maintenance of anaesthesia was performed with sevoflurane before and after CPB, respectively with fentanyl and propofol in continuous intravenous administration during CPB. The depth of anaesthesia was monitored using a bispectral index to avoid anaesthetic underdosage or overdosage, and echocardiography was used throughout the perioperative period to assess the volemic status and cardiac output and to guide fluid administration and vasoactive therapy. The CPB circuit was primed with 1000 ml of crystalloid solution (Ringer lactate) and 500 ml of 5% succinylated gelatine for all patients included in the study. The viscosity of the priming solution was measured at 38 degrees Celsius, with a value of 0.75 cP. Mean blood pressure values above 65 mmHg during CPB were preferentially maintained by increasing pump flow to an index of 2.8 litres/m^2/minute, followed by the administration of vasoconstrictor medication. For each patient included in the study, the demographics (age, sex, height, weight, body mass index), presence of cardiovascular risk factors (smoking, hypertension, diabetes, dyslipidaemia), type of surgery, urgency of surgery, EuroSCORE II risk score, duration of CPB, duration of aortic cross-clamping, duration during CPB in which mean blood pressure was less than 65 mmHg, duration of postoperative mechanical ventilation and duration of ICU stay were recorded. In the study protocol, ten distinct time points were defined for patient assessment: T0 - preoperative time, T1 - before initiating the CPB, T2 - the end of CPB, T3 - the end of surgery/ ICU admission, T4 - T9 - days 1-6 postoperatively. For each of these time points the following values were recorded: temperature, blood pH, serum lactate, haemoglobin, serum urea, serum creatinine, C-reactive protein, PV, total protein and plasma fibrinogen concentration. For each interval between two consecutive time points (T1-T0, T2-T1, T3-T2, T4-T3, T5-T4, T6-T5, T7-T6, T8-T7, T9-T8) the type and volume of administered fluid (crystalloid, colloid, allogenic blood products, fibrinogen), diuresis, haemofiltration use and haemofiltrate volume were recorded. The APACHE II severity score was calculated for T3 time point (ICU admission), and the SOFA score was calculated for each time point from T3 onwards. The total protein concentration, plasma fibrinogen and PV value were determined on both EDTA anticoagulant and citrate anticoagulant sampling tubes to obtain quantitative results for all three parameters, irrespective of the anticoagulant used in the sampling tube. The PV values were determined on a VROC automatic viscometer (RheoSense, California, USA) at patient temperature using the viscometer-adapted temperature stabilizer (RheoSense, California, USA).

The characteristics of the patient cohort were analysed for electively operated patients compared to emergency operated patients. The statistically significant differences between these two groups were observed in the percentage of active smoking patients (13.95%, n=6 versus 57.14%, n=4, p=0.02) and the APACHE II score at ICU admission (9.6 ± 3.0 versus 9.1 ± 3.0, p=0.01). No other statistically significant differences in preoperative, intraoperative or postoperative characteristics of elective or emergency patients were revealed.

Chapter 5 describes the perioperative trajectory, coefficients of variation and percentage changes in PV values, total protein concentration, fibrinogen concentration and haemoglobin values in patients undergoing cardiac surgery.

The lowest absolute values for PV (EDTA: 1.3 ± 0.15 cP, citrate: 1.23 ± 0.15 cP), total protein (EDTA: 4.37 ± 0.75 g/dL, citrate: 3.83 ± 0.65 g/dL), fibrinogen (EDTA: 1.96 ± 0.46 g/L, citrate: 2.06 ± 0.59 g/L) and haemoglobin (8.42 ± 1.27 g/dL) were observed at the end of CB, following acute normovolemic haemodilution during CPB. Starting from the preoperative time point, PV reached a statistically significantly lower value at the end of CPB (RANOVA, $p<0.001$), then progressively increasing until postoperative day 6 and reaching a value that was not statistically significantly higher than the value at the preoperative time point. The total protein concentration was statistically significantly lower at the end of CPB (RANOVA, $p<0.001$) compared to the preoperative time point, then progressively increasing postoperatively to a statistically significantly lower value compared to the preoperative time point (RANOVA, $p<0.001$). This is consistent with the presence of acute haemodilution during CPB. Compared to the preoperative value, fibrinogen concentration was statistically significantly lower at the end of CPB (RANOVA, $p<0.001$) and reached a statistically significantly higher value on postoperative day 2 (RANOVA, $p<0.001$), thereafter the trajectory having a plateaued appearance. This appearance is consecutive to the presence of CPB-associated inflammatory syndrome in the first postoperative days. The haemoglobin concentration reached the absolute minimum mean value at the end of CPB, and the values subsequently stabilized until the end of the postoperative follow-up period, with a value statistically significantly lower compared to the preoperative time point (RANOVA, $p<0.001$). This trajectory reflects intraoperative haemodilution, perioperative bleeding and inflammation-induced anaemia in the postoperative period. PV, total protein, fibrinogen and haemoglobin exhibit similar trajectories in the context of cardiac surgery with CPB,

reflecting changes induced by the presence of haemodilution and inflammatory syndrome in the perioperative period of cardiac surgery (Figures 1a and 1b). The PV values were statistically significantly higher for patients operated on in emergency compared to those operated on electively for the preoperative time point only for determinations performed on EDTA anticoagulant medium (1.49 ± 0.14 cP versus 1.71 ± 0.21 cP, p=0.04), and the difference was not observed for any other subsequent time point (T1-T9). Also, no statistically significant difference was observed for any of the T0-T9 time points in the PV value between patients who received intraoperative haemofiltration and patients who didn't receive intraoperative haemofiltration.

During the perioperative period, very small values were demonstrated for the interindividual coefficients of variation and percentage changes in PV, both for the preoperative time and following acute intraoperative haemodilution and the inflammatory syndrome in the postoperative period (Table 1). The overall coefficient of variation of fibrinogen was the only one statistically significantly higher compared to the overall coefficient of variation of PV (ANOVA, $p<0.05$). The coefficients of variation were not statistically significantly different for total protein and haemoglobin compared to PV coefficients of variation at preoperative and postoperative time points, but only at the end of CPB (ANOVA, $p<0.05$) due to acute haemodilution. The fibrinogen coefficients of variation were statistically significantly higher compared to PV coefficients of variation preoperatively (ANOVA, $p<0.05$), at the end of CPB (ANOVA, $p<0.05$) and postoperatively (ANOVA, $p<0.05$). these differences were observed for both EDTA and citrate measurements.

PV showed the lowest overall percentage changes (EDTA: 6.50%, citrate: 5.10%) compared to total protein (EDTA: 18.80%, citrate 17.80%, RANOVA, $p<0.001$), fibrinogen (EDTA 36.20%, citrate 47.30%, RANOVA, $p<0.001$) and haemoglobin (27.40%, RANOVA, $p<0.001$) for both EDTA and citrate measurements.

These aspects confirm data from the experimental literature and indicate that PV is tightly controlled within a narrow range of values, even in the context of acute haemodilution, the presence of inflammatory syndrome and increased variations in PV determinants (total protein concentration and fibrinogen concentration). The reasons and factors that determine this tight control of the PV value are not known, but some experimental data report protein conformation, pH and temperature as possible factors involved in viscosity change [36].

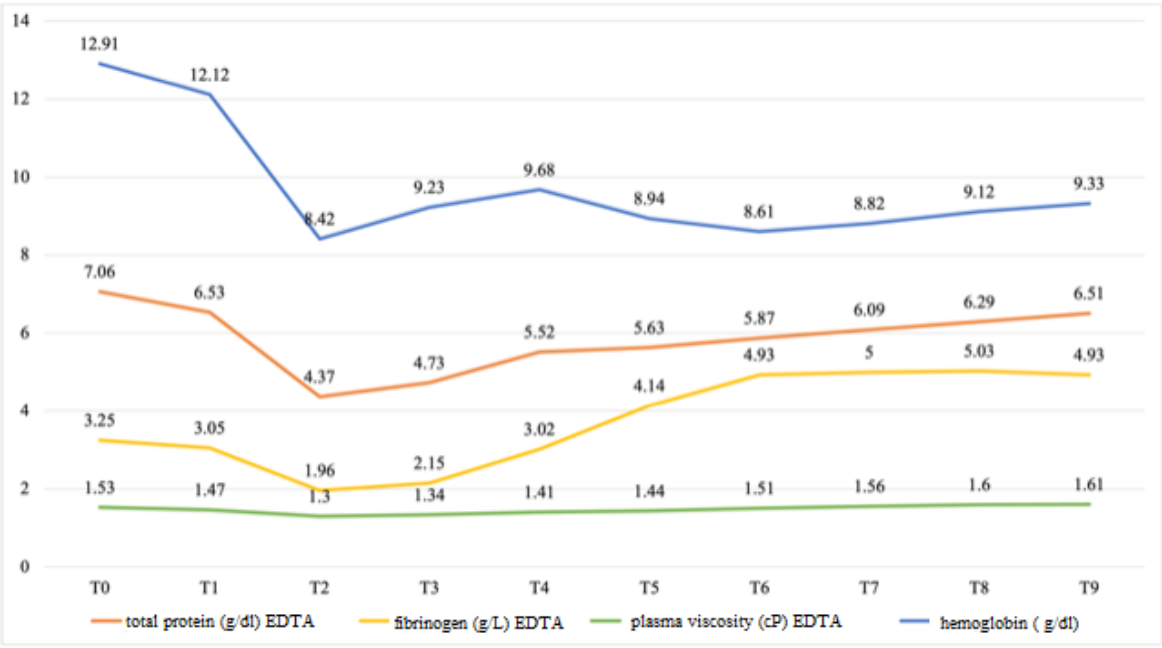

Figure 1a. Perioperative trajectories of haemoglobin, VP, total protein and fibrinogen (EDTA)

Legend: T0 – preoperative; T1 – start CPB; T2 – end CPB; T3 – end of surgery; T4-T9 – postoperative day 1-6

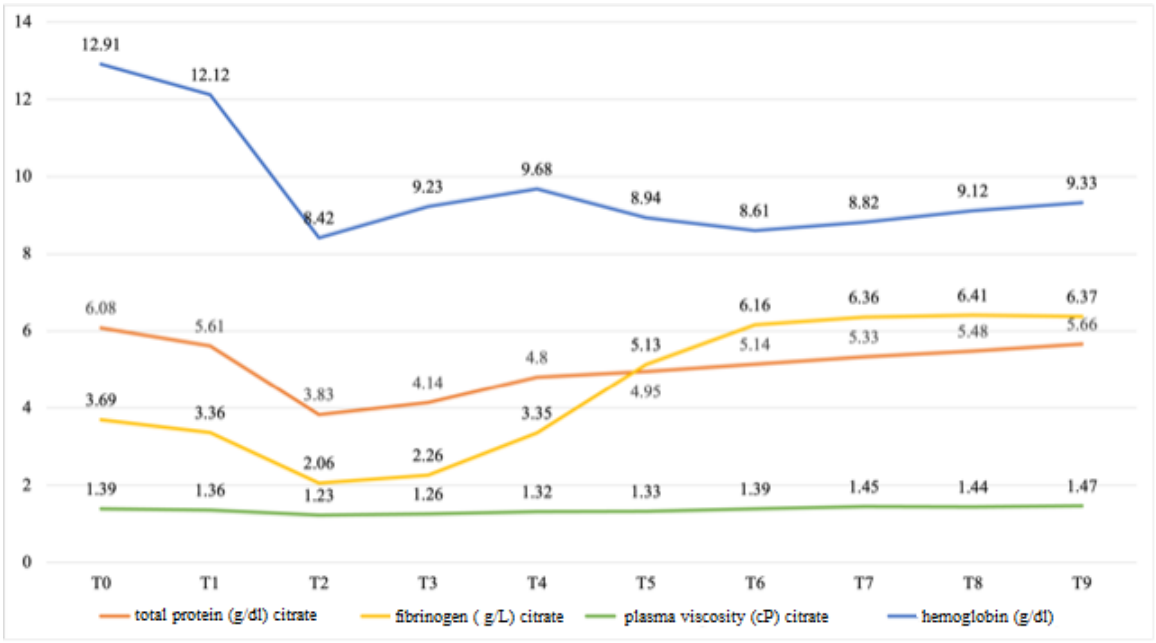

Figure 1b. Perioperative trajectories of haemoglobin, VP, total protein and fibrinogen (citrate)

Legend: T0 – preoperative; T1 – start CPB; T2 – end CPB; T3 – end of surgery; T4-T9 – postoperative day 1-6

Table 1. Coefficients of variation (%) for PV, total protein, fibrinogen and haemoglobin for T1-T9 time points compared to T0 time point

Time / Variable	T0 (preop)	T1 (start CPB)	T2 (end CPB)	T3 (end surgery)	T4 (1st POD)	T5 (2nd POD)	T6 (3rd POD)	T7 (4th POD)	T8 (5th POD)	T9 (6th POD)	Mean ± SD
EDTA plasma viscosity (%)	10.44	11.34	11.89	9.91	13.45	11.25	11.39	12.34	11.06	9.98	11.3 ± 1.08
p value (versus T0)[1]	-	0.56	0.37	0.71	0.08	0.60	0.54	0.25	0.69	0.76	-
Citrate plasma viscosity (%)	9.99	10.65	12.52	11.25	12.99	11.68	10.26	16.18	15.28	10.44	12.12 ± 2.14
p value (versus T0)[1]	-	0.65	0.12	0.41	0.07	0.28	0.85	**0.004**	**0.005**	0.76	-
EDTA total protein (%)	8.52	9.23	17.11[#]	13.53[#]	14.63	11.88	9.25	10.45	9.74	10.36	11.47 ± 2.79
p value (versus T0)[1]	-	0.58	**<0.001**	**0.001**	**0.003**	**0.023**	0.57	0.16	9.35	0.17	-
Citrate total protein (%)	8.27	9.17	16.88[#]	14.01[#]	14.39	11.42	8.39	10.56[#]	9.78[#]	10.72	11.35 ± 2.85
p value (versus T0)[1]	-	0.47	**<0.001**	**0.003**	**0.002**	**0.002**	0.92	0.09	0.24	0.07	-

EDTA fibrinogen (%)	17.48#	15.83#	23.37#	20.70#	18.59#	12.76	15.37#	13.36	14.02#	15.06#	**16.65 ± 3.39***
p value (versus T0)[1]	-	0.50	0.05	0.25	0.67	**0.02**	0.38	0.08	0.13	0.31	**-**
Citrate fibrinogen (%)	20.40#	19.94#	28.56#	24.16#	20.66#	16.97#	17.49#	16.67	17.44#	18.26#	**20.05 ± 3.76***
p value (versus T0)[1]	-	0.95	**0.02**	0.26	0.93	0.21	0.30	0.17	0.29	0.45	-
Haemoglobin (%)	13.30	15.36#	15.00#	13.48#	12.55	12.35	11.07	10.15	9.25	9.10	12.16 ± 2.22
p value (versus T0)[1]	-	0.32	0.41	0.92	0.69	0.61	0.21	0.06	**0.01**	**0.01**	-

Abbreviations: SD = standard deviation; EDTA = Ethylenediaminetetraacetic acid; 1st POD-6th POD – postoperative day 1-6.

[1]The p value refers to comparisons with preoperative coefficient of variation for each time point, for each variable separately. *Statistically different global coefficient of variation compared to global coefficients of variation for PV, total protein and haemoglobin ($p<0.05$). #Statistically different coefficient of variation compared to coefficient of variation for PV at the same time point. The coefficients of variation for all variables for all time points were compared using one-way ANOVA test.

Chapter 6 describes the sources of variation of the PV value for preoperative, end of CPB and day 6 postoperatively. Patients operated on in emergency had statistically significantly higher preoperative PV values compared to electively operated patients (EDTA: 1.49 ± 0.14 cP versus 1.71 ± 0.21 cP, p=0.04). The urgency of surgery may indicate the presence of a large number of therapeutically uncontrolled cardiovascular risk factors, the cumulative effect of which results in an increased PV value. Contrary to data reported in the literature, no statistically significant differences were observed in this cohort between preoperative PV values according to age, sex or preoperative presence of cardiovascular risk factors such as active smoking, hypertension, dyslipidaemia or diabetes. Furthermore, the presence of cardiovascular risk factors was not associated with preoperative PV value. The total protein concentration was the only factor associated with PV values preoperatively (EDTA: B=0.052, 95% CI [0.008-0.169], p=0.028; citrate: B=0.095, 95% CI [0.015-0.176], p=0.022), at the end of CPB (EDTA: B=0.105, 95% CI [0.003-0.210], p=0.043; citrate: B=0.087, 95% CI [0.018-0.155], p=0.014) and postoperatively (EDTA: B=0.130, 95% CI [0.070-0.189], <0.001; citrate: B=0.112, 95% CI [0.042-0.182], p=0.002) in multivariate linear regression analysis. The total protein concentration was also the main determinant of PV value in the linear mixed model analysis. In univariate linear regression analysis, fibrinogen concentration was associated with the PV value preoperatively, at the end of CPB and postoperatively, but this relationship did not hold in multivariate linear regression analysis. This result is contradictory to data in the literature showing that the PV value may be influenced by fibrinogen concentration in the inflammatory syndrome in some animal species [33]. CPB duration and intraoperative haemofiltrate volume were associated with the PV value in univariate linear regression analysis, but the association did not hold in multivariate linear regression analysis. Given that CPB and haemofiltration play an important role in the degree of intraoperative haemodilution, the absence of these associations should be interpreted with caution given the possible lack of statistical power of the study. The value of C-reactive protein as an indicator of postoperative inflammatory syndrome was not associated with the PV value for the postoperative time point, but the absence of this association may be related to the possible lack of statistical power of the study.

Chapter 7 assesses the impact of the PV value on postoperative outcome in cardiac surgery patients. The study does not find a statistically significant difference in terms of length of stay in the ICU, peak perioperative creatinine value or lactate value at the end of

CPB between patients with PV values less than 1.2 cP and patients with PV values higher than 1.2 cP at the end of CPB. Also, no significant difference was observed between the 25% and 75% quartiles of the ICU length of stay when compared according to the PV values at T0, T2 and T9. The lack of these statistically significant differences can be explained by the small number of patients included in the study and the fact that, due to the exploratory nature of the main objective, no statistical power analysis was performed prior to the investigation. The lack of a significant relationship between the 1.2 cP critical value of PV and the postoperative outcome of patients undergoing cardiac surgery also suggests that a large number of patients or the presence of extreme pathological conditions (massive haemodilution, exsanguination), similar to those in experimental studies, which are difficult to replicate in clinical trials, would be required in the attempt to demonstrate this.

The choice of the ideal calcium chelator for measuring PV, total protein concentration and fibrinogen concentration from the same sample tube remains an open discussion. Both samples using citrate and samples using EDTA as an anticoagulant can be used for measuring fibrinogen concentration and total protein concentration. The application of correction coefficients is necessary for the measurement of total protein concentration for determinations on citrate anticoagulant and for the measurement of fibrinogen concentration for determinations on EDTA anticoagulant, respectively [37]. The PV value is not significantly influenced by sampling on EDTA or citrate anticoagulant [37]. Although differences in PV, total protein and fibrinogen values measured from tubes with EDTA or citrate anticoagulant were identified in this study, the perioperative trajectories of the three parameters analysed were similar irrespective of the anticoagulant used in the sampling tube. Moreover, total protein concentration was the only factor linked with the perioperative PV value in multivariate linear regression analysis and in mixed linear model analysis, regardless of the calcium chelator used for sampling and processing. Most PV values lower than 1.2 cP were measured in samples using citrate anticoagulant. These aspects may be important for the methodology of future studies investigating the relationship between the PV values and microcirculatory changes or postoperative course of patients.

Conclusions and Personal Contributions

This thesis reports the first study in the literature describing the trajectory of PV in the context of haemodilution and CPB-associated inflammatory syndrome. The study demonstrates that PV is tightly controlled within a narrow range of values even in the presence of these two acute events, but the factors or mechanisms that determine the tight control of the PV value, irrespective of variations in total protein concentration and fibrinogen concentration, are not known.

The total protein concentration is the main determinant of the PV value. This implies that a decrease in total protein concentration can be considered a surrogate for a decrease in the PV value in the perioperative period of cardiac surgery.

Although a significant relationship between the 1.2 cP critical value of PV and the occurrence of organ dysfunction in the postoperative period has not been demonstrated, FCD and other parameters used to assess microcirculation could be altered following changes in PV, before microcirculation alteration is evident clinically or by laboratory determinations of tissue hypoperfusion markers and thus before the onset of organ dysfunction.

The results of the present study will facilitate the design of observational/interventional studies that will analyse the statistical relationship between absolute or relative changes in PV, FCD as an indicator of microcirculatory conditions, and organ dysfunction.

Selective bibliography

1. Cecconi M, De Backer D, Antonelli M, Beale R, Bakker J, Hofer C, Jaeschke R, Mebazaa A, Pinsky MR, Teboul JL, Vincent JL, Rhodes A. Consensus on circulatory shock and hemodynamic monitoring. Task force of the European Society of Intensive Care Medicine. Intensive Care Med. 2014 Dec;40(12):1795-815.
2. Ince C. Hemodynamic coherence and the rationale for monitoring the microcirculation. Crit Care. 2015;19 Suppl 3(Suppl 3):S8.
3. Kara A, Akin S, Ince C. The response of the microcirculation to cardiac surgery. Curr Opin Anaesthesiol. 2016 Feb;29(1):85-93.

4. Baskurt OK, Meiselman HJ. Blood rheology and hemodynamics. Semin Thromb Hemost. 2003 Oct;29(5):435-50.
5. Cabrales P, Tsai AG. Plasma viscosity regulates systemic and microvascular perfusion during acute extreme anemic conditions. Am J Physiol Heart Circ Physiol. 2006 Nov;291(5):H2445-52.
6. Tsai AG, Friesenecker B, McCarthy M, Sakai H, Intaglietta M. Plasma viscosity regulates capillary perfusion during extreme hemodilution in hamster skinfold model. Am J Physiol. 1998 Dec;275(6):H2170-80.
7. Cowan AQ, Cho DJ, Rosenson RS. Importance of blood rheology in the pathophysiology of atherothrombosis. Cardiovasc Drugs Ther. 2012 Aug;26(4):339-48.
8. Danesh J, Collins R, Peto R, Lowe GD. Haematocrit, viscosity, erythrocyte sedimentation rate: meta-analyses of prospective studies of coronary heart disease. Eur Heart J. 2000 Apr;21(7):515-20.
9. Brun JF, Varlet-Marie E, Connes P, Aloulou I. Hemorheological alterations related to training and overtraining. Biorheology. 2010;47(2):95-115.
10. De Backer D, Creteur J, Dubois MJ, Sakr Y, Vincent JL. Microvascular alterations in patients with acute severe heart failure and cardiogenic shock. Am Heart J. 2004 Jan;147(1):91-9.
11. Segal SS. Regulation of blood flow in the microcirculation. Microcirculation. 2005 Jan-Feb;12(1):33-45.
12. Davis MJ. Perspective: physiological role(s) of the vascular myogenic response. Microcirculation. 2012 Feb;19(2):99-114.
13. Balligand JL, Feron O, Dessy C. eNOS activation by physical forces: from short-term regulation of contraction to chronic remodeling of cardiovascular tissues. Physiol Rev. 2009 Apr;89(2):481-534.
14. De Backer D, Ortiz JA, Salgado D. Coupling microcirculation to systemic hemodynamics. Curr Opin Crit Care. 2010 Jun;16(3):250-4.
15. De Backer D, Donadello K, Favory R. Link between coagulation abnormalities and microcirculatory dysfunction in critically ill patients. Curr Opin Anaesthesiol. 2009 Apr;22(2):150-4.
16. De Backer D, Hollenberg S, Boerma C, Goedhart P, Büchele G, Ospina-Tascon G, Dobbe I, Ince C. How to evaluate the microcirculation: report of a round table conference. Crit Care. 2007;11(5):R101.

17. Johansson PI, Stensballe J, Ostrowski SR. Shock induced endotheliopathy (SHINE) in acute critical illness - a unifying pathophysiologic mechanism. Crit Care. 2017 Feb 9;21(1):25.
18. De Backer D, Donadello K, Sakr Y, Ospina-Tascon G, Salgado D, Scolletta S, Vincent JL. Microcirculatory alterations in patients with severe sepsis: impact of time of assessment and relationship with outcome. Crit Care Med. 2013 Mar;41(3):791-9.
19. De Santis P, De Fazio C, Franchi F, Bond O, Vincent JL, Creteur J, Taccone FS, Scolletta S. Incoherence between Systemic Hemodynamic and Microcirculatory Response to Fluid Challenge in Critically Ill Patients. J Clin Med. 2021 Feb 1;10(3):507.
20. Ince C, Boerma EC, Cecconi M, De Backer D, Shapiro NI, Duranteau J, Pinsky MR, Artigas A, Teboul JL, Reiss IKM, Aldecoa C, Hutchings SD, Donati A, Maggiorini M, Taccone FS, Hernandez G, Payen D, Tibboel D, Martin DS, Zarbock A, Monnet X, Dubin A, Bakker J, Vincent JL, Scheeren TWL; Cardiovascular Dynamics Section of the ESICM. Second consensus on the assessment of sublingual microcirculation in critically ill patients: results from a task force of the European Society of Intensive Care Medicine. Intensive Care Med. 2018 Mar;44(3):281-299.
21. Cabrales P, Martini J, Intaglietta M, Tsai AG. Blood viscosity maintains microvascular conditions during normovolemic anemia independent of blood oxygen-carrying capacity. Am J Physiol Heart Circ Physiol. 2006 Aug;291(2):H581-90.
22. Cabrales P, Tsai AG, Intaglietta M. Microvascular pressure and functional capillary density in extreme hemodilution with low- and high-viscosity dextran and a low-viscosity Hb-based O2 carrier. Am J Physiol Heart Circ Physiol. 2004 Jul;287(1):H363-73.
23. Dubin A, Pozo MO, Casabella CA, Murias G, Pálizas F Jr, Moseinco MC, Kanoore Edul VS, Pálizas F, Estenssoro E, Ince C. Comparison of 6% hydroxyethyl starch 130/0.4 and saline solution for resuscitation of the microcirculation during the early goal-directed therapy of septic patients. J Crit Care. 2010 Dec;25(4):659.e1-8.
24. Koning NJ, Vonk AB, van Barneveld LJ, Beishuizen A, Atasever B, van den Brom CE, Boer C. Pulsatile flow during cardiopulmonary bypass preserves postoperative

microcirculatory perfusion irrespective of systemic hemodynamics. J Appl Physiol (1985). 2012 May;112(10):1727-34.

25. Mathie RT, Ohri SK, Keogh BE, Williams J, Siney L, Griffith TM. Nitric oxide activity in patients undergoing cardiopulmonary bypass. J Thorac Cardiovasc Surg. 1996 Nov;112(5):1394-5.
26. Ospina-Tascon G, Neves AP, Occhipinti G, Donadello K, Büchele G, Simion D, Chierego ML, Silva TO, Fonseca A, Vincent JL, De Backer D. Effects of fluids on microvascular perfusion in patients with severe sepsis. Intensive Care Med. 2010 Jun;36(6):949-55.
27. Cabrales P, Tsai AG, Frangos JA, Briceño JC, Intaglietta M. Oxygen delivery and consumption in the microcirculation after extreme hemodilution with perfluorocarbons. Am J Physiol Heart Circ Physiol. 2004 Jul;287(1):H320-30.
28. Habib RH, Zacharias A, Schwann TA, Riordan CJ, Engoren M, Durham SJ, Shah A. Role of hemodilutional anemia and transfusion during cardiopulmonary bypass in renal injury after coronary revascularization: implications on operative outcome. Crit Care Med. 2005 Aug;33(8):1749-56.
29. Gaffney AM, Sladen RN. Acute kidney injury in cardiac surgery. Curr Opin Anaesthesiol. 2015 Feb;28(1):50-9.
30. Verheij J, van Lingen A, Raijmakers PG, Rijnsburger ER, Veerman DP, Wisselink W, Girbes AR, Groeneveld AB. Effect of fluid loading with saline or colloids on pulmonary permeability, oedema and lung injury score after cardiac and major vascular surgery. Br J Anaesth. 2006 Jan;96(1):21-30.
31. Aird WC. The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. Blood. 2003 May 15;101(10):3765-77.
32. Rehm M, Bruegger D, Christ F, Conzen P, Thiel M, Jacob M, Chappell D, Stoeckelhuber M, Welsch U, Reichart B, Peter K, Becker BF. Shedding of the endothelial glycocalyx in patients undergoing major vascular surgery with global and regional ischemia. Circulation. 2007 Oct 23;116(17):1896-906.
33. Johnn H, Phipps C, Gascoyne S, Hawkey C, Rampling M. A comparison of the viscometric properties of the blood from a wide range of mammals. Clin Hemorheol Microcirc. 2003;12(1):639-647.
34. Cabrales P, Tsai AG, Intaglietta M. Increased plasma viscosity prolongs microhemodynamic conditions during small volume resuscitation from hemorrhagic shock. Resuscitation. 2008 Jun;77(3):379-86.

35. Lowe GD, Lee AJ, Rumley A, Price JF, Fowkes FG. Blood viscosity and risk of cardiovascular events: the Edinburgh Artery Study. Br J Haematol. 1997 Jan;96(1):168-73.
36. Masuelli MA, Sansone MG. Hydrodynamic properties of gelatin-studies from intrinsic viscosity measurements. Products and Applications of Biopolymers. InTech. 2012 Mar; 8(1):85-116.
37. Baskurt OK, Boynard M, Cokelet GC, Connes P, Cooke BM, Forconi S, Liao F, Hardeman MR, Jung F, Meiselman HJ, Nash G, Nemeth N, Neu B, Sandhagen B, Shin S, Thurston G, Wautier JL; International Expert Panel for Standardization of Hemorheological Methods. New guidelines for hemorheological laboratory techniques. Clin Hemorheol Microcirc. 2009;42(2):75-97.

List of published articles related to the present research

Valeanu L, Bubenek-Turconi SI, Ginghina C, Balan C. Hemodynamic Monitoring in Sepsis-A Conceptual Framework of Macro- and Microcirculatory Alterations. Diagnostics (Basel). 2021 Aug 28;11(9):1559.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8469937/
Literature review from General part, chapter 2.
Published in Diagnostics; SCIE (Web of Science) indexed; ISSN: 2075-4418; 2021 Impact Factor: 3.992.

Valeanu L, Andrei S, Ginghina C, Robu C, Ciurciun A, Balan C, Stefan M, Stoian A, Stanculea I, Cheta A, Dima L, Stiru O, Filipescu D, Bubenek-Turconi SI, Longrois D. Perioperative trajectory of plasma viscosity: A prospective, observational, exploratory study in cardiac surgery. Microcirculation. 2022 Jul;29(4-5):e12777.
https://onlinelibrary.wiley.com/doi/10.1111/micc.12777
Prospective, observational study; data from chapters 3-8.
Published in Microcirculation; SCIE (Web of Science) indexed; ISSN: 1549-8719; 2021 Impact Factor: 2,679.