


BMJ Open Prevention of vasoplegia with CytoSorb in heart failure patients undergoing cardiac surgery (CytoSorb-HF trial): protocol for a randomised controlled trial

Olga Papazisi ¹, Eline F Bruggemans,¹ Remco R Berendsen,² Juan D V Hugo,¹ Jan H N Lindeman,³ Saskia L M A Beeres,⁴ M Sesmu Arbous,⁵ Wilbert B van den Hout,⁶ Bart J A Mertens,⁶ Can Ince,⁷ Robert J M Klautz,¹ Meindert Palmen¹

To cite: Papazisi O, Bruggemans EF, Berendsen RR, *et al.* Prevention of vasoplegia with CytoSorb in heart failure patients undergoing cardiac surgery (CytoSorb-HF trial): protocol for a randomised controlled trial. *BMJ Open* 2022;**12**:e061337. doi:10.1136/bmjopen-2022-061337

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2022-061337>).

Received 21 February 2022
Accepted 12 August 2022



© Author(s) (or their employer(s)) 2022. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

Correspondence to
Dr Meindert Palmen;
M.Palmen@lumc.nl

ABSTRACT

Introduction Vasoplegia is a common complication after cardiac surgery and is associated with poor prognosis. It is characterised by refractory hypotension despite normal or even increased cardiac output. The pathophysiology is complex and includes the systemic inflammatory response caused by cardiopulmonary bypass (CPB) and surgical trauma. Patients with end-stage heart failure (HF) are at increased risk for developing vasoplegia. The CytoSorb adsorber is a relatively new haemoadsorption device which can remove circulating inflammatory mediators in a concentration based manner. The CytoSorb-HF trial aims to evaluate the efficacy of CytoSorb haemoadsorption in limiting the systemic inflammatory response and preventing postoperative vasoplegia in HF patients undergoing cardiac surgery with CPB.

Methods and analysis This is an investigator-initiated, single-centre, randomised, controlled clinical trial. In total 36 HF patients undergoing elective cardiac surgery with an expected CPB duration of more than 120 min will be randomised to receive CytoSorb haemoadsorption along with standard surgical treatment or standard surgical treatment alone. The primary endpoint is the change in systemic vascular resistance index with phenylephrine challenge after CPB. Secondary endpoints include inflammatory markers, sublingual microcirculation parameters and 30-day clinical indices. In addition, we will assess the cost-effectiveness of using the CytoSorb adsorber. Vascular reactivity in response to phenylephrine challenge will be assessed after induction, after CPB and on postoperative day 1. At the same time points, and before induction and on postoperative day 4 (5 time points in total), blood samples will be collected and the sublingual microcirculation will be recorded. Study participants will be followed up until day 30.

Ethics and dissemination The trial protocol was approved by the Medical Ethical Committee of Leiden The Hague Delft (METC LDD, registration number P20.039). The results of the trial will be published in peer-reviewed medical journals and through scientific conferences.

Trial registration number NCT04812717.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This is the first randomised controlled trial evaluating the efficacy of CytoSorb in preventing vasoplegia in heart failure patients undergoing cardiac surgery with cardiopulmonary bypass.
- ⇒ Postoperative vasoplegia is associated with poor outcomes highlighting the importance of new therapeutic or preventive options for this complication.
- ⇒ Except for the perfusionists, patients, clinicians and non-clinician investigators involved in the trial will be blinded for treatment allocation to minimise potential bias.
- ⇒ The effect of CytoSorb will be investigated also at the microcirculatory level.
- ⇒ The trial involves only one centre which might limit the generalisability of the results.

INTRODUCTION

Vasoplegia is a common complication after cardiac surgery with cardiopulmonary bypass (CPB) with an incidence that ranges between 5% and 47%, depending on the population being investigated and the definition used.¹⁻⁴ It is characterised by low systemic vascular resistance (SVR) which results in severe hypotension in the presence of normal or even increased cardiac output (CO) and blunted or no response to administration of vasopressors.⁵ The complication is associated with increased morbidity and mortality rates and, consequently, has important negative consequences for patients and healthcare costs.^{2,6}

The precise aetiology of postoperative vasoplegia is still unclear. However, different mechanisms are thought to be involved.⁵ The combination of exposure of blood to the foreign surfaces of CPB and surgical trauma triggers a systemic inflammatory response, which is considered a causative factor in the development of vasoplegia. The sequential

release of numerous inflammatory mediators leads to the inactivation of vasoconstrictor mechanisms and the concurrent activation of vasodilatory pathways that may lead to systemic hypotension and subsequent potential organ injury.⁷

Patients with end-stage heart failure (HF) undergoing cardiac surgery with CPB are known to be more susceptible to vasoplegia than patients without HF.⁸ More specifically, left ventricular ejection fraction <35%–40% has been reported to be an independent predictor of vasoplegia after cardiac surgery with CPB.^{1,9} A pre-existing, increased inflammatory profile along with the compensatory chronic activation of the sympathetic nervous system and the renin-angiotensin-aldosterone system may be responsible for the development of postoperative vasoplegia in HF patients.^{10–12}

Recently, we demonstrated in the VASOR study performed at our institution¹³ that HF patients exhibit a diminished vascular responsiveness to the administration of phenylephrine (α_1 -agonist) already before surgery compared with patients without HF. Further, we showed that the vascular responsiveness is almost completely abolished in HF patients after the use of CPB. We speculate that the results of this study reflect a possible downregulation and/or desensitisation of vascular α_1 -adrenoreceptors due to chronic endogenous adrenergic stimulation in HF patients, as it has already been documented for β_1 -adrenoreceptors and β_2 -adrenoreceptors.¹⁴ We hypothesise that this reduced vascular responsiveness may render HF patients more sensitive to the systemic inflammatory response during cardiac surgery. Thus, minimising the systemic inflammatory response could be a treatment strategy to mitigate vasoplegia in this patient population.

The CytoSorb adsorber (CytoSorbents Corporation, New Jersey, USA) is a haemoadsorption device that was approved for use in Europe in 2011. It is a single-use device that contains polymer beads that adsorb cytokines as blood passes through the device. The use of the device has already been tested in multiple studies which report its safety and efficacy in cytokine reduction and, consequently, inflammation reduction.^{15–17} However, until the time this protocol was written, no studies had been conducted to assess the efficacy of the use of CytoSorb in HF patients undergoing cardiac surgery.

Trial objectives

Primary objective: to evaluate the efficacy of CytoSorb use in HF patients undergoing cardiac surgery in improving vascular responsiveness after CPB and, consequently, in preventing postoperative vasoplegia.

Secondary objectives:

- ▶ To investigate the performance of the device in reducing inflammatory mediators.
- ▶ To investigate the performance of the device in improving clinical outcomes.
- ▶ To investigate the performance of the device in improving microcirculation.

- ▶ To investigate the cost-effectiveness of using the device.

METHODS AND ANALYSIS

The trial protocol is written in accordance with the ‘Standard Protocol Items: Recommendations for Interventional Trials’ checklist¹⁸ (online supplemental file 1).

Trial design and study setting

This is an investigator-initiated, single-centre, randomised controlled clinical trial in patients with HF who undergo cardiac surgery with CPB. The trial will be conducted at Leiden University Medical Center (LUMC).

Trial population

Patients with HF planned for cardiac surgery with CPB with an anticipated duration of at least 120 min will be considered for participation in the trial. Detailed inclusion and exclusion criteria are given below.

Inclusion criteria

- ▶ Diagnosed with HF in line with the European Society of Cardiology guidelines.¹⁹
- ▶ Left ventricular ejection fraction $\leq 35\%$.
- ▶ Undergoing cardiac surgery with CPB with an anticipated duration of >120 min.
- ▶ Age ≥ 18 years.

Exclusion criteria

- ▶ Mentally incapacitated.
- ▶ Emergency operation.
- ▶ Need for preoperative vasopressor support and/or moderate or high dosages of intravenous inotropic support (>4 gamma dobutamine or dopamine).
- ▶ Severe tricuspid regurgitation.
- ▶ Daily use of nitroglycerine or isosorbide dinitrate.
- ▶ Use of alpha blockers.
- ▶ Being heparin-induced thrombocytopenia positive and citrate regional anticoagulation is unavailable as an alternative anticoagulation method.
- ▶ Platelet count $< 20 \times 10^9/L$.

Randomisation and blinding

Patients will be randomised to receive either CytoSorb haemoadsorption along with standard surgical treatment (intervention) or standard surgical treatment without CytoSorb (control) in a 1:1 ratio, using block randomisation with random block sizes of 4 and 6. Randomisation will be performed in Castor EDC (Amsterdam, the Netherlands) by the responsible perfusionist (JDVH). Castor uses a validated random block randomisation model which ensures true randomness during the allocation procedure. Patients, clinicians (surgeons, anaesthesiologists and other practitioners) and non-clinician investigators will be blinded to treatment allocation until after statistical analysis. Perfusionists will hide the CytoSorb device (or the absence of it) from the sight of the surgeons and anaesthesiologists. Therefore, blinding

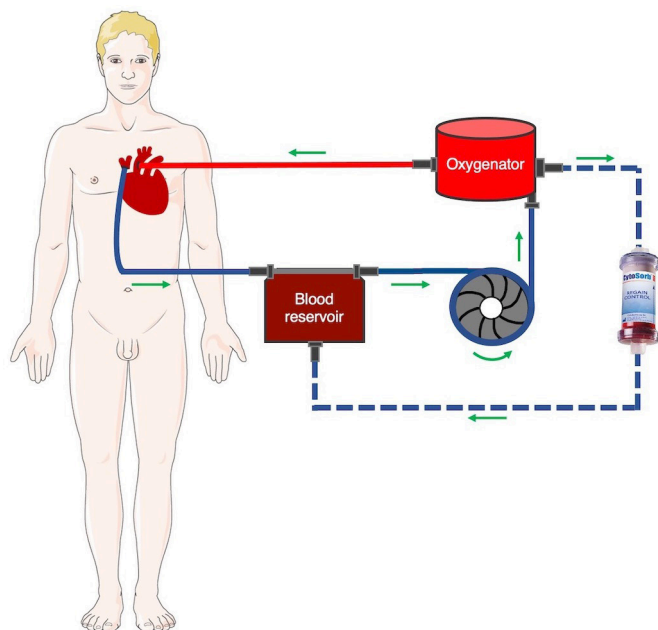


Figure 1 CytoSorb integration in the cardiopulmonary bypass system.

of perfusionists during CytoSorb use is not feasible. No sham device will be used.

Anaesthetics and haemodynamic monitoring

A standardised anaesthetic protocol will be used. Patients will be given propofol, remifentanyl and sufentanyl using target-controlled infusion. Administration of ketamine and sevoflurane is not allowed. For measurement of change in systemic vascular resistance index (delta SVR_i), the main study parameter, all patients will receive a pulmonary artery catheter. The catheter will be placed after induction and will be connected to a HemoSphere advanced monitoring system (Edwards LifeSciences, Irvine, California, USA).

Intervention

The CytoSorb device will be incorporated as a parallel shunt off of the main CPB system by perfusionists trained in the use of the device (figure 1).

Study time points and procedures

Vascular reactivity in response to phenylephrine challenge will be assessed in all trial participants after induction, after termination of CPB and on postoperative day 1. Taking of blood samples and assessment of sublingual microcirculation will be at the same time points and, additionally, before induction and on postoperative day 4 (5 time points in total). Clinical outcomes will be collected until postoperative day 30. The trial schedule can be found in figure 2.

Phenylephrine challenge

A bolus of 2 µg/kg phenylephrine will be given intravenously (same protocol as in the VASOR study) to measure its effect on SVR_i, mean arterial pressure (MAP) and other

haemodynamic parameters. When the treating physician decides that it is unsafe for the patient to administer a vasoconstrictor, the challenge will not be performed.

Blood samples

At each time point, blood samples will be collected into two tubes of 10 mL to analyse the inflammatory markers interleukin (IL)-6, IL-8 and IL-10. The samples will be centrifuged (1550g, 10 min, 4°C) and the plasma and serum will be stored at -80°C until analysis. Additional blood analysis other than prespecified may be planned based on the findings of this study.

Sublingual microcirculation

Sublingual microcirculation measurements will be performed using incident dark field imaging (Cytocam, Braedius Medical, Huizen, The Netherlands). On each time point, three image sequences at three different sublingual spots will be recorded per patient by two trained professionals. Each video clip of the microcirculatory flow will be assessed for adequate quality using the microcirculation image quality score proposed by Massey and Shapiro.²⁰ The completely automated MicroTools Software and the semi-automated Automated Vascular Analysis software V.3.2 (MicroVision Medical, Amsterdam, the Netherlands) will be used to obtain the microcirculatory parameters.^{21 22} The mean of the three measurements per spot will be noted.

Study parameters

Primary endpoints

- ▶ Delta SVR_i with phenylephrine challenge (defined as the SVR_i after phenylephrine administration minus the SVR_i before the challenge) after CPB.
- ▶ The occurrence of vasoplegia, defined as the continuous need of vasopressors (norepinephrine ≥ 0.2 µg/kg/min and/or terlipressin (any dose)) combined with a cardiac index (CI) ≥ 2.2 L/min/m² for at least 12 consecutive hours, starting within the first 3 days postoperatively.

Secondary endpoints

- ▶ Delta SVR_i with phenylephrine challenge on postoperative day 1.
- ▶ Total administered dosage of vasopressors.
- ▶ Change in interleukin IL-6, IL-8, IL-10 levels.
- ▶ Change in sublingual microcirculation parameters (microvascular flow index (MFI), capillary density, functional capillary density, total vessel density, proportion of perfused vessels, perfused vessel density, rolling leucocytes, mean cell velocity, capillary haematocrit, red blood cell velocity, heterogeneity index (calculated as the difference between the highest MFI minus the lowest MFI and divided by the mean MFI)).
- ▶ Change in MAP with phenylephrine challenge after CPB and on postoperative day 1.
- ▶ Hours on mechanical ventilation.
- ▶ Hours on mechanical circulatory support.
- ▶ Hours on postoperative renal replacement therapy.

each treatment group will have 90% power to detect a difference in means of 400 dyn s/cm⁵ assuming that the common SD is 350 dyn s/cm⁵²³ and when using a 0.05 two-sided significance level. To compensate for possible loss of data due to failing of the vasoconstriction test, one extra patient will be included in each group, resulting in a total sample size of 36 patients.

Statistical analysis

The intention-to-treat principle will be applied in all analyses. No missing outcome data will be imputed. All analyses will be performed using IBM SPSS Statistics for Windows, V.25.0 (IBM Corp, Armonk, New York, USA). A p value <0.05 will be considered statistically significant (two-sided).

Primary and secondary efficacy analysis

For comparison of primary and secondary continuous endpoints, the Student's t-test for independent samples or the Mann-Whitney U test will be used, where appropriate. For discontinuous endpoints, the Pearson χ^2 test or the Fisher's exact test will be used, where appropriate. Longitudinal data will be analysed using the linear mixed-effects model approach.

Cost-effectiveness analysis

A cost analysis will be performed, estimating costs during the index hospitalisation in the LUMC. Costs will include the CytoSorb use, vasopressor medication, blood transfusion products, amount of used resuscitation fluids, duration of surgery, ICU stay and non-ICU hospital stay. The CytoSorb device and medication will be valued using market prices. For other healthcare, reference prices will be used from the Dutch guidelines for economic evaluations in healthcare. Also, a cost-effectiveness analysis will be performed relating CytoSorb use costs to the occurrence of postoperative vasoplegia ('costs per prevented patient with vasoplegia').

Patient and public involvement

Patients and the public are not involved in the trial, including the trial design, conduct, evaluation and dissemination.

ETHICS AND DISSEMINATION

The current trial protocol was approved by the Medical Ethical Committee Leiden The Hague Delft (in Dutch: Medisch Ethische Toetsingscommissie Leiden Den Haag Delft (METC LDD), registration number P20.039). The initial trial protocol was approved on 27 November 2020. Prior to patient enrolment, the protocol was amended to include the sublingual microcirculation measurements and version 2.0 was approved by the METC LDD on 13 August 2021. The trial will be conducted in agreement with the Declaration of Helsinki (October 2013) and in accordance with the Medical Research Involving Human Subjects Act (in Dutch: Wet medisch-wetenschappelijk onderzoek met mensen (WMO)) and

Good Clinical Practice. Subsequent protocol amendments will be submitted to the METC LDD and registered on ClinicalTrials.gov. The LUMC has a liability insurance and, in addition, a medical research subject insurance which are both in accordance with the WMO.

Recruitment and consent

Eligible patients will receive oral and written information about the trial and will be given at least 24 hours for consideration (online supplemental file 2). Participation will be voluntary and written informed consent will be obtained the day before surgery by the Principal Investigator (MP) or the operating surgeon. Study participants can withdraw their consent at any time and without any consequences. Individuals that withdraw before data collection has started, will be replaced.

Data management

Handling of data complies with the General Data Protection Regulation (in Dutch: Algemene verordening gegevensbescherming). Data collection will be pseudonymised and the code key will be stored on a secured server from the LUMC that is backed up daily. Trial data will be stored and maintained in a database created in Castor EDC. Castor EDC complies with ICH E6 (R2) on Good Clinical Practice. Everyone involved in the trial will have authorised access to the data with own accounts and user rights. Reading rights will be allowed to persons carrying out data quality inspections. Data monitoring is provided throughout the study period by independent monitors of the department of Good Research Practice of the LUMC.

Safety monitoring and adverse events

A Data Safety Monitoring Board was not deemed necessary since this is a clinical trial evaluating a CE-marked medical device in the intended patient population and without known device-related complications. Serious adverse events (SAEs) will be reported through the web portal ToetsingOnline to the METC LDD within 7 days after the responsible investigator has first knowledge of the SAE. Adverse events and protocol deviations will be recorded. The Principal Investigator will submit a summary of the trial status, including SAE reports, to the METC LDD once a year.

Dissemination

The results of this trial will be published in peer-reviewed medical journals and presented at scientific conferences. Deidentified (including patient codes) trial datasets will be made available from the Principal Investigator on reasonable request. A data transfer agreement between the LUMC and the receiving institution will cover the transfer of the data.

Trial status

The first patient was enrolled in the trial on 27 October 2021. Study enrolment is currently limited due to the COVID-19 pandemic. Under normal circumstances, the

recruitment rate is expected to be approximately two patients per month.

DISCUSSION

Vasoplegia is a serious complication after cardiac surgery in patients with end-stage HF. In recent retrospective studies at our institution, vasoplegia occurred in 19%–23% of HF patients undergoing cardiac surgery with CPB, depending on the (sub)population studied, and was a significant contributor to mortality.⁶ The increasing prevalence of end-stage HF and the advent of more surgical options for this patient population highlight the importance of developing new strategies for the prevention or treatment of this postoperative complication. The CytoSorb adsorber, a haemoadsorption device capable of removing circulating inflammatory mediators, has shown promising results in a variety of patient populations. However, up until this study protocol was written, no study existed that had tested the efficacy of CytoSorb use in end-stage HF patients undergoing cardiac surgery with CPB. Therefore, with this randomised controlled clinical trial we aim to investigate the efficacy and cost-effectiveness of CytoSorb use in preventing vasoplegia and improving clinical outcomes in this fragile patient population.

Author affiliations

¹Department of Cardiothoracic Surgery, Leiden University Medical Center, Leiden, The Netherlands

²Department of Anaesthesiology, Leiden University Medical Center, Leiden, The Netherlands

³Department of Vascular Surgery, Leiden University Medical Center, Leiden, The Netherlands

⁴Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands

⁵Department of Intensive Care, Leiden University Medical Center, Leiden, The Netherlands

⁶Department of Biomedical Data Sciences, Leiden University Medical Center, Leiden, The Netherlands

⁷Department of Intensive Care, Laboratory of Translational Intensive Care, Erasmus University Rotterdam, Rotterdam, The Netherlands

Contributors MP and OP conceptualised the study. OP, EFB, RRB, JHNL and MP drafted the protocol. MP obtained funding. JDVH conceived the blinding procedure for non-perfusionists. OP, EFB and BJAM wrote the statistical analysis plan for the primary and secondary efficacy analysis. WBvdH wrote the analysis plan for the cost-effectiveness analysis. OP, EFB, RRB, JDVH, JHNL, SLMAB, MSA, WBvdH, BJAM, CI, RJMK and MP contributed to refinement of the protocol and approved the final manuscript.

Funding This trial is financially supported by the Zabawas Foundation and by CytoSorbents Europe GmbH. Neither of the two funders had any role in the trial design. Data collection, analysis, interpretation of the results and dissemination will be done by the trial's investigators independently from any potential conflicting interest of CytoSorbents Europe GmbH.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been

peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iD

Olga Papazisi <http://orcid.org/0000-0001-5048-5016>

REFERENCES

- Sun X, Zhang L, Hill PC, *et al*. Is incidence of postoperative vasoplegic syndrome different between off-pump and on-pump coronary artery bypass grafting surgery? *Eur J Cardiothorac Surg* 2008;34:820–5.
- van Vessel ME, Palmén M, Couperus LE, *et al*. Incidence and predictors of vasoplegia after heart failure surgery. *Eur J Cardiothorac Surg* 2017;51:532–8.
- Kortekaas KA, Lindeman JHN, Reinders MEJ, *et al*. Preexisting endothelial cell activation predicts vasoplegia after mitral valve surgery. *Interact Cardiovasc Thorac Surg* 2013;17:523–30.
- Asleh R, Alnsasra H, Daly RC, *et al*. Predictors and clinical outcomes of vasoplegia in patients bridged to heart transplantation with continuous-flow left ventricular assist devices. *J Am Heart Assoc* 2019;8:e013108.
- Orozco Vinasco DM, Triana Schoonewolff CA, Orozco Vinasco AC. Vasoplegic syndrome in cardiac surgery: definitions, pathophysiology, diagnostic approach and management. *Rev Esp Anestesiol Reanim* 2019;66:277–87.
- van Vessel ME, Palmén M, Couperus LE, *et al*. Vasoplegia after surgical left ventricular restoration: 2-year follow-up. *Ann Thorac Surg* 2018;106:1371–8.
- Landry DW, Oliver JA. The pathogenesis of vasodilatory shock. *N Engl J Med* 2001;345:588–95.
- Kortekaas KA, Lindeman JHN, Versteegh MIM, *et al*. Preexisting heart failure is an underestimated risk factor in cardiac surgery. *Neth Heart J* 2012;20:202–7.
- Carrel T, Englberger L, Mohacsi P, *et al*. Low systemic vascular resistance after cardiopulmonary bypass: incidence, etiology, and clinical importance. *J Card Surg* 2000;15:347–53.
- Adamo L, Rocha-Resende C, Prabhu SD, *et al*. Reappraising the role of inflammation in heart failure. *Nat Rev Cardiol* 2020;17:269–85.
- Kortekaas KA, Lindeman JH, Versteegh MI, *et al*. Heart failure determines the myocardial inflammatory response to injury. *Eur J Heart Fail* 2013;15:400–7.
- Colson PH, Bernard C, Struck J, *et al*. Post cardiac surgery vasoplegia is associated with high preoperative copeptin plasma concentration. *Crit Care* 2011;15:R255.
- Papazisi O, van Vessel ME, Beeres SL, *et al*. Abstract 15823: Diminished vasoresponsiveness in patients with heart failure. *Circulation* 2020;142.
- Ruffolo RR, Kopia G. Importance of receptor regulation in the pathophysiology and therapy of congestive heart failure. *Am J Med* 1986;80:67–72.
- Saller T, Hagl C, Woitsch S, *et al*. Haemadsorption improves intraoperative haemodynamics and metabolic changes during aortic surgery with hypothermic circulatory arrest. *Eur J Cardiothorac Surg* 2019;56:731–7.
- Nemeth E, Kovacs E, Racz K, *et al*. Impact of intraoperative cytokine adsorption on outcome of patients undergoing orthotopic heart transplantation—an observational study. *Clin Transplant* 2018;32:e13211.
- Träger K, Fritzlér D, Fischer G, *et al*. Treatment of post-cardiopulmonary bypass SIRS by hemoabsorption: a case series. *Int J Artif Organs* 2016;39:141–6.
- Chan A-W, Tetzlaff JM, Altman DG, *et al*. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158:200–7.

- 19 McDonagh TA, Metra M, Adamo M. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: developed by the task force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J* 2021;42:3599–726.
- 20 Massey MJ, Shapiro NI. A guide to human in vivo microcirculatory flow image analysis. *Crit Care* 2016;20:35.
- 21 Hilty MP, Guerci P, Ince Y, *et al.* MicroTools enables automated quantification of capillary density and red blood cell velocity in handheld vital microscopy. *Commun Biol* 2019;2:217.
- 22 Dobbe JGG, Streekstra GJ, Atasever B, *et al.* Measurement of functional microcirculatory geometry and velocity distributions using automated image analysis. *Med Biol Eng Comput* 2008;46:659–70.
- 23 Schwinn DA, McIntyre RW, Hawkins ED, *et al.* Alpha 1-adrenergic responsiveness during coronary artery bypass surgery: effect of preoperative ejection fraction. *Anesthesiology* 1988;69:206–17.